Emerging Infectious Diseases

Zoonotic Infections

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Reviewer Appreciation

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Correction
Vol. 27, No. 10
The name of author Xiaohui Wang was misspelled in Emergomyces orientalis Emergomycosis Diagnosed by Metagenomic Next-Generation Sequencing
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Centers for Disease Control and Prevention
Clinical Characteristics of Corynebacterium Bacteremia Caused by Different Species, Japan, 2014–2020

Ryosuke Yamamuro, Naoto Hosokawa, Yoshihito Otsuka, Ryosuke Osawa

In support of improving patient care, this activity has been planned and implemented by Medscape, LLC and Emerging Infectious Diseases. Medscape, LLC is jointly accredited by the Accreditation Council for Continuing Medical Education (ACCME), the Accreditation Council for Pharmacy Education (ACPE), and the American Nurses Credentialing Center (ANCC), to provide continuing education for the healthcare team.

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Learning Objectives

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

- Describe the proportion of true bacteremia and differences in clinical characteristics of patients with bacteremia from C. striatum, C. jeikeium, and other Corynebacterium species, based on a retrospective medical record review
- Describe differences in mortality and antimicrobial susceptibility in patients with bacteremia from C. striatum, C. jeikeium, and other Corynebacterium species, based on a retrospective medical record review
- Describe clinical implications of differences in clinical characteristics of patients with bacteremia from C. striatum, C. jeikeium, and other Corynebacterium species, based on a retrospective medical record review

CME Editor

P. Lynne Stockton Taylor, VMD, MS, ELS(D), Technical Writer/Editor, Emerging Infectious Diseases. Disclosure: P. Lynne Stockton Taylor, VMD, MS, ELS(D), has disclosed no relevant financial relationships.

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Corynebacterium bacteria are club-shaped gram-positive rods that are ubiquitous in the environment. Because Corynebacterium species other than C. diphtheriae colonize skin and mucous membranes in humans, Corynebacterium is typically considered a clinically nonsignificant contaminant in cultures (1). Recently, the frequency of detecting C. striatum and C. jeikeium as causative agents of severe bloodstream infections (2,3), infective endocarditis, pneumonia, meningitis, and skin and soft tissue infections (SSTIs) has increased (4). Furthermore, these 2 species have been identified most frequently in cultures of clinical specimens, mainly blood, pus, urine, and pleural effusion (5).

Studies that have identified Corynebacterium infections or bacteremia to the species level are limited, and most are case reports (6). The largest study to date of Corynebacterium bacteremia investigated 98 cases; however, the species were not identified (7). The largest study that identified Corynebacterium species included 30 cases of true bacteremia in 339 patients with positive blood cultures (8). In our study, we aimed to determine the differences in characteristics and clinical presentations for patients with bacteremia caused by C. striatum, C. jeikeium, or other species of Corynebacterium.

Materials and Methods

Study Design

We retrospectively reviewed electronic medical records and clinical microbiology records of patients with positive blood cultures for Corynebacterium spp. in Kameda Medical Center (Chiba, Japan) during January 2014–May 2020. This facility is an 865-bed, tertiary-care general medical center that provides a wide variety of services including general medicine, surgery, oncology, cardiothoracic surgery, hematopoietic stem cell transplantation, and renal transplantation to ≈310,000 persons each year. All patients with blood cultures positive for any organism are automatically referred to the infectious diseases department for consultation. Board-certified infectious disease physicians evaluate the patients and document the consultation report in medical records. The study protocol was reviewed and approved by the Kameda Medical Center Institutional Review Board (reference no. 20-046). The need for written informed consent was waived. The study complied with the principles of the Declaration of Helsinki.

Study Population

We included all patients at the hospital who had blood cultures positive for Corynebacterium spp. during the study period. We collected data about age, sex, underlying conditions, clinical diagnosis, 90-day mortality rates, species of Corynebacterium, and antimicrobial susceptibility. If the same patient had multiple episodes of Corynebacterium bacteremia during the study period, we included only the first episode.

Definitions

We defined a case as true bacteremia when 2 sets of blood cultures from a patient with signs of infection were positive for Corynebacterium spp. or when 1 set of blood cultures and a clinically relevant specimen from another site (e.g., urine or sputum) where the infection was thought to exist (on the basis of signs/symptoms and examination findings) were both positive for the same species of Corynebacterium. For patients with only 1 set of blood cultures in which Corynebacterium spp. were detected and for whom bacteremia was clinically suspected, new blood cultures were performed, and reevaluated as necessary, before antimicrobial agents were initiated. These patients were carefully followed by our infectious disease physicians to ensure the absence of infection. This definition was based on a previous study (9). Catheter-related bloodstream infection (CRBSI) was considered definite for patients who met 1 of the following 3 criteria: 1) ≥1 set of blood cultures and semiquantitative cultures of a catheter segment (>15 CFUs/plate) were both positive for the same Corynebacterium species; 2) peripheral blood cultures and blood cultures from a catheter lumen were both positive for the same species of Corynebacterium, and its differential time to positivity was >2 hours (10); or 3)
2 sets of blood cultures were positive for *Corynebacterium* species, and signs of inflammation or purulence were present at the catheter insertion site (11). Diagnosis of other focal infections were based on the US Centers for Disease Control and Prevention National Healthcare Safety Network criteria (12).

We classified a case as no focus when physical examination by infectious disease physicians revealed no localized signs of infection, urinalysis was negative for pyuria or bacteriuria, chest images (radiographs or computed tomography scans) showed no infiltrates or masses, and the case still satisfied the criteria for true bacteremia. Chronic kidney disease was defined as being present when serum creatinine level was >2.0 mg/dL. Liver disease was defined as presence of liver cirrhosis or chronic hepatitis B or C.

**Laboratory Methods**

We used RapID CB Plus (Kyokuto Pharmaceutical Industrial Co. Ltd., https://www.kyokutoseiyaku.co.jp) for bacterial identification during January 2014–May 2015. This kit correctly identifies 95% of *Corynebacterium* isolates to the species level (13). Starting in June 2015, we identified strains by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and a Bruker MALDI Biotyper (Bruker Daltonics GmbH, https://www.bruker.com). We used score cutoff values according to recommendations proposed by the manufacturer (≥2.0). For some cases in which no identification or ambiguous identification was achieved by these methods, we confirmed identification by using 16S rRNA gene sequence analysis. We performed antimicrobial susceptibility tests by broth microdilution, using Clinical and Laboratory Standards Institute (CLSI, https://clsi.org) M45 A2:2ED 2010 during January 2014–December 2016 and CLSI M45 3rd edition from January 2017 on.

**Statistical Analyses**

We used Fisher exact or Pearson χ² tests to compare categorical variables. For continuous variables, we used Mann–Whitney U or paired t-tests, and for estimating survival probabilities we used Kaplan-Meier curves. We estimated and compared the cumulative incidence of mortality by using the log-rank test and compared differences in antimicrobial susceptibility between *Corynebacterium* species by using Fisher exact or Pearson χ² tests. We considered p<0.05 to indicate statistical significance. We performed all statistical analyses by using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (The R Foundation, https://www.r-project.org) (14).

**Results**

**Proportion of True Bacteremia Cases**

Of 115 patients in this study, *C. striatum* was detected in 67 (58%), *C. jeikeium* in 14 (12%), and other *Corynebacterium* species in 34 (30%) patients. The category of other consisted of 15 species (Table 1). In total, there were 60 cases of true bacteremia and 55 cases of contamination, resulting in 52% of patients having true bacteremia. Of the 60 patients with true bacteremia, 55 had ≥2 sets of positive blood cultures with *Corynebacterium* spp.; 5 had 1 set of positive blood cultures but met the definition of true bacteremia in our study. Of 115 patients, ≥2 genera of bacteria were detected in blood culture from only 1 patient; this patient had diverticulitis and bacteremia caused by *Corynebacterium* spp. and *Escherichia coli*. The patient recovered after receiving treatment.

<table>
<thead>
<tr>
<th><em>Corynebacterium</em> species</th>
<th>Total, n = 115</th>
<th>True bacteremia, n = 60</th>
<th>Contamination, n = 55</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. striatum</em></td>
<td>67</td>
<td>47</td>
<td>20</td>
</tr>
<tr>
<td><em>C. jeikeium</em></td>
<td>14</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Other, total</td>
<td>34</td>
<td>3</td>
<td>31</td>
</tr>
<tr>
<td><em>C. accolens</em></td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>C. arfemans</em></td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td><em>C. amycolatum</em></td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td><em>C. aurimucosum</em></td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td><em>C. coyleae</em></td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>C. glucuronolyticum</em></td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>C. minutissimum</em></td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td><em>C. mucifaciens</em></td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>C. pseudodiphtheriticum</em></td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>C. resists</em></td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><em>C. riegelii</em></td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>C. simulans</em></td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td><em>C. singulare</em></td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><em>C. tuberculostearicum</em></td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><em>C. urealyticum</em></td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

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for *E. coli* bacteremia alone; *Corynebacterium* spp. were considered to be contaminants. The percentages of true bacteremia cases caused by *C. striatum* (70%) and *C. jeikeium* (71%) were significantly higher than those for other species (9%; p<0.001 for each) (Table 2).

**Clinical Diagnosis and Underlying Diseases**

Hematologic malignancy was the most common underlying disease (33%), especially in 64% of patients with *C. jeikeium* bacteremia, followed by solid tumors (24%) and diabetes mellitus (23%) (Table 2). *C. striatum* and *C. jeikeium* were more frequently detected than other species in patients with hematologic malignancy (p = 0.036 and p<0.001, respectively) and neutropenia (p<0.01 and p<0.001, respectively). Of the 60 patients with true bacteremia, 25 (42%) had infection at an unknown site; 17 (28%) had CRBSI; and 18 (30%) had infection at other foci, including SSTI, pyelonephritis, pneumonia, empyema, infective endocarditis, vertebral osteomyelitis, central venous port infection, and spontaneous bacterial peritonitis.

**Mortality Rates**

Mortality rates among patients with true bacteremia were 34% among those with bacteremia caused by *C. striatum*, 30% by *C. jeikeium*, and 0 by other species of *Corynebacterium*. (Figure). We observed no significant differences in survival rates between these groups (C. striatum p = 0.25 and C. jeikeium p = 0.32). Six patients experienced a fulminant course of illness that resulted in death within 7 days; for all 6 patients, the causative organism was *C. striatum*.

**Antimicrobial Susceptibility**

All tested strains of *Corynebacterium*, regardless of species, were susceptible to vancomycin, linezolid, and minocycline (Table 3). *C. striatum* and *C. jeikeium* were less susceptible than other species to penicillin (p<0.001 for each), ceftriaxone (p<0.001 for each), meropenem (p<0.001 for each), erythromycin (p<0.01 for each), and ciprofloxacin (p<0.001 for *C. striatum* and p = 0.02 for *C. jeikeium*).

**Discussion**

With regard to the characteristics of *Corynebacterium* bacteremia at the species level, we report 3 major findings. First, *C. striatum* and *C. jeikeium* each caused true bacteremia more frequently than did other *Corynebacterium* species. Second, hematologic malignancies were the most common underlying disease in patients with *Corynebacterium* bacteremia (33%).

**Table 2. Clinical diagnosis and characteristics of patients with Corynebacterium species detected in blood culture, Japan, 2014–2020***

<table>
<thead>
<tr>
<th>Variable</th>
<th>All, n = 115</th>
<th>C. striatum, %, n = 67</th>
<th>C. jeikeium, %, n = 14</th>
<th>Other species, %, n = 34</th>
<th>C. striatum vs. other species p</th>
<th>C. jeikeium vs. other species p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>71</td>
<td>71</td>
<td>66</td>
<td>77</td>
<td>0.055</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>80 (70)</td>
<td>51 (76)</td>
<td>13 (93)</td>
<td>16 (47)</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>F</td>
<td>35 (30)</td>
<td>16 (24)</td>
<td>1 (7)</td>
<td>18 (53)</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Underlying disease, no. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>27 (23)</td>
<td>14 (20)</td>
<td>2 (14)</td>
<td>11 (32)</td>
<td>0.309</td>
<td>0.292</td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>16 (14)</td>
<td>9 (13)</td>
<td>2 (14)</td>
<td>5 (15)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Liver disease</td>
<td>4 (4)</td>
<td>4 (6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.299</td>
<td>NA</td>
</tr>
<tr>
<td>Solid tumor</td>
<td>27 (24)</td>
<td>13 (19)</td>
<td>4 (29)</td>
<td>10 (29)</td>
<td>0.378</td>
<td>1</td>
</tr>
<tr>
<td>Leukemia†</td>
<td>20 (17)</td>
<td>11 (16)</td>
<td>8 (57)</td>
<td>1 (3)</td>
<td>0.056</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Malignant lymphoma‡</td>
<td>14 (12)</td>
<td>8 (12)</td>
<td>3 (21)</td>
<td>3 (9)</td>
<td>0.537</td>
<td>0.171</td>
</tr>
<tr>
<td>Hematologic malignancy§</td>
<td>38 (33)</td>
<td>24 (36)</td>
<td>9 (64)</td>
<td>5 (15)</td>
<td>0.036</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Underlying condition, no. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutropenia, &lt;500 cells/mm³</td>
<td>29 (25)</td>
<td>19 (28)</td>
<td>8 (57)</td>
<td>2 (6)</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Corticosteroid</td>
<td>8 (7)</td>
<td>5 (6)</td>
<td>2 (14)</td>
<td>1 (3)</td>
<td>0.661</td>
<td>0.2</td>
</tr>
<tr>
<td>Chemotherapy, within 3 mo</td>
<td>41 (36)</td>
<td>23 (34)</td>
<td>10 (71)</td>
<td>8 (24)</td>
<td>0.377</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Clinical diagnosis, no. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>True bacteremia</td>
<td>60 (52)</td>
<td>47 (70)</td>
<td>10 (71)</td>
<td>3 (9)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No focus</td>
<td>25 (22)</td>
<td>19 (29)</td>
<td>6 (43)</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CRBSI</td>
<td>17 (15)</td>
<td>13 (19)</td>
<td>3 (21)</td>
<td>1 (3)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Other focus¶</td>
<td>18 (16)</td>
<td>15 (22)</td>
<td>1 (7)</td>
<td>2 (6)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Contamination</td>
<td>55 (48)</td>
<td>20 (30)</td>
<td>4 (29)</td>
<td>31 (91)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*ALL, acute lymphoid leukemia; AML, acute myeloid leukemia; CRBSI, catheter-related blood stream infection; DLBCL, diffuse large B cell lymphoma; MDS, myelodysplastic syndrome; MM, multiple myeloma; NA, not applicable; ND, not done; IVLBC, intravascular large B cell lymphoma; PTCL, peripheral T-cell lymphoma.

†No. cases: AML, 17; ALL, 3.
‡No. cases: DLBCL, n = 8: PCTL, n = 3; IVLBC, n = 1; lymphoplasmacytic lymphoma, n = 1; Burkitt lymphoma, n = 1.
§No. cases: leukemia, n = 20; lymphoma, n = 14; MM, n = 3; MDS, n = 1; myelofibrosis, n = 2.
¶No. cases: pyelonephritis, n = 5; skin and soft tissue infection, n = 4; empyema, n = 2; pneumonia, n = 1; prostatitis, n = 1; infective endocarditis, n = 1; osteomyelitis, n = 1; vascular graft infection, n = 1; central venous port infection, n = 1; spontaneous bacterial peritonitis, n = 1.
Third, although the most common sources of infection were of unknown origin and CRBSI, other sources (e.g., pyelonephritis, SSTI, and empyema) accounted for 30% of true bacteremia cases.

The strengths of our study include having had infectious disease specialists assess infection sites and classify cases as true bacteremia to ensure study quality. Furthermore, detailed clinical and microbiological data were available because we included all cases of Corynebacterium bacteremia in our center over 6 years.

A previous study reported that contamination rates varied among species of Corynebacterium and that C. jeikeium caused true bacteremia more frequently than other species (8). The overall contamination rates of 48% in all patients treated in our study approximated those for 2 previous studies in Japan (46% and 42%) (9,15). However, higher contamination rates were reported in a study performed in Sweden (8), where 93% of these cases were considered to be contaminations and C. afermentans accounted for 14%, C. aurimucosum for 7%, and C. amycolatum for 6% of the total Corynebacterium species detected in blood culture. Our study also demonstrated a high contamination rate of 93% for those species, but our frequency of detection was less than that in the previous study; detection rates in our study were 5% for C. afermentans, 3% for C. aurimucosum, and 3% for C. amycolatum. The difference in contamination rates in both studies may be underpinned by regional differences in the epidemiology of Corynebacterium species. It is plausible that the study populations may differ because the study in Sweden was population based, whereas our study was performed in a tertiary-care hospital. Furthermore, indications for blood culture may differ between these studies.

Bacteremia with C. striatum or C. jeikeium, the most frequently identified species in our study, seemed to be more associated with a higher 90-day mortality rate when compared with other species, although we observed no significant difference. Factors associated with a poor prognosis for Corynebacterium spp. bacteremia are mixed infection, chronic kidney disease, and lack of a central venous catheter (16). However, we are unaware of any study that has reported on differences in mortality rate among patients with infections by different species of Corynebacterium. One study reported that C. striatum formed biofilms on polyurethane catheters in vitro and hypothesized that biofilm may contribute to the establishment of hospital-acquired infections (17). Indeed, biofilm formation has been associated with true bacteremia in another study (18). C. jeikeium has also been reported to form biofilm, which can promote opportunistic infections (19). Although further study is needed, the tendency for C. striatum and C. jeikeium to form biofilm and their association with true bacteremia may be a reason for worse outcomes compared with outcomes for infection with other Corynebacterium species.

All strains of Corynebacterium spp. detected in this study were sensitive to vancomycin, minocycline, and linezolid. A previous study reported that most isolates were resistant to penicillin, ciprofloxacin, and tetracycline, and in contrast, all isolates were sensitive to observed no significant difference. Factors associated with a poor prognosis for Corynebacterium spp. bacteremia are mixed infection, chronic kidney disease, and lack of a central venous catheter (16). However, we are unaware of any study that has reported on differences in mortality rate among patients with infections by different species of Corynebacterium. One study reported that C. striatum formed biofilms on polyurethane catheters in vitro and hypothesized that biofilm may contribute to the establishment of hospital-acquired infections (17). Indeed, biofilm formation has been associated with true bacteremia in another study (18). C. jeikeium has also been reported to form biofilm, which can promote opportunistic infections (19). Although further study is needed, the tendency for C. striatum and C. jeikeium to form biofilm and their association with true bacteremia may be a reason for worse outcomes compared with outcomes for infection with other Corynebacterium species.

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### Table 3. Antimicrobial susceptibility testing of Corynebacterium species isolated from blood culture, Japan, 2014–2020*

<table>
<thead>
<tr>
<th>Species</th>
<th>Susceptible/tested (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PEN</td>
</tr>
<tr>
<td></td>
<td>(21)</td>
</tr>
<tr>
<td>C. jeikeium, n = 14</td>
<td>0/14</td>
</tr>
<tr>
<td></td>
<td>(0)</td>
</tr>
<tr>
<td>Other species, n = 34</td>
<td>27/34</td>
</tr>
<tr>
<td></td>
<td>(79)</td>
</tr>
<tr>
<td></td>
<td>(36)</td>
</tr>
</tbody>
</table>

*CIP, ciprofloxacin; CLI, clindamycin; CRO, ceftriaxone; ERY, erythromycin; GEN, gentamicin; LZD, linezolid; MEM, meropenem; MIN, minocycline; PEN, penicillin; VAN, vancomycin.
†Antimicrobial susceptibility testing for linezolid was only performed if requested by physicians.
vancomycin (20). In another study, only a few strains of *C. jeikeium* were resistant to doxycycline (21). The results of our study are consistent with those reports.

The most common underlying disease in patients with *Corynebacterium* bacteremia in our study was hematologic malignancy (33%). Among bacteremic patients with hematologic malignancies, the second most common gram-positive bacteria were *Corynebacterium* spp. (22). *C. striatum* was more likely to cause bacteremia in patients with malignancies or neutropenia (15), and *C. jeikeium* also caused bacteremia, frequently in patients with neutropenia or a history of previous antimicrobial treatment (23). The reason for the higher frequency of *Corynebacterium* bacteremia in patients with hematologic malignancies remains unknown. Among patients with hematologic malignancies, the reported rate of skin or rectal colonization with *Corynebacterium* spp. was 41% (24). We hypothesize that skin and mucosal barrier failures resulting from intense chemotherapy, chronic indwelling infusion catheters, and increased colonization may put patients with hematologic malignancies at a higher risk for *Corynebacterium* bacteremia.

Although the most common sources for *Corynebacterium* bacteremia were unknown or CRBSI, other sources accounted for 30% (18/60 cases), including lower respiratory tract infections, urinary tract infections, and SSTIs. Previous studies have reported that *C. striatum* can cause pneumonia (25), urinary tract infections, and intra-abdominal infections (4). Case studies have also reported *C. jeikeium* as being responsible for infective endocarditis (6), pacemaker infections (26), and prosthetic joint infections (27). *Corynebacterium* spp. are often reported as coryneform and not fully identified unless they are from sterile specimens because they colonize the skin and are ubiquitous in the environment. We emphasize the value of actively identifying coryneforms in specimens, even if they are not sterile (e.g., sputum or urine), especially in suspected cases of *Corynebacterium* bacteremia.

The first limitation of our study was that we used RapID CB Plus and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry mainly for identification and performed 16s rRNA sequencing analysis for only a subset of cases. In conclusion, the proportion of cases of true bacteremia caused by *C. striatum* or *C. jeikeium* was higher than that caused by other *Corynebacterium* species, and the mortality rate for true bacteremia was ≤30%. *C. striatum* and *C. jeikeium* were frequently detected in patients with hematologic malignancies and neutropenia. Healthcare providers should give special consideration to these 2 species of *Corynebacterium* and consider the possibility of true bacteremia rather than contamination when they are detected in blood cultures, especially in patients with hematologic malignancies.

**Acknowledgments**

We thank Muneyoshi Kimura and Atsushi Shiraishi for their support with statistical analyses. We also thank the medical technologists in the Kameda Medical Center for their invaluable technical assistance.

**About the Author**

Dr. Yamamuro is a medical doctor at Kameda Medical Center. His primary research interests include opportunistic infections and immune mechanism in hematologic transplant patients.

**References**


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Early case detection is important to control and prevent infectious disease outbreaks (1). The 5 identified purposes for early detection surveillance are detecting the first case of the disease in a population previously free, detecting new cases in an area already infected, early detection of an abnormal increase in the level of a disease normally present at a base level, screening for individual cases of noncommunicable diseases, and the first detection of an invasive species in an area previously free of that species (2). The International Health Regulations (2005) (3) impose obligation on countries to develop, strengthen, and maintain their capacities to detect, verify, assess, report, and respond to any events that may constitute a public health risk and thereby prevent international spread. Public health surveillance systems are poorly developed in many low-income and middle-income countries, as demonstrated by recent Ebola outbreaks, which had devastating consequences in the health and economy of several countries (4–7).

Ebola virus disease (EVD), if not detected and reported early, can rapidly spread and result in high rates of illness and death (8,9). In recent years, the world has faced the 2 largest EVD epidemics in recorded history, both of which were declared public health emergencies of international
correlation between the director-general of the World Health Organization (WHO).

EVD case definitions are crucial surveillance tools, both for referring suspected cases and as screening tools to aid admission and laboratory testing decisions at health facilities (10). WHO has developed standard case definitions for alert, suspected, probable, and confirmed cases in the context of routine and community-based surveillance (11,12) (Appendix Table, https://wwwnc.cdc.gov/EID/article/27/12/21-0290-App1.pdf).

Insufficient command of these case definitions at the community and health facility level has resulted in late detection of EVD outbreaks. For instance, recent epidemics in both West Africa and the Democratic Republic of the Congo (DRC) were officially declared 3 months after the effective start of the epidemics (13,14). The epidemic in DRC was the second largest EVD outbreak ever documented after the West Africa EVD epidemic (2013–2016); a total of 3,481 cases (3,323 confirmed and 158 probable) and 2,299 deaths were recorded in August 2018–June 2020 in North Kivu, Ituri, and South Kivu Provinces. This outbreak was particularly complex because it occurred in an active conflict zone (15). Public health performance indicators at the beginning of this EVD response were poor, including many community deaths, poor contact tracing, and delays between symptom onset and case isolation. A decline in incidence toward the end of 2019 was thought to be the result of improvement in the quality of surveillance activities, including prompt investigation, early detection and isolation of cases, enhanced community-based surveillance, rapid follow-up of high-risk contacts, and an adaptive vaccination strategy (16).

Soon after the declaration of the 10th EVD outbreak in the DRC, an Early Warning, Alert and Response System (EWARS) was implemented throughout North Kivu and Ituri Provinces, to report, collect, investigate, validate, and take early action (isolation, safe burial, or referral) on alerts that met the suspected case definition for EVD. We describe and evaluate this system as implemented in the subcoordination of Beni, established to manage the response across several health zones.

Methods

Description of the EWARS

The Alert Unit was the core functional unit around which the EWARS was organized (Figure 1); it was composed of an overall operational leader who coordinated activities, a database and information administrator, a case management leader, a Safe and Dignified Burial (SDB) leader, 3 telephone operators, 1 alert monitoring officer, 1 database manager, 1 data clerk, and 1 archivist. The main role of the Alert Unit was to gather and scan alerts from various sources, coordinate the field investigations with the rapid intervention teams, and, if relevant, organize the referral and ambulance transfer or safe burial in collaboration with the case management or SDB team. All alerts and their outcomes were entered and archived into paper-based alert and investigation forms and a Microsoft Excel database (https://www.microsoft.com). There were 4 main sources of alerts: community, in which community health workers, community members, and political and administrative authorities raised alerts; active case finding conducted in health facilities and other structures (pharmacies, churches, traditional practitioners); surveillance sites, including contact tracing teams, vaccination sites, and points of entry/points of control (PoE/PoC); and finally, public and private health facilities that ensured passive reporting.

Two toll-free numbers were activated on August 26, 2018, to enable rapid and easy alert reporting from all the sources. Calls were directed to telephone operators in the Alert Unit, which was operational 24 hours per day, 7 days per week; a smaller team for night shifts comprised the operations leader and phone managers only. In active case finding, passive reporting, or PoE/PoC, the alert notifier completed an alert form, or for community and contact tracing alerts, the telephone operator or alert monitoring officer completed the form. When telephone operators received the alerts, they checked for duplication and conducted preliminary triage to prioritize them by epidemiologic and clinical factors. Rapid intervention teams were then notified to investigate the alert onsite.

Rapid intervention teams were made up of a field epidemiologist, an infection prevention and control (IPC) officer, a communication officer, and a psychosocial worker. All these response pillars were positioned in each health area covered by the alert system, from which a senior epidemiologist would organize rapid intervention teams. The investigation consisted of a detailed history, assessment of the epidemiologic link, clinical symptoms for validation against the suspected case definition (17), and initial listing of contacts. Investigation forms were stored in the Alert Unit, and copies were sent to Ebola treatment centers (ETC) for patients requiring admission. The rapid intervention team validated or invalidated the alert on
the basis of the investigation findings and provided immediate feedback to the Alert Unit contact persons, who coordinated the next steps.

For invalidated alerts, the family can proceed with ordinary burial of deceased patients, whereas living patients were referred to public healthcare facilities for free healthcare. Living patients with validated alerts were immediately transferred to a transit center, isolation center, or ETC, depending on the patient’s condition and location. There was no additional validation at triage in ETC. To reduce the risk that a transfer would refuse a patient, the intervention team would propose 2 options according to patient condition and preference: transfer the patient by ambulance or by motorcycles driven by Ebola survivors. After admission to the isolation center, patients followed the suspected case management algorithm: blood samples were taken and tested by using GeneXpert (Cepheid, https://www.cepheid.com) within 3 hours after admission. Cases confirmed by PCR were immediately admitted to an ETC for treatment. Those patients with an
initial negative test were discharged pending a second negative result 72 hours later.

The SDB team were notified of validated alerts of deceased patients, then joined the rapid intervention team onsite to engage with the family. The body was secured and a swab sample taken and sent to the laboratory for testing. With family consent, SDB proceeded immediately. However, if the family refused, the body was kept at the morgue until the laboratory result was available. If the result was negative, the body was returned to the family to proceed with ordinary burial; if the results were positive, SDB was mandatory and enforced by authorities.

Evaluation Approach and Data Sources and Indicators
We conducted a quantitative evaluation according to guidelines published by WHO (18) and the US Centers for Disease Control and Prevention (CDC) (19). We used the anonymized Alert Unit database, covering the health zones of Beni, Mutwanga, and Oicha, during August 5, 2018–June 30, 2020, to assess EWARS using the EVD suspected case definition as the standard. An alert was considered validated if it met the definition of an alert case by community-based surveillance or the definition of a suspected case by mobile teams or health stations or centers (12). An investigator would validate a suspected case on the evidence of clinical signs in the patient (Appendix Figure).

To assess the true sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) would require laboratory testing for all patients, which would not have been feasible. We calculated sensitivity as the proportion of alerts validated among all alerts meeting the suspected case definition, specificity as the proportion of invalidated alerts among all alerts not meeting the suspected case definition, PPV as the proportion of invalidated alerts among all alerts not meeting the suspected case definition, NPV as the proportion of alerts that met the suspected case definition among all validated alerts, and PPV as the proportion of alerts that did not meet the suspected case definition among all invalidated alerts. We assessed timeliness as the median, range, and interquartile range (IQR) of the delay between the transmission of alert to the Alert Unit and the start of the onsite investigation. We evaluated representativeness through the geographic and demographic coverage of the alerts by comparing alert incidence by sex, age group, and health zone. We appraised usefulness by considering the number of confirmed and probable cases that were detected through the alert system. Finally, we assessed stability by considering how the system was operating over time, disruptions, and sustainability of functioning beyond the emergency response phase, notably in relation with costs and human resources. We conducted all analyses using R statistical software version 4.0.3 (20).

Results
Outcomes of EWARS
During the study period, 195,601 alerts were received; 194,768 (99.6%) from the health zones of Beni, Mutwanga, and Oicha, and 833 (0.4%) from other health zones (Figure 2). A small number (52,240, 2.7%) were reports of community deaths.

On average, there were 280 alerts/day (range 2–955, median 127 alerts/day), although this value greatly varied over time. The number of daily alerts increased progressively, from 6 at the outset in August 2018 to a peak of 922 at the beginning of March 2020. We observed multiple sudden, short-lived decreases in the daily number of alerts, particularly in mid-November 2019 and early April 2020, coinciding with security incidents (see Stability) (Figure 3).

A total of 30,728 (15.8%) alerts were validated as suspected cases. Among those, 801 (2.6%) were finally classified as confirmed cases and 3 (<0.1%) as probable cases. No invalidated alerts became confirmed cases; the information recorded the first time remains in the database, and a new alert with the same information could be quickly detected.

Most (62.6%) alerts were raised by active case finding teams, followed by passive reporting from health facilities (19.0%), and community alerts (15.0%). The remainder (3.6%) originated from other sources (Table 1).

Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value
We excluded 434 alerts (0.2%) that were not investigated and 201 alerts (0.1%; 197 invalidated and 4 validated) that could not be classified according to the case definition because of missing data. A total of 17,927 (9.2%) alerts met the EVD suspected case definition. Sensitivity was 84.6% (95% CI 84.1%–85.1%) and specificity 91.2% (95% CI 91.0%–91.3%). PPV was 49.4% (95% CI 48.8%–49.9%) and NPV 98.3% (95% CI 98.2%–98.4%) (Table 2).

Indicators varied with time, health zone, and source of notification (Table 3). Overall, sensitivity increased over time, and specificity remained high throughout the study period. PPV decreased while NPV increased, which is consistent with the outbreak
dynamics and the decrease in incidence toward the end of the epidemic (Appendix Figure).

Sensitivity was higher for alerts arising from surveillance sites (98.0%, 95% CI 97.4%–98.7%), community alerts (91.4%, 95% CI 90.1%–92.7%), and active case finding (87.5%, 95% CI 86.9%–88.1%) and lower for those arising from passive reporting from health facilities (65.4%, 95% CI 63.8%–67.0%). Conversely, specificity was highest in health facilities (96.2%, 95% CI 96.0%–96.4%), and was high (>90%) for all other sources except surveillance sites. Sensitivity was higher in Beni (94.8%, 95% CI 94.4%–95.2%) than in Mutwanga (54.9%, 95% CI 52.4%–57.3%) and Oicha (64.3%, 95% CI 62.8%–65.8%), but specificity was higher in Mutwanga (96.4%, 95% CI 96%–96.7%) and Oicha (93.3%, 95% CI 92.8–93.8).

Timeliness
An investigation was initiated within 2 hours from the time of alert for 188,184 (96.6%) alerts. The median time from alert transmission to the arrival of the investigation team on site was 11 minutes (IQR 10–15 minutes). Information about the time of investigation was not available for 3,475 (1.8%) alerts.

Timeliness of responses varied over time; substantial delays were observed at the outset of the system implementation, with greatest delays in Mutwanga (Figure 4). We saw no marked difference in timeliness by source of notification.

Representativeness
We observed substantial variations in the alert incidence between the health zones. On average, there were 241 (range 2–789) alerts/day in Beni, 42.3 (range 1–181) alerts/day in Mutwanga, and 25.4 (range 1–138) alerts/day in Oicha. The alert incidence in the population followed a similar pattern: an average of 36 alerts/1,000 inhabitants/week in Beni, 2.5 alerts/1,000 inhabitants/week in Oicha, and 2.4 alerts/1,000 inhabitants/week in Mutwanga. In Beni,
Ebola EWARS, DRC

The incidence of alerts increased progressively from the outset (Figure 5). However, in Mutwanga and Oicha, incidence remained low until the beginning of November 2019, when it rapidly increased following community transmission.

We observed more alerts among female (56.3%) than male (43.4%) patients. Children <5 years of age were the most represented (23.6%), followed by patients 20–29 years of age (18.9%) and 10–19 years of age (18.9%); these percentages approximate the age and sex breakdown of the local population, with the exception of children 5–9 years of age, who were underrepresented (11%).

Usefulness and Cost
The EWARS system led to the detection of 801 confirmed and 3 probable cases, which equates to 242 alerts notified and 38 alerts validated for each case detected by the system. The total direct and indirect costs associated with EWARS implementation and maintenance was US $353,525 over the 2-year period of operation (Table 4), yielding a minimum value of US $1.8/alert and US $438/case detected.

Stability
The alert system operated 24 hours per day, 7 days per week, including a minimal night team to ensure continuity. Continuous communication and reporting of alerts was possible by a comprehensive and stable mobile phone coverage covering all health areas. As such, alerts were collected and analyzed on a continuous basis, and reports were produced and distributed daily. However, despite the continuous availability of human resources and communication networks, the system was severely disrupted by security incidents. Security incidents coincided with decreases in the number of alerts, affecting both the reporting and investigation of alerts (Figure 3). The Alert Unit ceased operations following the standard 90-day period of heightened surveillance after the declaration of the end of the outbreak, as determined by WHO (21).

Discussion
During August 2018–June 2020, EWARS led to the notification and investigation of 194,768 alerts and the detection of 801 confirmed and 3 probable EVD cases. The evaluation showed an overall good performance of the system regarding the main attributes we assessed, highlighting the many strengths of such a system. However, it also revealed disparities in performance between the health zones covered by the system, reflecting differences in the timing of implementation and, most notably, unequal operating conditions (e.g., security incidents).

This system encompassed both event-based and indicator-based surveillance (22,23), resulting
in 7.8% of alerts meeting the definition of EVD suspected case, 4-fold higher than the event-based surveillance system at the community level during the Ebola outbreak in Sierra Leone in 2014–2016, and a 6-fold higher 49.4% PPV (24). Approximately 92% of our alerts did not meet the suspected case definition because of a time lag of days between symptom onset, on which the alert launch was based, and the symptoms that were actually present in these patients during investigation.

Although the overall proportion of detected cases among alerts was low (0.4% of all alerts), EWARS aimed to be highly sensitive; actions taken around those confirmed cases successfully interrupted transmission chains and prevented further spread of the disease. Indeed, the system showed a high sensitivity and specificity (>80%) and a low PPV, which reflects the low EVD incidence in the population. All health areas covered by the system reported alerts that did not differ greatly from the population structure, thus suggesting a good demographic representativeness. The system presented prompt timeliness of investigation of alerts throughout its 2 years of operation. Finally, the minimum cost per alert or cases was relatively low compared with that for a nationwide telephone alert system established for rapid notification and response during the 2014–2015 Ebola disease epidemic in Sierra Leone (25).

This good performance of EWARS can be explained by the intensive, comprehensive, and continuous reporting flow. First, the system relied on the use of various sources of alerts, involving both passive and active case reporting from the community, health structures, and other surveillance sites. Second, it built upon a stable and extensive telephone network further supported by toll-free numbers, a means of communication that is easily accessible, acceptable, and already commonly used by all stakeholders involved in surveillance. Third, it adopted a decentralized approach for the organization of the investigation teams, which enabled comprehensive coverage of all health areas and prompt reactivity for early action. The existence of a dedicated team at the subcoordination level further supported the coordination of activities at the local level while aiding in the centralization and consolidation of the information circuit. The unceasing availability of all key actors of the reporting system (surveillance, investigation teams, alert unit, and case management/SDB) ensured the continuous reporting and actions around alerts in timely manner. How fast a system detects and responds effectively

Table 1. Characteristics of Ebola virus disease alerts received in Beni subcoordination, Democratic Republic of the Congo, August 5, 2018–June 30, 2020

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%) alerts, n = 194,768</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td></td>
</tr>
<tr>
<td>2018</td>
<td>3,211 (1.6)</td>
</tr>
<tr>
<td>2019</td>
<td>67,579 (34.7)</td>
</tr>
<tr>
<td>2020</td>
<td>123,978 (63.7)</td>
</tr>
<tr>
<td>Final alert status</td>
<td></td>
</tr>
<tr>
<td>Invalidated</td>
<td>163,606 (84.0)</td>
</tr>
<tr>
<td>Validated</td>
<td>30,728 (15.8)</td>
</tr>
<tr>
<td>Not investigated</td>
<td>434 (0.2)</td>
</tr>
<tr>
<td>Alert initial status</td>
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<tr>
<td>Deceased</td>
<td>5,230 (2.7)</td>
</tr>
<tr>
<td>Alive</td>
<td>189,538 (97)</td>
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<td>Final case classification</td>
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<td>Not a case</td>
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<tr>
<td>Confirmed case</td>
<td>801 (0.4)</td>
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<tr>
<td>Probable</td>
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<td>Active case finding</td>
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<td>Health structure</td>
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<td>Community</td>
<td>28,928 (15.0)</td>
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<td>Beni</td>
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<td>Mutwanga</td>
<td>12,891 (6.6)</td>
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<td>Oicha</td>
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<td>Known contact of confirmed or probable case</td>
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</tr>
<tr>
<td>Unknown</td>
<td>44 (0.1)</td>
</tr>
</tbody>
</table>

Table 2. Evaluation results and overall characteristics of Ebola virus disease alerts from EWARS, Democratic Republic of the Congo, August 5, 2018–June 30, 2020*0

<table>
<thead>
<tr>
<th>Alert system</th>
<th>Suspected case definition</th>
<th>% (95% CI)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Validated</td>
<td>No. met</td>
<td>No. unmet</td>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Validated</td>
<td>15,163</td>
<td>15,561</td>
<td>30,724</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invalidated</td>
<td>2,764</td>
<td>160,645</td>
<td>163,409</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15,245</td>
<td>184,104</td>
<td>199,349</td>
<td>84.6 (84.1–85.1)</td>
<td>91.2 (91.0–91.3)</td>
<td>49.4 (48.8–49.9)</td>
</tr>
</tbody>
</table>

*Total excludes 434 (0.2%) alerts that were not investigated and 201 (0.1%) alerts that could not be classified according to the case definition due to missing data. EWARS, Early Warning, Alert and Response System; NPV, negative predictive value; PPV, positive predictive value.

SYNOPSIS

Known contact of confirmed or probable case

No. (%) alerts, n = 194,768
to a threat is the optimal measure of performance. Continuously evaluating and improving timeliness can identify performance bottlenecks and help to accelerate progress, improving detection speed and response quality (26).

The alert system performed better in Beni for all attributes we studied. In Mutwanga and Oicha, sensitivity was <80%, alert incidence was low (even after an increase in the number of daily alerts in late 2019), geographic coverage appeared less comprehensive as many health areas reported few alerts, and delays in investigation were longer, particularly at the outset. Mutwanga and Oicha are 2 rural health zones located at the epicenter of nonstate armed groups’ territories, which greatly affected the operations. Surveillance and investigation activities faced regular security incidents and restrictions, long distances to alert sites, and poor road networks in many health areas. In this context, the alert system was initially implemented in Beni and progressively extended and strengthened in Mutwanga and Oicha. For example, in the early phase, rapid intervention teams were staffed in the Beni subcoordination office only, such that alert investigations in Mutwanga and Oicha suffered longer delays. Surveillance and reporting capacities were also weaker in Mutwanga and Oicha. In November 2019, a training of response personnel (registered nurses, supervisors, and investigators) was organized to address the low incidence of alerts; to strengthen data management capacities, data managers were deployed, leading to a rapid increase in alerts from these health zones.

Despite the effects of security incidents, the EWARS continued to operate throughout the whole period, managing an increasing volume of alerts, leading to the detection of hundreds of cases. In a context of limited surveillance capacities and weak health systems, such an intensive and steadily reporting alert system was vital for the early detection of cases and interruption of the spread of the disease in the population. However, the system was conceived and implemented in an ad hoc manner within the frame-
work of the Ebola outbreak response, which limited its sustainability beyond the resources and time period of the outbreak response. The financial, logistical, and human resources needed to implement and maintain the system were made possible by dedicated response funds and the time-bound engagement of both national support teams and international financial and technical partners. The EWARS ceased operations within 12 weeks of the declared end of the outbreak. The long-term sustainability of systems such as EWARS remains unknown. An additional limitation was the challenge in assessing overall performance measures of the system, such as completeness, acceptability, and flexibility. We evaluated EWARS with regard to its objectives, but we could not extrapolate the effects of the system on the overall outbreak dynamics.

In conclusion, the magnitude and duration of the 10th and largest Ebola outbreak in DRC, occurring in an active conflict zone, highlighted the need for prompt, functional, and effective infectious disease surveillance systems. We have demonstrated that the EWARS implemented was a cost-effective component of this surveillance system. Our findings underscore the importance of early-warning systems, along with the necessity of ensuring efficiency and sustainability beyond the duration of the emergency response phase. As such, Integrated Disease Surveillance and Response is a relevant framework to further strengthen the International Health Regulations (2005)

**Table 4. Costs for EWARS in Beni, Mutwanga, and Oicha, Democratic Republic of the Congo, August 2018–June 2020***

<table>
<thead>
<tr>
<th>Health zone</th>
<th>Item</th>
<th>Implementation period costs, USD</th>
<th>Total cost, USD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beni</td>
<td>Prime staff for alerts management teams</td>
<td>6,000</td>
<td>43,600</td>
</tr>
<tr>
<td>Prime staff for data managers</td>
<td>900</td>
<td>10,350</td>
<td>11,400</td>
</tr>
<tr>
<td>Ambulance rental</td>
<td>6,000</td>
<td>72,000</td>
<td>144,000</td>
</tr>
<tr>
<td>Fuel</td>
<td>9,600</td>
<td>36,000</td>
<td>63,600</td>
</tr>
<tr>
<td>Purchase of telephones</td>
<td>175</td>
<td>NA</td>
<td>175</td>
</tr>
<tr>
<td>Purchase of materials†</td>
<td>5,500</td>
<td>12,000</td>
<td>18,450</td>
</tr>
<tr>
<td>Communication credit</td>
<td>750</td>
<td>3,600</td>
<td>6,600</td>
</tr>
<tr>
<td>Green numbers‡</td>
<td>15,200</td>
<td>15,200</td>
<td>30,400</td>
</tr>
<tr>
<td>Oicha</td>
<td>Prime for alerts management teams</td>
<td>NA</td>
<td>18,450</td>
</tr>
<tr>
<td>Prime for data managers</td>
<td>12,150</td>
<td>5,400</td>
<td>12,600</td>
</tr>
<tr>
<td>Communication credit</td>
<td>1,200</td>
<td>600</td>
<td>1,800</td>
</tr>
<tr>
<td>Mutwanga</td>
<td>Prime for alerts management teams</td>
<td>NA</td>
<td>12,600</td>
</tr>
<tr>
<td>Prime for data managers</td>
<td>7,200</td>
<td>5,400</td>
<td>12,600</td>
</tr>
<tr>
<td>Communication credit</td>
<td>800</td>
<td>600</td>
<td>1,400</td>
</tr>
<tr>
<td>Total</td>
<td>44,125</td>
<td>208,300</td>
<td>353,525</td>
</tr>
</tbody>
</table>

*Expenditures included direct and indirect costs. EWARS, Early Warning, Alert and Response System; NA, not applicable.
†Flip charts, markers, printed forms.
‡Telephone numbers 0820800001 and 0999009405, which health workers and community members could use for no charge. WHO covered this expense.
capacities (27,28). The need to evaluate and learn from field implementation of surveillance systems in infectious disease outbreaks, even in such difficult contexts, is an opportunity to better understand response efforts and improve future responses (29).

Acknowledgments
We thank the alert unit team and all the actors who have been involved in the EWARS in Beni, Mutwanga, and Oicha, DRC.

About the Author
Dr. Keita is a medical doctor and public health specialist, currently working as technical officer for risk assessment with the WHO Regional Office for Africa. During the 10th EVD outbreak in DRC, he was deployed as senior field coordinator in Beni. His research interests include infectious diseases epidemiology, risk assessment, public health response in humanitarian and emergency settings, health systems strengthening, healthcare management, and global health policy.

References


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May 2021

COVID-19

• Coordinated Strategy for a Modeling-Based Decision Support Tool for COVID-19, Utah, USA

• Clinical Laboratory Perspective on Human Infections Caused by Unusual Nonhemolytic, Lancefield Group B Streptococcus halichoeri

• Case Series of Laboratory-Associated Zika Virus Disease, United States, 2016–2019

• Successful Control of an Onboard COVID-19 Outbreak Using the Cruise Ship as a Quarantine Facility, Western Australia

• Whole-Genome Sequencing of Shiga Toxin–Producing Escherichia coli OX18 from a Fatal Hemolytic Uremic Syndrome Case

• Coccidioidomycosis and COVID-19 Co-Infection, United States, 2020

• Epidemiologic History and Genetic Diversity Origins of Chikungunya and Dengue Viruses, Paraguay

• Herd Immunity against Severe Acute Respiratory Syndrome Coronavirus 2 Infection in 10 Communities, Qatar

• Use of Genomics to Track Coronavirus Disease Outbreaks, New Zealand

• Engineered NS1 for Sensitive, Specific Zika Diagnosis from Patient Serology

• Active Case Finding of Current Bornavirus Infections in Human Encephalitis Cases of Unknown Etiology, Germany, 2018–2020

• Transmission of Severe Acute Respiratory Syndrome Coronavirus 2 during Border Quarantine and Air Travel, New Zealand (Aotearoa)

• Monitoring SARS-CoV-2 Circulation and Diversity through Community Wastewater Sequencing, the Netherlands and Belgium

• Serologic Screening of Severe Acute Respiratory Syndrome Coronavirus 2 Infection in Cats and Dogs during First Coronavirus Disease Wave, the Netherlands

• Symptom Diary–Based Analysis of COVID-19 Disease Course, Germany, 2020

• Global Trends in Norovirus Genotype Distribution among Children with Acute Gastroenteritis

• Genetic Evidence and Host Immune Response in Persons Reinfected with SARS-CoV-2, Brazil

• Prevalence and Clinical Profile of SARS-CoV-2 Infection among Farmworkers, California, June–November 2020

To revisit the May 2021 issue, go to: https://wwwnc.cdc.gov/eid/articles/issue/27/5/table-of-contents
Outcomes and costs of coronavirus disease (COVID-19) contact tracing are limited. During March–May 2020, we constructed transmission chains from 184 index cases and 1,499 contacts in Salt Lake County, Utah, USA, to assess outcomes and estimate staff time and salaries. We estimated 1,102 staff hours and $29,234 spent investigating index cases and contacts. Among contacts, 374 (25%) had COVID-19; secondary case detection rate was $=31\%$ among first-generation contacts, $=16\%$ among second- and third-generation contacts, and $=12\%$ among fourth-, fifth-, and sixth-generation contacts. At initial interview, 51% (187/370) of contacts were COVID-19–positive; 35% (98/277) became positive during 14-day quarantine. Median time from symptom onset to investigation was 7 days for index cases and 4 days for first-generation contacts. Contact tracing reduced the number of cases between contact generations and time between symptom onset and investigation but required substantial resources. Our findings can help jurisdictions allocate resources for contact tracing.

By July 2021, >33 million cases of coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), were documented in the United States, and most cases involved contact tracing by health departments (1). Preventing SARS-CoV-2 transmission through contact tracing requires rapid diagnosis, immediate isolation of cases, and rigorous tracking and precautionary isolation of close contacts (2–4). Because SARS-CoV-2 appears to be most transmissible before and immediately after symptom onset, clinical and transmission studies have shown that timely identification of cases and contacts is essential to preventing transmission (5–7). In addition, mathematical models have shown contact tracing, when implemented with other mitigation measures, can effectively reduce community spread of SARS-CoV-2 (8,9).

Evaluations of contact tracing for tuberculosis and HIV have found that contact tracing is an effective and sustainable approach to transmission reduction when disease prevalence is low but that contact tracing becomes less cost-effective as disease prevalence increases compared with other approaches, such as provider-initiated testing and intensified case finding (10,11). Programmatic data on outcomes and costs of contact tracing for COVID-19 are limited but essential for aiding public health agencies in designing or improving existing contact tracing programs (12). We aimed to quantify contact tracing efforts in Salt Lake County, Utah, USA, to examine how contact tracing affected case-finding, evaluate key contact tracing time intervals, and estimate the staff time and salary costs required to conduct investigations.

Methods

We examined persons with laboratory-confirmed or probable COVID-19 cases and their close contacts
retrospectively by using Salt Lake County Health Department (SLCoHD) surveillance data. We quantified the yield from each index case that generated a contact investigation and created transmission chains. We also examined 25 index cases and close contacts prospectively to estimate staff time and salary cost spent in contact tracing efforts.

SLCoHD Contact Tracing Procedures and Testing Guidelines

During March 12–May 3, 2020, SLCoHD staff traced all reported case-patients with laboratory-confirmed SARS-CoV-2 infection and their close contacts. Close contacts of any confirmed or probable case-patients were traced until no further symptomatic or positive contacts could be identified. Early in the study period, state guidelines called for prioritizing testing symptomatic close contacts of confirmed COVID-19 case-patients. Later in the study period, testing was available to anyone with approval from their healthcare provider.

Definitions

We defined a confirmed COVID-19 case as detection of SARS-CoV-2 RNA by real-time reverse transcription PCR (13). According to the Council of State and Territorial Epidemiologists definition, a probable case is one that meets clinical criteria and epidemiologic evidence with no confirmatory laboratory testing performed for COVID-19, meets presumptive laboratory evidence and either clinical criteria or epidemiologic evidence, or meets vital records criteria with no confirmatory laboratory testing performed for COVID-19 (13). We defined a probable case as a symptomatic close contact to a confirmed case-patient. We defined close contacts as anyone ≤6 feet of a confirmed case-patient or a symptomatic contact to a confirmed case-patient (i.e., a probable case) for ≥15 minutes, ≥2 days before the case-patient’s symptom onset and until the case-patient began strict isolation or until the contact’s last exposure to the case.

Index Case Identification and Transmission Chains

SLCoHD staff conducted contact tracing investigations via telephone interview. Interviews included 5 components: providing isolation or quarantine guidance; monitoring contacts for 14 days after their last exposure to a case, with the option for daily phone calls or text messages; entering demographic data for contacts into the Utah National Electronic Disease Surveillance System (EpiTrax, https://epi.health.utah.gov/utah-national-electronic-disease-surveillance-system-ut-nedss) for linkage and tracking; community notifications, including notifying businesses, workplaces, event venues, churches, or persons who might have been exposed to confirmed cases; and providing resources, such as information on housing or financial support, SARS-CoV-2 testing locations, and where and when to seek medical care.

We grouped contacts into 3 main categories: confirmed cases, probable cases, and contacts under observation. We further divided the 3 categories into 8 subclassifications: confirmed cases comprised index, symptomatic positive, and asymptomatic positive cases; probable cases comprised untested but symptomatic persons; and contacts under observation comprised persons who were asymptomatic not tested, symptomatic negative, or asymptomatic negative, as well as unknown status cases (Appendix, https://wwwnc.cdc.gov/EID/article/27/12/21-1505-App1.pdf). Status of probable cases and contacts under observation could change during the quarantine period; for instance, a probable case could become a symptomatic positive case if the contact had a SARS-CoV-2-positive test result during the quarantine period.

Data Source

We used EpiTrax surveillance data to retrospectively construct COVID-19 transmission chains for all confirmed index case-patients and contacts. We abstracted demographics, exposure history, SARS-CoV-2 test results, symptoms, and underlying conditions for confirmed or probable cases. We also abstracted investigation notes and applicable dates for last exposure to the confirmed or probable case, symptom onset, symptom resolution, initial health department contact, COVID-19 tests, monitoring period, hospital admission and discharge, and death. We also identified each contact’s relationship to their respective index case-patient, such as household or nonhousehold contact and generation of contact (first through sixth generation).

We chose a priori to systematically select 10% of laboratory-confirmed cases diagnosed during March 12–May 3, 2020, in Salt Lake County. However, during that period, the number of cases identified in Salt Lake County grew. Our final sample represented 8% of the total 2,757 cases.

Effort Time and Cost

We selected 25 index case-patients and prospectively documented the time spent interviewing them and their 144 contacts, from time of initial health department interaction with the index case-patient to the end of each contact’s 14-day monitoring period. Interviewers prospectively recorded time needed to complete all 5 investigation components for the selected index case-patients. We grouped contacts into 1 of the 8 subclassifications and applied a β-PERT
distribution to Monte Carlo simulation to estimate time and staff salary required to conduct contact tracing investigations for each of the 8 disease statuses (Appendix). We used the minimum, mean, and maximum time documented investigating each of the 8 disease subclassifications as parameters for the simulation (Appendix). We estimated salary cost by multiplying the median wage of all staff involved in contact tracing by the total number of hours spent on the contact tracing investigation (Appendix). Costs comprised time spent conducting all interviews (i.e., cost per index case and cost per contact, including those that were ultimately unreachable or out of jurisdiction) and for community notifications. We excluded nonstandardized costs, such as overhead, overtime, and time and costs for trainings.

Data Management and Analysis
To quantify contact tracing efforts, we evaluated the number of contacts yielded and investigated from each index case. We did not reclassify symptomatic contacts to an index case-patient if their symptom onset date was earlier than their respective index case-patient, but we did include them in the analysis. We used R (R Foundation for Statistical Computing, https://www.r-project.org) and Stata (StataCorp LLC, https://www.stata.com) software for data management and descriptive analysis. We calculated 95% CIs for estimated time intervals between events, such as symptom onset, testing, and initial contact, and for estimated cost per type of case or contact investigation. This activity was reviewed by the Centers for Disease Control and Prevention and was conducted consistent with its policy and applicable federal laws (14–19).

Results

Index Case Identification and Contact Tracing
Of the 229 cases identified from the line list, 45 were excluded; 12 were excluded because the case-patient was a contact of a previously included index case and 33 because of incomplete data (Figure 1). Our final analysis included 184 index cases and 1,499 linked contacts. Among linked contacts, 922 were first-generation, 387 second-generation, 99 third-generation, 39 fourth-generation, 49 fifth-generation, and 3 sixth-generation contacts. Third-, fourth-, fifth-, and sixth-generation contacts were directly or indirectly linked to first-generation contacts of patients who tested positive, who had confirmed cases, or who had symptomatic but untested probable cases (Figure 1). Among 184 index case-patients, 153 (83%) did not have known contact with a laboratory-confirmed COVID-19 case-patient. Across all generations, we identified a median of 5 (range 0–97) contacts and a mean of 2.03 confirmed and probable secondary cases for each index case (Table 1). Of 1,499 contacts, 96 were unreachable; 89 were unreachable or did not have adequate information to trace, and 7 were out of jurisdiction and did not have final disease status. Of 1,499 contacts, 374 (25%) became confirmed or probable cases, of which 285 (19%) were confirmed and 89 (6%) were probable. The rate of secondary case detection was ≈31% among first-generation contacts; ≈16% among both second- and third-generation contacts; and ≈12% among fourth-, fifth-, and sixth-generation contacts.

Disease Status at Initiation and End of the Contact’s Monitoring Period
Among 1,499 contacts, 277/1,027 (27%) were tested during their monitoring period (Figure 2). Of the 277 tested contacts, 98 (35%) were SARS-CoV-2-positive after initial health department interaction. Among the 362 (24%) SARS-CoV-2-negative contacts, 183 (51%) had tested negative before their initial health department interview and 179 (49%) tested negative after the initial interview.

The proportion of household contacts who were symptomatic and positive increased from 11% at initial health department interaction to 18% after the monitoring period (Figure 2). When comparing the final disease status of contacts exposed within their household versus outside of their household, more contacts exposed within their households received testing (23% vs. 13%) (data not shown).

Key COVID-19–Associated Dates
The median time from symptom onset to initial health department interaction was 7 days (interquartile range [IQR] 4–10 days) for index cases compared with 4 days (IQR 1–7.25 days) for first-generation contacts (Figure 3; Appendix). The median time from laboratory PCR test collection to initial interview was 2 days (IQR 2–4 days) for index case-patients compared with 0 days (IQR 2–4 days) for first-generation contacts. Index case-patients generally started isolation on the day of the initial SLCoHD interview (median 0 days, IQR 0–3 days). First-generation contacts reported having quarantined themselves for a median of 0 days (IQR 0–5 days) before initial interview. First-generation contacts reported a date of last exposure as a median of 4 days (IQR 0–7 days) before the initial interview; household contacts reported a median of 1 day (IQR 0–5 days), and nonhousehold contacts reported a median of 6 days (IQR 4–9 days). The
time between last exposure to isolation decreased for each subsequent generation (Appendix). Among 270 contacts who reported ongoing exposure, such as persons who could not or did not isolate, 96% were household contacts.

**Effort and Staffing Cost**

We calculated time and salary cost (in USD) required to conduct contact tracing (Figure 4). Total time required to investigate 184 index cases and their 1,499 contacts was 1,102 staff hours at a total cost of $29,234 (Appendix). Median time and cost spent investigating an index case and all successive generations of contacts was 4.16 hours (95% CI 4.06–4.72 hours) at $107.22 (95% CI $92.60–$120.70).

Time and costs varied depending on the status of the contact. For each index case, the median investigation time was 79.23 (95% CI 76.56–81.40) minutes and...
median cost was $33.67 (95% CI $32.34–$35.22). Negative asymptomatic cases required the least amount of staff time, 21.50 (95% CI 21.05–22.08) minutes costing a median of $9.29 (95% CI $9.07–$9.50). The total time spent on community notification for exposure to a confirmed case was 84.13 hours (Figure 4). Each notification took a median of 34.67 (95% CI 32.45–37.78) minutes, including 121 (66%) index case-patients who requested work excuse letters and 14 (7.6%) index case-patients who requested notifications to community locations, such as medical facilities, event venues, churches, and grocery stores. The average gross hourly wage for salaried epidemiologists, nurses, and office support staff involved in contact tracing efforts was $29.52 (range $23.61–$35.42) (Appendix Table 4).

**Discussion**

Our analysis of contact tracing of 184 index cases and 1,499 close contacts in Salt Lake County, Utah, highlights the substantial cost and time needed for these investigations. In addition, we found that, for successive generations of contacts traced, fewer cases were identified, and the time between symptom onset and SARS-CoV-2 testing decreased. However, changing quarantine or social distancing guidance during the investigation period also might have resulted in fewer cases in later generations. These findings highlight the effectiveness of contact tracing to guide control measures and reduce onward transmission of SARS-CoV-2. Other jurisdictions can use these findings to examine their contact tracing yields, effort, and key COVID-19–associated time intervals to help guide programmatic changes.

Contact tracing is resource intensive (8). Every index case investigated produced a transmission chain containing a median of 5 linked contacts. The median time to investigate these transmission chains was 4.16 (95% CI 4.06–4.72) hours at a cost of $107.22 (95% CI $92.60–$120.70). During the study period, 2,757 COVID-19 cases in Salt Lake County required investigation period also might have resulted in few-

## Table. Number of contacts identified and COVID-19 status by generation among persons during COVID-19 contact tracing, Salt Lake County, Utah, USA, March–May 2020*

<table>
<thead>
<tr>
<th>Generation</th>
<th>Final status†</th>
<th>No. contacts/no. index cases investigated‡</th>
<th>No. contacts traced to identify 1 case (mean)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>All, n = 1,499</td>
<td>Confirmed case</td>
<td>285 (19)</td>
<td>1.55</td>
</tr>
<tr>
<td></td>
<td>Probable case</td>
<td>89 (6)</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>Not a case</td>
<td>1,029 (69)</td>
<td>5.59</td>
</tr>
<tr>
<td></td>
<td>Unreachable or out of jurisdiction</td>
<td>96 (6)</td>
<td>0.52</td>
</tr>
<tr>
<td>First, n = 922</td>
<td>Confirmed case</td>
<td>223 (24)</td>
<td>1.21</td>
</tr>
<tr>
<td></td>
<td>Probable case</td>
<td>63 (7)</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>Not a case</td>
<td>568 (64)</td>
<td>3.20</td>
</tr>
<tr>
<td></td>
<td>Unreachable or out of jurisdiction</td>
<td>48 (5)</td>
<td>0.26</td>
</tr>
<tr>
<td>Second, n = 387</td>
<td>Confirmed case</td>
<td>43 (11)</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>Probable case</td>
<td>17 (4)</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>Not a case</td>
<td>304 (79)</td>
<td>1.65</td>
</tr>
<tr>
<td></td>
<td>Unreachable or out of jurisdiction</td>
<td>23 (6)</td>
<td>0.13</td>
</tr>
<tr>
<td>Third, n = 99</td>
<td>Confirmed case</td>
<td>10 (10)</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Probable case</td>
<td>7 (7)</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Not a case</td>
<td>73 (74)</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>Unreachable or out of jurisdiction</td>
<td>9 (9)</td>
<td>0.05</td>
</tr>
<tr>
<td>Fourth–sixth, n = 91</td>
<td>Confirmed case</td>
<td>9 (10)</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Probable case</td>
<td>2 (2)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Not a case</td>
<td>64 (70)</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>Unreachable or out of jurisdiction</td>
<td>16 (18)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

*COVID-19, coronavirus disease; NA, not applicable.
†Contacts were categorized as follows: confirmed cases comprised symptomatic-positive persons, asymptomatic-positive persons, and persons with unknown symptoms who tested positive; probable cases comprised symptomatic persons who were not tested; not a case comprised asymptomatic persons who were not tested, asymptomatic-negative persons, and symptomatic-negative persons. See Appendix (https://wwnc.cdc.gov/EID/article/27/12/21-0505-App1.pdf) for each generation breakdown by final status.
‡The number of contacts per index case investigated was calculated by dividing the number of contacts in each category by the 184 index cases.
§The number of contacts traced to find a confirmed or probable case in each generation was calculated by dividing the total number of contacts (n = 1,499) by the number of cases in each category.
confirmed COVID-19 case-patient. The prevalence of cases without an identified epidemiologic link raises concerns over unrecognized transmission (23), which suggests contact tracing efforts alone might not be sufficient to stop disease transmission.

Our contact tracing yields, laboratory confirmation of infection among 19% of contacts, were higher than those in South Korea (4%), and Shenzhen (15%) and Guangzhou (17%) in China (2,7,24). Consistent with findings from recent studies (1,2,24,25), we found household contacts were infected at a higher rate (32%) than nonhousehold contacts (16%). The finding of higher infection rates among household contacts reinforces the importance of evaluating prevention measures, such as using hotels for contacts unable to separate themselves from other household members (26). Compared with index cases (n = 184), confirmed secondary cases (n = 285) identified through contact tracing generated about one fourth of the contacts and less than one fifth of the secondary cases. During the study period, testing capacity was limited, delaying health department notifications and initiation of contact tracing investigations, which might have increased yields because case-patients spent more time not knowing their infection status (8). In addition, because primarily symptomatic persons received testing, positive results might have resulted in higher rates and thus higher yields.

Modeling shows the probability of COVID-19 control decreases with long delays from symptom onset to case isolation, fewer cases ascertained by contact tracing, and increasing transmission before symptom onset (8). Thus, time intervals between symptom onset, laboratory testing, and initial health department interview provide insight into the efficiency of contact tracing investigations (27). One study found that contact tracing for COVID-19 reduced the time to test confirmation by 2.3 days and time to contact isolation by 1.9 days (24). Similarly, we observed a 3-day decrease in the time from symptom onset to initial health department interview starting with first-generation contacts and noted to be the same or further decreasing in most subsequent generations. The time interval from symptom onset to initial health department interview was longer than that from symptom onset to first positive test or from symptom onset to isolation initiation. This time interval decreased between the first-generation and sixth-generation contacts; later generation contacts might have had more opportunity to follow health department recommendations and for the health department to promptly recommend testing when indicated. Although the usefulness of contact tracing in the setting of sustained SARS-CoV-2 transmission has been questioned (28,29), consistent with other studies, our findings show that contact tracing reduced transmission; only one fourth of contacts traced and

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**Figure 2.** Coronavirus disease status at initial health department interaction and after 14-day monitoring period, Salt Lake County, Utah, USA, March–May 2020. Numbers in the center signify the change in status from initial interaction by health department after the monitoring period. Numbers on left and right represent total (% of cases in each group. The median monitoring period was the time from initial health department interview to 14 days after the last exposure to the index case. Colors represent disease status classification category.
quarantined experienced COVID-19–like symptoms or tested SARS-CoV-2–positive.

New technologies, such as mobile telephone application–based symptom monitoring and electronic contact tracing platforms, might alleviate some of the burden needed to carry out investigations. In Utah, contacts could opt to receive daily phone calls or text message notifications. Text messaging might improve efficiency by decreasing time for contact follow-up, but it requires additional resources, a robust information technology infrastructure, and strong data protection safeguards (21). Smartphone technology is another powerful tool for contact tracing; a widely accepted smartphone application that does not have major privacy concerns, including the collection of personal data such as location, might prove useful (30). In addition, technology such as point-of-care SARS-CoV-2 testing, where results can be obtained within 48 hours, could reduce laboratory turnaround time. Rapid tests aid in quickly identifying index cases and contacts to implement isolation protocols (J. Joung et al., unpub. data, https://doi.org/10.1101/2020.05.04.20091231) and could improve contact tracing metrics. Online platforms that can identify how cases and contacts are linked, such as MicrobeTrace (https://microbetrace.cdc.gov/MicrobeTrace), also could aid in the management of investigations by reducing duplicative efforts, thereby improving efficiency.

The ongoing COVID-19 pandemic and emergence of the SARS-CoV-2 B.1.617.2 (Delta) variant have demonstrated the need for continuing layered prevention strategies, including contact tracing (31). Our findings can help local and state jurisdictions

Figure 3. Box-and-whisker plots showing time from key coronavirus disease contact tracing–associated dates to other key dates, Salt Lake County, Utah, USA, March–May 2020. A) Days from symptom onset to PCR testing; B) days from PCR testing to initial interaction with Salt Lake County Health Department (SLCoHD); C) days from symptom onset to initial interaction with SLCoHD; D) days from last day of exposure to a confirmed or probable case to initial interaction with SLCoHD; E) days from monitoring start date to initial interaction with SLCoHD. The all contacts category includes contacts with an unknown relationship to a confirmed or probable case. Dotted red lines represent when the Salt Lake County Health Department had initial interactions with cases or contacts. Vertical lines within boxes indicate the median, left and right box edges indicate the interquartile range (IQR), and whiskers indicate the lower extreme and upper extreme quartiles; black dots indicate outliers. Negative values along the x-axis indicate that the second event happened before the first event.
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determine the cost, effort, and yields associated with implementing a comprehensive contact tracing program, factors that are crucial for guiding policy decisions. Our data, coupled with further cost studies, can help inform resource allocation, including staffing needs and roles, technology requirements, and strategies to evaluate cost-effectiveness. In addition, our findings can be used to develop mathematical models to determine the need to scale up contact tracing to focus on all cases and contacts or to scale down and focus only on first-, second-, and third-generation contacts, as well as to decide who to interview, such as high-risk contacts or household contacts.

Our study’s first limitation is that our approach might not be generalizable because Utah’s surveillance system enables linkage between cases and contacts, which might not be available in other jurisdictions; differences could also exist in contact

Figure 4. Estimated median time and cost spent educating, interviewing, and charting index cases and their contacts by final coronavirus disease status, Salt Lake County, Utah, USA, March–May 2020. Community notifications consisted of notifying businesses or persons that might have been exposed to the confirmed case such as in a workplace, at a wedding, or in a church. Asterisk (*) indicates case; dagger (†) indicates probable case. A) Median time in minutes and 95% CIs are reported above each bar. B) Median cost in USD and 95% CIs are reported above each bar.
participation across jurisdictions. Second, during March 2020, testing was available only for persons meeting initial COVID-19 symptom criteria (Appendix), which might have reduced case identification and the ability to test contacts. Third, interventions such as social distancing guidance and stay-at-home orders introduced during March–May 2020 might have decreased transmission. Fourth, information was derived from interviews, which have a potential for recall bias, including naming all contacts (32). Fifth, costs of contact tracing are underestimated because we could not account for overtime benefits, such as time-and-a-half pay; overhead, such as staff health insurance and facility utility costs; staff training time; time spent providing services to the community, such as time to drop off masks; and other expenditures. Sixth, we could not track how many persons complied with recommendations to self-isolate or quarantine; the ability to determine whether cases and contacts complied with recommendations would aid in further quantifying contact tracing yield and effort. Finally, patients who do not seek care, potentially because of presumptomatic or asymptomatic infection, are a further challenge to preventing additional cases because SARS-CoV-2 shedding is highest early in illness (8). We found that 2% of asymptomatic contacts tested SARS-CoV-2-positive and 76% of asymptomatic contacts were not tested. Therefore, the attack rate might have been underestimated given the large proportion of asymptomatic contacts who did not get tested.

In conclusion, our analysis highlights the importance of contact tracing to reduce transmission of SARS-CoV-2. However, the effectiveness of contact tracing is contingent upon availability of substantial resources and rapid testing capacity. Persons should seek testing as soon as they experience COVID-19-like symptoms and begin isolation while results are pending. Because of early viral shedding, health department messaging should strongly direct contacts to obtain testing when possible, especially contacts with a higher risk for exposure, such as caregivers within households, populations in congregate settings, and contacts with underlying conditions; or for contacts who have an occupation requiring them to be in contact with other vulnerable persons, such as long-term care facility workers, day-care workers, and those who work with unvaccinated persons (33,34). Contact tracing metrics evaluated in this study can help other jurisdictions design, improve, and scale up contact tracing programs as needed for their specific epidemiologic contexts. Health departments should consider adjusting their approach to contact tracing as the situation evolves and adopting new technologies as these become available.

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We thank Andrew Hill and Prabasaj Paul for their critical review of this analysis and Dagmar Vitek for supporting and approving this evaluation. We thank the Salt Lake County residents for their cooperation working with the health department in their contact tracing efforts. We thank the Salt Lake County Health Department and Utah Department of Health for their participation and involvement in this project, which contributed to its success; we would not have been able to carry out this evaluation without their support.

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References
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Transmission of Severe Acute Respiratory Syndrome Coronavirus 2 in Households with Children, Southwest Germany, May–August 2020


Resolving the role of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmission in households with members from different generations is crucial for containing the current pandemic. We conducted a large-scale, multicenter, cross-sectional seroepidemiologic household transmission study in southwest Germany during May 11–August 1, 2020. We included 1,625 study participants from 405 households that each had ≥1 child and 1 reverse transcription PCR–confirmed SARS-CoV-2–infected index case-patient. The overall secondary attack rate was 31.6% and was significantly higher in exposed adults (37.5%) than in children (24.6%–29.2%; p = <0.015); the rate was also significantly higher when the index case-patient was ≥60 years of age (72.9%; p = 0.039). Other risk factors for infectiousness of the index case-patient were SARS-CoV-2–seropositivity (odds ratio [OR] 27.8, 95% CI 8.26–93.5), fever (OR 1.93, 95% CI 1.14–3.31), and cough (OR 2.07, 95% CI 1.21–3.53). Secondary infections in household contacts generate a substantial disease burden.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly spread globally since its emergence in December 2019. As of March 2021, >120 million infections have been reported, and >2.7 million deaths have been attributed to the novel coronavirus disease (COVID-19) (1). The severity of COVID-19 and the risk for a complicated course of illness or death increase with age (2,3). In terms of SARS-CoV-2 transmission, conjectures early in the pandemic were that asymptomatic (i.e., healthy) but infectious children played a particularly substantial role. The underlying assumption that children were drivers of the pandemic was based on experience with seasonal influenza virus. Consequently, closures of schools and preschools were among the earliest nonpharmaceutical interventions for transmission (4). However, the role of children in the transmission of SARS-CoV-2 remains controversial (5–7).

Next to superspreading events (8), intrahousehold transmission of SARS-CoV-2 is a major driver of the pandemic (9). A recent systematic review and meta-analysis based on reverse transcription PCR

**Ethics**

The study protocol was approved by the independent ethics committees of the Medical Faculty Heidelberg (approval no. S-294/2020), Medical Faculty Tübingen (approval no. 293/2020BO2), University of Ulm (approval no. 152/20), and University of Freiburg (approval no. 256/20_201553). The study was conducted according to the Declaration of Helsinki. Written informed consent was obtained from all household members and parents or guardians; children gave consent when appropriate for their age.

**Eligibility Criteria and Study Procedure**

Households were eligible for enrollment if they met all of these inclusion criteria: SARS-CoV-2 detection by RT-PCR from a nasopharyngeal or oropharyngeal swab specimen in ≥1 household member, ≥1 household member <18 years of age, residency in the state of Baden-Württemberg, and all household members having been officially released from quarantine. Key exclusion criteria were lack of written consent and insufficient knowledge of the German language.

Questionnaire items were number of household members and, for each member, age, sex, and whether they had ever tested positive for SARS-CoV-2. We asked participants reporting an RT-PCR-confirmed SARS-CoV-2 infection for the date when the positive specimen was collected, COVID-19–related symptoms (fever, cough, diarrhea, or dysgeusia), and whether they were hospitalized for COVID-19. We defined the index case-patient as the household member with the first SARS-CoV-2 RNA–positive specimen collected. We validated this definition in a subset of 54 households from 1 study center for which additional questionnaire information on transmission routes from nonhousehold contacts with COVID-19 were available. In 52 (96.3%) of 54 households, the definition of the index case based on timing of the RT-PCR test was consistent with the definition based on this anamnestic information. The RT-PCR test was performed within 24 hours, and a positive test result immediately triggered a strict home isolation and
quarantine for all household members for ≥14 days unless hospitalization was required.

**Laboratory Analysis**

We sent blood samples to the respective diagnostic laboratories in the 4 study centers, and serum was prepared on the same day. Samples were either immediately analyzed or stored at 4°C until further processing. Samples were analyzed for IgG reactive to the S1 domain of the viral spike glycoprotein and the SARS-CoV-2 nucleocapsid (N) protein. Antibodies reactive to the N protein were measured either with the Elecsys Anti-SARS-CoV-2 IgG/IgM ECLIA test kit (Roche, https://www.roche.com) processed on a Roche Cobas e601 or e411 module (in Heidelberg, Tübingen, and Ulm), or by recomWell SARS-CoV-2 IgG ELISA (Mikrogen Diagnostik, https://www.mikrogen.de/start.html) run on a BEP III analyzer in (Freiburg). SARS-CoV-2 IgG for the S1 domain of the spike protein were measured with the Euroimmun Anti-SARS-CoV-2-ELISA (IgG) test kit (Euroimmun, https://www.euroimmun.com) in Freiburg and Ulm. In Heidelberg and Tübingen, IgG/IgM directed against the receptor-binding domain of S1 were analyzed with the SARS-CoV-2 Total (COV2T) CLIA Assay (Siemens Healthineers, https://www.siemens-healthineers.com) on a Siemens ADVIA Centaur XP analyzer.

We categorized serum samples with concordant results in both assays as seropositive or seronegative. In case of discordant results, we performed additional, study site–specific measurements. These measurements were a neutralization assay (Tübingen) (22); the Euroimmun Anti-SARS-CoV-2-ELISA (IgG) (Euroimmun) (Heidelberg); the Elecsys Anti-SARS-CoV-2 IgG/IgM ECLIA (Roche) (Freiburg); or the ARCHITECT SARS-CoV-2 IgG, a test for IgG against the viral N protein (Abbott Laboratories, https://www.abbott.com) on an Abbott ARCHITECT 1000 instrument (Ulm). We classified serum samples with a positive reaction in the additional assay as seropositive.

**Statistical Analysis**

We performed analyses with R version 4.0.0 (R Foundation for Statistical Computing, https://www.r-project.org). We present results for continuous variables as mean with SD (for data with normal distribution) or median with interquartile ranges (IQR) and minimum and maximum values, unless stated otherwise. SARS-CoV-2 seropositivity served as a proxy for previous infection. We calculated the observed SAR by dividing the number of exposed SARS-CoV-2 IgG-positive household members by all exposed household members. To model and predict SAR, we used generalized linear mixed-effects logistic regression models (GLMM) with a logit function and the dependent variable “SARS-CoV-2 infection (yes/no)” of exposed household members and the predictors age of index case-patient, age of exposed household member, sex of index case-patient, sex of exposed household member, household size, and SARS-CoV-2–seropositivity in the index case.

We used a generalized linear mixed-effects model tree (23) to detect subgroup interactions in SAR of exposed household members (R package glmertree). This method uses model-based recursive partitioning to detect subgroup interactions and a GLMM to estimate the random-effects parameters (23). No a priori formulated hypotheses were tested, and therefore all p values and CIs are reported as descriptive measures. We compiled a more detailed description of GLMM models, simulations, violin plots, and R code (Appendix, https://wwwnc.cdc.gov/EID/article/27/12/21-0978-App1.pdf).

**Results**

**Study Population**

We enrolled 473 households during May 11–August 1, 2020 (Appendix Figure). We excluded households in which the index case could not be determined (n = 61). SARS-CoV-2–seropositivity plateaued at ≈30 days after a positive RT-PCR test for SARS-CoV-2 RNA (Appendix Table 1). To reduce the probability of negative serologic results because of imminent seroconversion, we excluded households that participated <30 days after a positive RT-PCR test of the index case (n = 7). A total of 405 households with 1,625 members (922 adults and 703 children) were available for final analysis (Table 1; Figure 1). The median age of index case-patients (n = 405) was 43.6 (range 1.36–71.5) years; 25 index case-patients (6.2%) were children. Among exposed household members (n = 1,220), 678 participants (55.6%) were children and 542 (44.4%) were adults. The sex distribution of index case-patients and exposed household members was balanced (Table 1).

**SARS-CoV-2 Seropositivity and Observed Secondary Attack Rates**

A total of 400 of 1,220 exposed household members tested positive for SARS-CoV-2 IgG and were categorized as previously infected (Figure 1), resulting in an overall observed SAR of 32.8%. Among the 405
index case-patients with RT-PCR–confirmed SARS-CoV-2 infection, 363 (89.6%) were seropositive and 42 (10.4%) were seronegative at the time of study participation. The rate of seropositivity in households with a seropositive index case-patient (393 of 1,090 [36.1%]) was 6-fold higher than the rate in households with a seronegative index case-patient (7 of 130 [5.4%]) (Table 2). The observed SAR in adults was 38.0% (206 of 542) compared with 28.6% (194 of 678) in children; it did not differ substantially among the 3 pediatric age groups (<6 years, 26.6%; 6–11.9 years, 30.7%; 12.0–17.9 years, 27.9%).

The observed SAR in exposed household members increased with the age of the index case-patient, from 13.3% for those <12 years of age to 71.4% for those ≥60 years of age (Table 2). The observed SAR in exposed male (32.5%) and female (33.1%) household members and in those with a male (33.9%) or female (31.6%) index case-patient were similar. Among the 405 index case-patients, 394 (97.3%) reported COVID-19–related symptoms and 9 (2.2%) were asymptomatic; no data were reported for 2 index case-patients. The most prevalent symptom was dysgeusia; cough, fever, and diarrhea were next most prevalent (Appendix Table 2). Overall symptoms (98.3% vs. 88.1%), and especially fever (58.4% vs. 33.3%) and dysgeusia (66.1% vs. 31.0%), were more prevalent in seropositive index case-patients than in seronegative index case-patients. A total of 22 (5.4%) index case-patients were hospitalized.

Risk Factors for SARS-CoV-2 Transmission

We used a linear mixed-effects logistic regression model to analyze these risk factors for virus transmission: age and sex of index case-patients and of exposed household members, household size, and SARS-CoV-2 seropositivity of the index case-patient (Table 2). SARS-CoV-2 seropositivity of the index case-patient was the risk factor most strongly associated with the SAR (odds ratio [OR] 27.8, 95% CI 8.26–93.5; p<0.001).

The predicted SAR in adults was higher than the predicted SARs in the 3 pediatric age groups, which were broadly similar (Table 2). Age of the index case-patient was also a risk factor for virus transmission. The predicted SAR in exposed household members was lowest when the index case-patient was <12 years of age (12.0%) and highest with an index case-patient ≥60 years of age (72.9%) and plateaued around 31% for index case-patients 12.0–59.9 years of age. It differed significantly between adults 18.0–59.9 years of age and those ≥60 years of age (OR 9.02, 95% CI 1.19–72.8; p = 0.039). Sex of the index case-patient and sex of the exposed household member were not associated with the SAR (Table 2). Larger households tended toward lower predicted SARs (Table 2); when we applied a Fisher exact test to the observed data, households with ≥4 household members were associated with a lower SAR (Appendix Table 3).

We compared the observed and predicted SAR associated with age of the index case-patient, age of

### Table 1. Demographic characteristics of study participants from 405 households, southwest Germany, May–August 2020

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total cohort</th>
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<th>Children</th>
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<td>703</td>
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<td>Range</td>
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<td>38.0–49.9</td>
<td>9.03–16.2</td>
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<tr>
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<tr>
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<td>5.58–13.8</td>
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**Sex**

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**Household size***

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**Region**

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<td>Children</td>
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</tr>
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</table>

*Includes household members who did not participate.
exposed household members, household size, and SARS-CoV-2 seropositivity of the index case-patient (Figure 2). We calculated the predicted SAR by using the generalized mixed-effects logistic regression model with simulations. In all 4 analyses, the observed and predicted SARs were almost identical, indicating that this logistic regression model was valid.

We used the same risk factors for a generalized linear mixed model binary decision tree to study subgroup interactions of risk factors for SAR. The most dominant risk factor for transmission was SARS-CoV-2-seropositivity of the index case-patient; the next most dominant risk factor was increased age of exposed household members (Figure 3, panel A). In an alternative generalized linear mixed model binary decision tree, only age of the index case-patient was considered a risk factor, and the age of exposed household members was fixed in each terminal node (Figure 3, panel B). In this model, the SAR increased with age of the index case-patient. Within each age group of index case-patient, the SAR also increased with age of the exposed household member (Figure 3, panel B). The observed SAR was 23.1% (80/346) if the index case-patient was <37.8 years of age, 34.7% (287/827) if the index case-patient was 37.8–57.9 years of age, and 70.2% (33/47) if the index case-patient was >57.9 years of age. However, cutoff values determined by generalized linear mixed-effects model trees are data-driven and should not be interpreted as fixed parameters. When we excluded the 42 households with a seronegative index case-patient and analyzed the remaining 363 households (Appendix Table 4) or included the time interval from positive SARS-CoV-2 RNA specimen collection in the index case-patient to the serologic assessment of the household (Appendix Table 5), we obtained comparable results.

We analyzed the COVID-19–related symptoms cough, fever, dysgeusia, and diarrhea, as well as hospitalization in the index case-patient, in an additional linear mixed-effect logistic regression model, consisting only of households with a symptomatic index case-patient with known hospitalization status.
(n = 393) and adjusted for age of the index case-patient. The occurrences of fever and cough, but not of diarrhea, dysgeusia, or hospitalization, were significantly associated with a higher predicted SAR (Table 3).

### Discussion

This large multicenter serologic SARS-CoV-2 household transmission study focusing on children revealed that the predicted SAR in household members <18 years of age is ≈8–13 percentage points lower than in adults. The predicted SAR also increased with increasing age of the index case-patient, which resulted in SARs of exposed household members ranging from 12.0% when the index case-patient was <12 years of age to 72.9% when the index case-patient was ≥60 years of age. The infectiousness of teenagers was similar to adults <60 years of age, and the predicted SAR was 31% in both groups.

Next to age, a systemic immune response after SARS-CoV-2 infection in the index case-patient, as indicated by circulating virus-specific antibodies, was strongly associated with the occurrence of secondary household cases. The biologic basis for the strikingly low SAR of 5.4% in households with a seronegative index case-patient (42/405) is unclear. Given the high specificity of SARS-CoV-2 RT-PCR testing, a proportion of 10% false-positive results is unlikely. Presumably the individual viral load is associated with both a stronger adaptive immune response and the extent of symptoms, which in turn increase virus transmission. Our observations that fever and dysgeusia were less prevalent in seronegative index case-patients and that presence of fever and cough in the index case-patient increases SAR in exposed household members are in line with this hypothesis. Furthermore, our findings are in accordance with other studies, in which specific SARS-CoV-2 antibodies were frequently absent in patients with mild symptoms (15). However, the hypothesis that SARS-CoV-2 transmission is more likely in cases with higher or persisting viral load in the nasopharynx has not been formally tested.

Our observation of a SAR ≈10 percentage points higher in adults than in children is consistent with household studies based on RT-PCR-confirmed SARS-CoV-2 infection (10). In contrast, previous household transmission studies based on SARS-CoV-2 serologic testing reported lower (18), similar (28%) (17), or higher (43%–52%) (19–21) SARs in children. However, these studies were relatively small.

A low proportion of pediatric index case-patients (6.2%) and an increasing SAR with increasing age of the index case-patient is in line with most previous serologic testing–based (24) or RT-PCR-based (10,13,25) household transmission studies.
comparing infectiousness of pediatric and adult index case-patients. In addition, Soriano-Arandes et al. (13) found fewer intrahousehold transmissions after reopening schools, whereas intraschool transmissions were rare events in several countries after schools reopened in 2020 (26–32). Keeping schools open with strict hygiene measures in place could reduce overall SARS-CoV-2 transmission because close intrahousehold contact is reduced and children might act as sentinels for household transmissions when regularly tested at school.

Lower SARs in children have been previously attributed to differences in contact patterns; for example, physical interactions between spouses might be more intimate than between children and adults (33). Accordingly, we hypothesized that among children, toddlers might have more frequent and close physical contact with their parents than older children and adolescents, which might result in a SAR inversely correlated with age. However, we found the SAR to be similar among toddlers, older children, and adolescents, which indicates that behavior might not have a major effect on virus transmission within families. In contrast, the lower susceptibility to SARS-CoV-2 in children points toward the possible role of developmental factors related to host resistance and immunity. Low expression levels of angiotensin-converting enzyme 2, the cellular entry receptor of SARS-CoV-2 in the nasal epithelium of children has been previously suggested as a mechanistic factor (34). Moreover, previous endemic coronavirus infections in children might provide some protection, as indicated by frequently

Figure 2. Observed and predicted SARs in household members exposed to severe acute respiratory syndrome coronavirus 2, southwest Germany, May–August 2020. SARs shown are associated with age of index case-patient (A), age of exposed household member (B), household size (C), and SARS-CoV-2 seropositivity of the index case-patient (D). The mean observed SAR is shown as a black dot. The mean (black triangles), interquartile range (white bars), maximum and minimum (ends of vertical black line), and distribution (gray shading) of the predicted SAR are shown in the violin plots. The predicted SARs were calculated from the generalized linear mixed-effects logistic regression model. SAR, secondary attack rate.
circulating cross-reacting antibodies (35) and SARS-CoV-2-reactive CD4+ T cells in ≤60% of unexposed children and adolescents (36). Furthermore, the innate immune response in children with SARS-CoV-2 exposure and infection might differ from that in adults, such as with respect to circulating neutrophil subsets, the induction of interferons (37), and cytokines (38).

The strengths of this study are its multicenter design, the high number of study participants, the application of robust statistical models, and a relatively low risk for recruitment bias, because potentially eligible households were invited through health authorities. Limitations are the high proportion of symptomatic index case-patients (97%) and adult (94%) index case-

Figure 3. Generalized linear mixed model binary decision trees in study of transmission of severe acute respiratory syndrome coronavirus 2 in households with children, southwest Germany, May–August 2020. A) Model incorporating the 2 most dominant effects (p<0.001) on the SAR of exposed household members, SARS-CoV-2 seropositivity of the index case-patient and age of exposed household members with a seronegative or a seropositive index case-patient. B) Model incorporating only age of the index case-patient as a risk factor; SAR was modeled by age of exposed household member within each node. In both panels, the observed SAR as a proportion of seropositive (black) and seronegative (gray) exposed household members with these characteristics are shown within final nodes and as a percentage with the total number of seropositive/total exposed household members in parentheses above each node. In panel B, the predicted SARs are indicated within each final node as a red dot and red straight line. SAR, secondary attack rate.
patients. Potential explanations are the limited diagnostic test capacities at the time of the study, which favored testing of symptomatic adults and those with work-related exposure (e.g., in the healthcare sector) or a history of travel to high-risk regions. In households with an asymptomatic index case-patient, symptomatic secondary case-patients were possibly mislabeled as index case-patients as a result of this testing policy. An overestimation of the SAR in adults and an underestimation in children is likely, because COVID-19-associated symptoms were a good predictor for SARS-CoV-2 infection in adults but not in children (16). COVID-19–related symptoms were not more frequently in seropositive children than seronegative children, and other respiratory viruses were >100 times more prevalent than SARS-CoV-2 in children with acute respiratory symptoms across Germany during February–May 2020 (39). Another factor that leads to an underestimation of the SAR is a negative SARS-CoV-2 IgG test result in ≤10% of previously infected participants, which might partly be because of false-negative test results and a physiologic reduction of SARS-CoV-2 IgG levels over time.

Other potential weaknesses of serologic testing–based household transmission studies are the difficulty of differentiating between secondary and tertiary transmission within the same household and the inability to rule out nonhousehold infections of exposed household members. This could result in an overestimation of the SAR per index case. The probability that 1 nonhousehold (community) index case-patient infected several household members is a potential bias of this study. However, the probability of SARS-CoV-2 infections from 2 different nonhousehold (community) index case-patients can be assumed to be low, because this study was performed shortly after the first pandemic wave and the strict government-imposed lockdown (Appendix Figure), when the SARS-CoV-2 seroprevalence in southwest Germany was as low as 1.8% in adults and 0.6% in children (16). Finally, the findings regarding SAR and its age dependency only apply to the SARS-CoV-2 variants circulating in Germany at that time and might not be translated to the more transmissible Delta variant.

In conclusion, this multicenter SARS-CoV-2 household transmission study focusing on children demonstrates that secondary infections in household contacts generate a substantial disease burden. Age is a risk factor both for infectiousness of index cases and susceptibility of exposed household members. Furthermore, fever and cough in index case-patients were associated with higher levels of infectiousness. Households can be expected to remain sites for SARS-CoV-2 transmission because home quarantine and home isolation are key measures in cases of suspected or confirmed infections in most countries.

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### Table 3. Association between coronavirus disease–related symptoms or hospitalization in index case-patients and secondary attack rates in exposed household members from 393 households with a symptomatic index case-patient whose hospitalization status is known, southwest Germany, May–August 2020

<table>
<thead>
<tr>
<th>Index case symptom</th>
<th>No. index cases, n = 393</th>
<th>No. exposed, n = 1,182</th>
<th>No. seropositive exposed, n = 390</th>
<th>Observed SAR, %</th>
<th>Predicted SAR, % (IQR)†</th>
<th>Odds ratio (95% CI)†</th>
<th>p value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>225</td>
<td>666</td>
<td>253</td>
<td>38.0</td>
<td>36.8 (11.8–58.4)</td>
<td>1.03 (1.04–3.31)</td>
<td>0.015</td>
</tr>
<tr>
<td>Cough</td>
<td>236</td>
<td>717</td>
<td>272</td>
<td>37.9</td>
<td>36.8 (11.7–58.2)</td>
<td>2.07 (1.21–3.53)</td>
<td>0.008</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>91</td>
<td>271</td>
<td>94</td>
<td>34.7</td>
<td>33.2 (9.3–54.5)</td>
<td>0.80 (0.43–1.49)</td>
<td>0.481</td>
</tr>
<tr>
<td>Dysgeusia</td>
<td>253</td>
<td>748</td>
<td>252</td>
<td>33.7</td>
<td>32.2 (10.4–48.7)</td>
<td>1.41 (0.82–2.43)</td>
<td>0.213</td>
</tr>
<tr>
<td>Hospitalization</td>
<td>22</td>
<td>67</td>
<td>30</td>
<td>44.8</td>
<td>43.9 (15.2–68.9)</td>
<td>1.22 (0.40–3.75)</td>
<td>0.726</td>
</tr>
</tbody>
</table>

†The predicted SARs, odds ratios, and p values were calculated from a multivariable generalized linear mixed-effects logistic regression model. The respective references were absence of the symptom or “hospitalization” and adjusted for age of index case-patient as a numeric variable (OR 1.04, 95% CI 1.01–1.06, p = 0.0055).

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The first laboratory-confirmed case of coronavirus disease (COVID-19) in South Africa was reported on March 5, 2020, and the country has since experienced 2 waves of COVID-19, the first peaking in July 2020 and the second in January 2021 (1). Across Africa, the second wave was more severe than the first (2), and specifically in South Africa, higher weekly incidence, hospitalizations, and deaths were reported for the second wave, compared with the first (3–5). The second wave in South Africa was coupled with the emergence of a new variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), B.1.351, also known as 501Y.V2 or Beta (6).

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections may be underestimated because of limited access to testing. We measured SARS-CoV-2 seroprevalence in South Africa every 2 months during July 2020–March 2021 in randomly selected household cohorts in 2 communities. We compared seroprevalence to reported laboratory-confirmed infections, hospitalizations, and deaths to calculate infection–case, infection–hospitalization, and infection–fatality ratios in 2 waves of infection.

Post–second wave seroprevalence ranged from 18% in the rural community children <5 years of age, to 59% in urban community adults 35–59 years of age. The second wave saw a shift in age distribution of case-patients in the urban community (from persons 35–59 years of age to persons at the extremes of age), higher attack rates in the rural community, and a higher infection–fatality ratio in the urban community. Approximately 95% of SARS-CoV-2 infections were not reported to national surveillance.
of age, with no differences based on sex (8). Whether HIV infection increases the risk for SARS-CoV-2 infection is still unclear, and results from studies thus far have varied (9,10).

We describe the seroprevalence of SARS-CoV-2 in 2 household cohorts in a rural and an urban community at 5 timepoints from July 2020 to March 2021, during 2 epidemic waves. We compare disease prevalence between the first and second wave by comparing the seroprevalence by wave to reported laboratory-confirmed infections, hospitalizations, and deaths within the respective districts.

Methods

Study Population
We conducted a prospective study on a randomly selected household cohort in a rural community (Agincourt, Ehlanzeni District, Mpumalanga Province) and an urban community (Jouberton, Dr. Kenneth Kaunda District, North West Province) as part of the Prospective Household Study of SARS-CoV-2, Influenza, and Respiratory Syncytial Virus Community Burden, Transmission Dynamics, and Viral Interaction (PHIRST-C) study in South Africa. Methods for the cohort study are detailed in the Appendix (https://wwwnc.cdc.gov/EID/article/27/12/21-1465-App1.pdf). Recruitment to this study began in July 2020, and follow-up will continue through August 2021. Households that previously participated in the PHIRST study during 2016–2018 (11,12) and additional randomly selected households were eligible. Households with ≥3 household members of any age were enrolled if ≥80% of members consented. The study was approved by the University of the Witwatersrand Human Research Ethics Committee (reference no. 150808). The US Centers for Disease Control and Prevention relied on local clearance (Institutional Review Board approval no. 6840).

Seroprevalence
We collected baseline data and blood (blood draw [BD] 1) at enrollment (July 20–September 17, 2020) and every 2 months thereafter: BD2, September 21–October 10; BD3, November 23–December 12, 2020; BD4, January 25–February 20, 2021; and BD5, March 22–April 11, 2021. We confirmed HIV status from medical records (if a person was HIV-infected) and by using a rapid test for participants with unknown or self-reported negative status. We determined previous SARS-CoV-2 infection by using the Roche Elecsys anti-SARS-CoV-2 assay (Roche Diagnostics, https://www.roche.ch/en/standorte/rotkreuz.htm) to detect antibodies against the SARS-CoV-2 nucleocapsid protein. We performed the assay on the Cobas e601 instrument (Roche Diagnostics), and we considered a cutoff index (COI) ≥1.0 as an indication of prior infection (i.e., seropositivity). We performed data analysis in Stata 14 (StataCorp, https://www.stata.com) (13). We adjusted seroprevalence estimates for sensitivity and specificity, as previously described (14), on the basis of the manufacturers’ reported 99.5% sensitivity and 99.8% specificity (15). We obtained seroprevalence 95% credible intervals (CrIs) by using Bayesian inference with 10,000 posterior draws (14). We used Pearson’s χ² test to assess the statistical significance of differences in SARS-CoV-2 seropositivity between the 2 communities and across BDs, waves of infection, and HIV status.

Calculation of Infection–Case Ratio, Infection–Hospitalization Ratio, and Infection–Fatality Ratio by Wave of Infection
To assess the prevalence of SARS-CoV-2 and compare the severity of illness between the 2 waves, we performed an ecologic study comparing estimated number of infections on the basis of seroprevalence in our cohort study to reported number of cases, hospitalizations, and in-hospital and excess deaths in the same district for each wave. We calculated the age- and sex-adjusted total number of infections, laboratory-confirmed cases, hospitalizations, deaths, infection–case ratio (ICR) (i.e., number of infections compared with laboratory-confirmed cases), IHR, and in-hospital and excess deaths IFR (Appendix) for the first (March 1–November 21, 2020) and second (November 22, 2020–March 27, 2021) wave of infection (Figure 1).

Comparison of Cases between First and Second Wave of Infection
We compared characteristics of participants who showed seroconversion during the first and second wave of infections by using unconditional logistic regression. We compared participants who showed seroconversion in wave 1 (BD3) with those who showed seroconversion in wave 2 (BD5, excluding BD3 seroconversions). For this analysis, we only included participants with a BD3 and BD5 paired serum sample. For the multivariable model, we assessed all variables that were significant at p<0.2 on univariate analysis and dropped nonsignificant factors (p>0.05) with manual backward elimination. We also compared the site, age, sex, and HIV status of persons with a BD
3+5 pair with those without a BD 3+5 pair by using logistic regression.

**Persistence of SARS-CoV-2 Antibodies**

For participants with 5 serum samples collected and who showed seroconversion during BD2 to BD5, we plotted COI values with the BD at which seroconversion took place as point 0. For participants who were seropositive at baseline, we plotted COI results from each BD. We calculated mean COI and the exact 95% CI at each point by using the Clopper–Pearson method. We assessed percentage of participants with COI \( \geq 1 \) at each subsequent BD as number of participants with COI \( \geq 1 \) divided by total number of participants who showed seroconversion during BD2 to BD5 with a serum sample at the timepoint.

**Results**

**Study Population**

In the rural community, we approached 185 households, 118 (64%) were enrolled, and 641/692 (92%) of household members consented, agreed to participate, or both. In the urban community, 352 households were approached, 114 (32%) enrolled, and 570/607 (93%) of household members consented, agreed to participate, or both. In both communities, the percentage of children, women or girls, and unemployed persons included in the cohort were higher than in district census data (Appendix Table 1). Median age was 13 (interquartile range 7–29) and 21 (interquartile range 10–43) years, and HIV prevalence was 14% (95% CI 11%–17%) in the rural community and 18% (95% CI 14%–21%) in the urban community.

**Seroprevalence**

Most (83% [n = 553]) participants who lived in the rural community and most (83% [n = 499]) who lived in the urban community had both BD3 and BD5 blood collected (Appendix). Seroprevalence, adjusted for assay sensitivity and specificity, in the rural community was lower at BD1 than in the urban community (1% [95% CrI 0%–2%] vs. 15% [95% CrI 12%–18%]; p<0.001), increasing after the first wave of infections (at BD3) to 7% (95% CrI 5%–9%) in the rural community and 27% (95% CrI 23%–31%) in the urban community (p<0.001) (Figure 2; Appendix). After the second wave (BD5), seroprevalence increased to 26% (95% CrI 22%–29%; p<0.001) in the rural community and to 41% (95% CrI 37%–45%; p<0.001) in the urban community (Appendix).

At BD5, seroprevalence was highest in the 19–34 years age group (37% [95% CrI 28%–47%]) in the rural community and the 35–59 years age group (59% [95% CrI 49%–68%]) in the urban community (Figure 2; Appendix Table 3). The seroprevalence was lowest in children <5 years of age, 18% (95% CrI 10%–26%) in the rural community and 28% (95% CrI 17%–41%) in the urban community.

At BD5, SARS-CoV-2 seroprevalence was similar between HIV-infected and HIV-uninfected participants (Appendix Table 4). Persons who were HIV-positive were not more likely to be seropositive (adjusted odds ratio 1.0 [95% CI 0.7–1.5]).

**Infection–Case Ratio, Infection–Hospitalization Ratio, and Infection–Fatality Ratio by District and Wave of Infection**

During the first wave of infections (BD3) the age- and sex-adjusted seroprevalence at the rural site was
SARS-CoV-2 Seroprevalence, South Africa

11.75% (95% CrI 3.42%–24.60%), resulting in an ICR of only 4.74% (95% CI 2.36%–15.62%). We observed a 0.64% (95% CI 0.34%–1.96%) IHR and an in-hospital IFR of 0.12% (95% CI 0.07%–0.31%) and an excess deaths IFR of 0.43% (95% CI 0.21%–1.47%) (Figure 3, 4).

The seroprevalence in the rural community was 22.43% (95% CrI 10.46%–37.67%) for the second wave. The ICR was 3.71% (95% CI 2.28%–7.68%), IHR was 0.61% (95% CI 0.40%–1.22%), in-hospital IFR was 0.18% (95% CI 0.12%–0.34%), and excess deaths IFR was 0.65% (95% CI 0.39%–1.39%) (Figure 3, 4).

In the urban community, the seroprevalence at BD3 was 29.58% (95% CrI 18.04%–43.20%). We found a 3.54% (95% CI 2.53%–5.55%) ICR and 1.93% (95% CI 1.41%–2.98%) IHR. The in-hospital IFR was 0.16% (95% CI 0.13%–0.23%) and excess deaths IFR was 0.12% (95% CI 0.09%–0.20%) (Figure 3, 4). During the second wave, the seroprevalence in the urban community was 15.19% (95% CrI 6.49%–26.96%), resulting in an ICR estimate of 3.67% (95% CI 2.21%–8.07%), an IHR of 2.29% (95% CI 1.39%–4.96%), an in-hospital IFR of 0.36% (95% CI 0.24%–0.72%), and an excess deaths IFR of 0.50% (95% CI 0.29%–1.17%) (Figure 3, 4). These estimates standardized to World Health Organization world population estimates are shown in Appendix Figure 2.

Comparison of Case-Patients between First and Second Wave of Infection

Compared with the urban community, persons in the rural community who showed seroconversion were 4.7 (95% CI 2.9–7.6) times more likely to show seroconversion during the second wave. Compared with persons 35–59 years of age, persons 5–12 years of age were 2.1 (95% CI 1.1–4.2) times more likely to show seroconversion in the second wave and persons ≥60 years of age were 2.8 (95% CI 1.1–7.0) times more likely to show seroconversion in the second wave (Table). When we stratified the analysis by site, this association was only detected in the urban community (Appendix Table 6). Persons who did not have a BD 3+5 pair were more likely to be <5 or 19–34 years of age (Appendix Table 7).

Persistence of SARS-CoV-2 Antibodies

Of the 72 participants who were seropositive at BD1 and with BDs 1–5 samples collected, 99% (71/72) still had a COI ≥1 by BD5. The mean COI at baseline for seropositive participants was 64, which increased to 125 at BD2 and dropped to 59 at BD5 (Figure 5, panel A). The participant who no longer had detectable SARS-CoV-2 antibodies at BD5 had a starting COI of 9. Of the 210 participants with BD 1–5 samples, 99% (167/169), 99% (70/71%), and 93% (41/44) still had a COI ≥1 in the first, second, and third BD after initial seroconversion, respectively (Figure 5, panel B). The participants who seroreverted had starting COIs ranging from 2 to 6, and none showed seroconversion again after reversion during the study period. The mean COI at the point of seroconversion was 48, which increased to 86 at the first BD after seroconversion and reduced to 61 at the third BD after seroconversion.

Discussion

We assessed SARS-CoV-2 seroprevalence in 1,211 persons living in 2 diverse communities in South Africa and show that laboratory-confirmed cases reported from study districts greatly underestimate the actual prevalence of SARS-CoV-2 infections. At baseline, seroprevalence was 1% and 15%, increasing to 7% and 27%, respectively, after the first wave, by March 2021. After the second epidemic wave, seroprevalence was

Figure 2. Seroprevalence of severe acute respiratory syndrome coronavirus 2 at each blood collection, by age group, in a rural community (A) and an urban community (B), South Africa, March 2020–March 2021.
26% in the rural community and 41% in the urban community. The highest seroprevalence was 59% in adults 35–59 years of age in the urban community, and the lowest was 18% in rural community children <5 years of age. During the second wave, compared with the first wave, the rural site was more affected, and infections in the second wave more likely affected children 5–12 and adults ≥60 years of age in the urban community. In the urban community, IFR was higher in the second wave (0.36%–0.50%) compared with the first (0.12%–0.16%), and numbers of infections were lower, suggesting possible increased severity associated with the emergence of novel variant B.1.351. Most persons who showed seroconversion maintained detectable SARS-CoV-2 antibodies in subsequent serum samples.

Low seropositivity was observed at the rural site at baseline, and seroprevalence remained low after the first wave of infections, reaching 7%, which was considerably lower than the 27% at the urban site at the same time. This observation could be related to the relatively isolated location and lower population density in the rural community compared with more densely populated urban community. Seroprevalence in the rural site increased to 26% after the second wave of infections within the district. This increase could have been attributable to possible increased transmissibility of the B.1.135 lineage that was circulating in the second wave (16), as well as additional transmission networks in the community during the December holiday period, when largescale urban-to-rural migration takes place as persons return home for year-end holidays. The urban site had fewer seroconversions in the second wave compared with the first, which may be attributable to existing immunity among persons in the community after the first wave.

Figure 3. Age- and sex-standardized number of severe acute respiratory syndrome coronavirus 2 infections, laboratory-confirmed diagnoses, hospitalizations, and deaths per 100,000 population in a rural community during infection wave 1 (A) and wave 2 (B) and an urban community during infection wave 1 (C) and wave 2 (D), South Africa, March 2020–March 2021. CrI, credible interval.
As seen in previous studies (8), adults had the highest seroprevalence levels, although a relatively high seroprevalence of 18% and 28% persisted in children <5 years of age at the rural and urban community, respectively.

When comparing the characteristics of persons infected in the second wave to those infected in the first, persons infected in the second wave were more likely to be from the rural site and to be <13 or >60 years of age, compared with persons 35–59 years of age. The shift in age groups affected was only detected in the urban community, possibly because of the large number of adults infected during wave 1, whereas the number of infections in wave 1 in the rural community was lower.

A study conducted among blood donors in South Africa during the second wave found a seroprevalence of 32%–63% in 5 provinces of South Africa that have both rural and urban communities (W. Sykes et al., unpub. data, https://doi.org/10.21203/rs.3.rs-233375/v1). In our study, we observed a seroprevalence in adults ranging from 25% to 37% in rural households, and from 35% to 59% in urban households, suggesting that seroprevalence is heterogeneous between communities. In Kenya, the seroprevalence in blood donors during the country’s first wave of infections was 4% and was also higher in urban communities (17). In a population-level household serosurvey conducted in Zambia during their first wave of infections, 11% of persons had evidence of SARS-CoV-2 infection (18).

Based on our estimates, only 4%–6% of cases were laboratory-confirmed, suggesting that substantially higher prevalence of infection was ascertained through serologic testing and that the differences may have been greater in the urban community than the rural community; however, more extensive studies are needed to assess whether this observation is consistent in other areas. Compared with the urban community, the rural community had less than half the rate of hospitalization (0.6% vs. 2.0%). These observations may be attributable to differences in referral and testing policies, health-seeking behavior, and access to care, as well as differences in circulating lineages within these districts.

A study comparing the severity of the first and second waves of infections in South Africa in hospitalized patients found a higher mortality rate in the second wave, compared with the first (19). At the urban site, the IFR was higher in the second wave (0.36%–0.50%) compared with the first (0.12%–0.16%), although no differences were observed in IHR between the 2 waves. The lower overall number of infections in the second wave in this site means that our finding of increased mortality is unlikely to be related to pressure on health services. The increased severity of the second wave may be related to increased severity of the B.1.135 variant, but further studies are needed to confirm this relation. The excess death IFR during the first wave in the urban site was smaller than the in-hospital IFR. This difference may be attributable to uncertainty on the process for excess death estimation, or that the 85% contribution of COVID-19 to excess deaths was an underestimation within the province. However, the in-hospital IFR followed the same trend of increase between wave 1 and 2 (0.16% to 0.36%). Although no significant increase was observed between the IFR in the rural community between wave
Among persons 12 years of age.
‡Self-reported history of asthma, lung disease, heart disease, stroke, spinal cord injury, epilepsy, organ transplant, immunosuppressive therapy, organ transplantation, cancer, liver disease, renal disease, or diabetes.
§Among persons ≥15 years of age.
¶Among persons ≥18 years of age.

1 and 2, the excess (maximum) IFR estimate (0.43%) was already high in wave 1, similar to the IFR for wave 2 in the urban community (0.50%). Considering the lower IHR in the rural community for both waves compared with the urban community, this observation may point toward lack of access to care or delays in seeking care. In-hospital SARS-CoV-2 mortality rates have previously also been shown to be higher in Mpumalanga Province where the rural community is located (19).

Our first wave in-hospital IFR estimates (0.12% rural, 0.16% urban) were similar to the age-adjusted 0.15% reported from India for the first wave of laboratory-confirmed deaths from SARS-CoV-2 infection (20). In addition, our first wave excess death IFR was higher in the rural (0.431%) and lower in the urban (0.12%) community compared with the age-adjusted 0.28% IFR excess deaths reported from Brazil during their first wave of infections (21).

Although previous studies have shown that antibodies against the SARS-CoV-2 nucleocapsid wanes more quickly than those against the spike protein (22,23), 93% of persons who showed seroconversion at BD2 still had detectable nucleocapsid antibodies 6 months later. Direct antigen-sandwich format assays, such as the Roche anti-N assay used in our study,
have been found to reliably detect antibodies in longitudinal samples (24). Of the few that seroreverted in this timeframe, the starting COI was low.

We did not observe a difference in SARS-CoV-2 seroprevalence in HIV-infected and HIV-uninfected persons at either site. Although our sample size was too small to detect small differences in a stratified analysis, we also did not observe a signal when using logistic regression. Because HIV causes immune suppression, a concern exists that HIV-infected persons may be more susceptible to SARS-CoV-2 infection (9,10). Although HIV infection may not increase susceptibility to infection, it has been demonstrated to be a risk factor for having onset of severe COVID-19 and death after infection (9,25).

Our study is limited by a small sample size, reducing the power for accurate seroprevalence estimates in small age strata, and inclusion of only 2 geographic sites, and therefore may not be representative of other districts and provinces in South Africa. Because we used seroprevalence to estimate infections by wave, we could have missed reinfections in the second wave. Based on data from the same cohort, these reinfections occurred in only a small portion (3%) of the cohort (C. Cohen et al., unpub. data, https://doi.org/10.1101/2021.07.20.21260855) and would have had a negligible influence on the infection ratios. ICR, IHR, and IFR formed part of an ecologic analysis, which is inherently prone to biases. Excess deaths in the first wave may be underestimated because the reporting period only started in June. Transmission dynamics within our cohort may not be similar to the transmission dynamics within the district. Seventeen percent of persons did not have a BD 3+5 pair, and bias could have been introduced if the seroprevalence were different for those without a BD 3+5 blood pair. Ongoing follow-up of this cohort will track future infections and monitor antibody waning, and compare these data to laboratory-confirmed infections and symptoms from twice-weekly follow-up.

A strength of our study is the collection of samples from prospectively followed-up persons from randomly selected households within the study communities and inclusion of persons of all ages. As a longitudinal study, our study provides the advantage

![Figure 5. Cutoff index (COI) on Roche Elecsys (Roche Diagnostics, https://www.roche.ch/en/standorte/rotkreuz.htm) anti-severe acute respiratory syndrome coronavirus 2 assay for persons with blood draws 1–5 samples who were seropositive at baseline (A) or showed seroconversion during blood draws 2–5, South Africa, July 2020–April 2021. Purple line indicates mean COI with 95% CIs. COI values in panel B are aligned to first draw before seroconversion, COI, cutoff index.](Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 27, No. 12, December 2021 3027)
of serial comparisons of antibody responses in relation to reported laboratory-confirmed SARS-CoV-2 infections within the community through 2 successive SARS-CoV-2 waves.

We estimate that ≈95% of SARS-CoV-2 infections in these 2 communities were not laboratory-confirmed and reported to the national surveillance system, which has major implications for contact tracing and isolation and other measures to contain infection. We observed heterogeneity between seroprevalence estimates based on pandemic wave, community, and age group, indicating the need for ongoing studies that include diverse settings.

Additional members of the PHIRST-C group who contributed to this manuscript were Kgauagelo Patricia Kgagago, Linda de Gouveia, Maimuna Carrim, Mignon du Plessis, Retshidisiswe Kotane, and Tumelo Moloantoa.

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The investigators welcome enquiries about possible collaborations and requests for access to the dataset. Data will be shared after approval of a proposal and with a signed data access agreement. Investigators interested in more details about this study, or in accessing these resources, should contact the corresponding author.

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Melioidosis is a severe, potentially life-threatening bacterial disease caused by *Burkholderia pseudomallei*, a gram-negative bacterium found in water and soil in tropical and subtropical environments worldwide (1). Melioidosis might manifest as localized, pulmonary, systemic, or disseminated infections. However, melioidosis symptoms are nonspecific, and it is often misdiagnosed (1,2). Exposure to *B. pseudomallei* occurs through inhalation of contaminated dust or water droplets, ingestion of contaminated water, and direct contact with contaminated water or soil, particularly through cuts or abrasions (3). The incubation period in acute cases ranges from 1 to 21 days, although activation of latent infections might develop many years later (4,5). Persons at greater risk for developing melioidosis include those with diabetes, liver disease, renal disease, chronic lung disease, thalassemia, cancer, and other immunocompromising conditions (6,7).

Melioidosis (formerly Whitmore’s disease) was described in 1912 (8), and cases were historically identified primarily in northern Australia and areas of Southeast Asia, such as Thailand, where melioidosis is hyperendemic (9). However, the known geographic range has expanded considerably in recent decades; the estimated global burden is 165,000 human cases/year (10). On the basis of clinical cases or environmental isolation, *B. pseudomallei* is now suspected to be endemic to the environment in parts of Central and South America, the Caribbean, Mexico, and potentially in areas of the continental United States, such as Texas (11–14). Despite increased recognition of the expansive range of *B. pseudomallei*, nearly all cases of melioidosis in the continental United States are related to international travel to areas to which *Burkholderia pseudomallei*, the bacterium that causes melioidosis, is endemic. We report the diagnosis and clinical course of melioidosis in a patient from the United States who had no international travel history and the public health investigation to determine the source of exposure. We tested environmental samples collected from the patient’s home for *B. pseudomallei* by PCR and culture. Whole-genome sequencing was conducted on PCR-positive environmental samples, and results were compared with sequences from the patient’s clinical specimen. Three PCR-positive environmental samples, all collected from a freshwater home aquarium that had contained imported tropical fish, were a genetic match to the clinical isolate from the patient. This finding suggests a novel route of exposure and a potential for importation of *B. pseudomallei*, a select agent, into the United States from disease-endemic areas. 

**Human Melioidosis Caused by Novel Transmission of Burkholderia pseudomallei from Freshwater Home Aquarium, United States**

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nearly all US cases are related to previous residence in or travel to disease-endemic areas outside the continental United States (15).

**Case Report**

In October 2019, the US Centers for Disease Control and Prevention (CDC) was notified by the Maryland Department of Health and through the CDC Laboratory Response Network (LRN) of a preliminary positive bacterial isolate of *B. pseudomallei* from a blood culture specimen from a Maryland resident. The clinical specimen had been forwarded to the Virginia Division of Consolidated Laboratory Services, where it was confirmed, and shipped to CDC for simultaneous confirmation and genomic analysis.

The patient, a 56-year-old woman, was hospitalized on September 20, 2019 (day 0; Figure), because of fever, cough, and chest pain with onset 2 days earlier. The patient had a history of polymyositis, rheumatoid arthritis, and diabetes mellitus, and reported to have stopped long-term immunosuppressive medications (methotrexate, azathioprine, and prednisone) 1 month before symptom onset.

A thoracic radiograph on day 0 showed a right perihilar and lower lobe infiltrate, consistent with pneumonia. A noncontrast computed tomography (CT) scan on day 3 showed air space consolidation in the right lower lobe consistent with pneumonia, additional bibasilar air space densities, and a small right pleural effusion. Other notable clinical laboratory results at presentation included an increased leukocyte count of 22,800 cells/µL (reference range 4,500–11,000 cells/µL) and a decreased sodium level of 125 mmol/L (reference range 135–145 mmol/L).

We obtained blood cultures on day 0 before administration of antimicrobial drugs. Gram-negative rods were identified in 4 initial blood cultures; the rods were subsequently identified as *B. pseudomallei*. Three additional blood cultures obtained on days 2–4 grew the same organism. The patient was given ceftriaxone and azithromycin on days 0–3, then on day 4, treatment was changed to meropenem after *B. pseudomallei* was identified. The patient showed gradual symptom improvement; fever resolved, and leukocyte count normalized. The intensive phase duration was extended because of persistent bacteremia. She was discharged on day 11 and continued taking intravenous meropenem as an outpatient. Antimicrobial drug susceptibility testing was not performed because the patient had been

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**Figure.** Clinical, laboratory, and epidemiologic timeline for a patient who had melioidosis, Maryland, USA, 2019. CDC, Centers for Disease Control and Prevention.
responding to treatment with meropenem before LRN confirmation of *B. pseudomallei*.

After 3 weeks of taking meropenem, the patient’s leukocyte count increased to 14,700 cells/µL, and fever developed (temperature 100.1°F [37.8°C]). A repeat CT scan showed a patchy opacity at the right lung base and bilateral interstitial process consistent with pneumonia, but a 45 × 24 mm mass in the right lung posteriorly could not be excluded and increased concern for pulmonary abscess. The patient was readmitted, sulfamethoxazole/trimethoprim was given, and treatment with meropenem continued. She clinically improved and was discharged after 1 week. Repeat, noncontrast, thoracic CT scans showed improvement of right basilar consolidation after 6 weeks of meropenem and sulfamethoxazole/trimethoprim and resolution after 10 weeks. The patient completed eradication therapy with a 10-week course of meropenem and 12-week course of sulfamethoxazole/trimethoprim.

**Methods**

**Case Investigation**

We conducted an initial epidemiologic investigation to assess the travel history of the patient and other possible exposures to *B. pseudomallei*. Interviews were conducted October–December 2019, with the patient and other household members. After establishing no international travel history and identification of positive environmental samples, the interviews focused on the freshwater home aquariums, tropical fish, and contact with the fish and aquariums of the patient.

**Environmental Sampling**

During November 2019, the investigation team visited the home of the patient to collect environmental samples and assess potential sources of *B. pseudomallei*. The patient had 2 freshwater aquariums (tanks A and B, both ≈5 liters). We collected bulk water samples (≈50 mL) from each aquarium and swab specimens of biofilms (3 from tank A and 2 from tank B). We collected an additional 16 samples, including swab specimens of all household faucets, soil from potted plants and around the property, 2 beauty products, and 4 liquid vaping products. During December 2019, the team collected 6 additional specimens: bulk water from tanks A and B, gravel from tank B, filters from tank B, dead fish carcasses from tank B, and a dry artificial plant that had been in tank B but was removed during August 2019. During the second visit, the Maryland Department of Health also performed decontamination, removal, and safe disposal of fish tank B from the home of the patient.

**Laboratory Confirmation**

The clinical isolate was confirmed as *B. pseudomallei* at the Virginia Division of Consolidated Laboratory Services and CDC by using the LRN algorithm, including biochemicals and PCR. All 29 environmental samples were sent to CDC for culture and identification. We directly inoculated all environmental samples into TBSS-C50 (Galimand) broth and incubated broths in a shaking incubator at 37°C for 6 days. We then cultured enriched broths onto Ashdown agar, and extracted DNA by using a QIAamp Fast Stool Mini Kit (QIAGEN, https://www.qiagen.com) or testing by real-time PCR. Suspected colonies from Ashdown agar were selected for further workup, and confirmation of isolates followed the LRN algorithm.

**Multilocus Sequence Typing and Whole-Genome Sequencing**

CDC performed multilocus sequence typing on isolate MD2019a (clinical). CDC also performed whole-genome sequencing (WGS) for isolates MD2019a (clinical), MD2019b (water from tank B collected during November 2019), and MD2019c (swab specimen of biofilms from tank B collected during November 2019) by using the Nextera FLEX Kit (https://www.illumina.com) for library preparation and the iSeq 100 instrument (Illumina) with a 2 × 151-bp kit. We analyzed draft genomes along with a reference panel of *B. pseudomallei* genomes from publicly available sources representing geographic diversity by using Parsnp in the HARVEST 1.3 suite (https://harvest.readthedocs.io/en/latest/content/parsnp.html) to detect single-nucleotide polymorphisms (SNPs).

**Results**

**Initial Investigation**

The initial epidemiologic investigation showed that the patient was a long-time resident of Maryland who had never traveled outside the continental United States. She reported previous pet ownership of reptiles and cats >5 years ago. She denied any other direct soil or environmental water exposure. She also denied use of herbal products or products known to be imported from Asia or Australia. She reported use of vaping products. No family members or close contacts had an illness similar to that of the patient.

Multilocus sequence typing yielded sequence type 369, which had been seen in examples from Malaysia, Thailand, and Vietnam (16). WGS of the
clinical isolate, MD2019a, showed that when compared to a panel of publicly available genomes composed to represent geographic diversity, it clustered with genomes from Southeast Asia predominantly associated with Singapore and Malaysia (Appendix Figure, https://wwwnc.cdc.gov/EID/article/27/12/21-1756-App1.pdf). The genetic link of the isolate to Southeast Asia, coupled with lack of international travel history for the patient, led the investigation team to conduct follow-up interviews and environmental sampling in the home of the patient.

Isolation of *B. pseudomallei* from Environmental Samples

Of the 23 environmental samples collected during November 2019, a total of 3 samples, all from tank B, were positive for *B. pseudomallei* by culture and real-time PCR. All other November 2019 samples were negative. The water and gravel samples from tank B collected during December 2019 were also positive for *B. pseudomallei* by PCR and culture. The artificial plant that was removed from the tank during August 2019 was positive by PCR, but no growth was observed in culture. The December 2019 water sample from tank A was negative.

Genetic Match of Aquarium Isolates with the Clinical Isolate

Comparison of the draft genome sequences between the clinical isolate (MD2019a) and the 2 aquarium samples from tank B (MD2019b and MD2019c) showed no SNPs between MD2019a and MD2019c, and only 1 SNP was detected between MD2019a and MD2019b. This finding indicates that all 3 isolates were clonal.

Follow-Up Patient Interviews

The patient reported she had purchased fish, aquariums, and associated supplies during July 2019 (Figure). She purchased the tanks and gravel substrate at a large retail store. She purchased 3 cherry barbs (*Puntius titteya*) for tank A from a retail pet store, and all were alive at the time of her interview. She purchased 3 fancy-tailed guppies (*Poecilia reticulata*) for tank A from a retail pet store, and they died during August 2019. During October 2019, after her illness onset, she purchased 3 tiger barbs (*Puntigrus tetrazona*) for tank B from the same store, and they died during November 2019.

The patient reported that the water in tank B was persistently cloudier than the water in tank A and more difficult to keep clean. She reported that she was the primary caretaker of the fish and recalled reaching bare hands and arms into the water and gravel of tank B to disrupt sediment while cleaning it, as recently as August 2019.

Discussion

This investigation of a patient who had melioidosis and no history of travel to disease-endemic areas provides strong evidence for documented transmission of *B. pseudomallei* from a freshwater home aquarium to a human. Although this type of transmission has not been described in the literature, contamination of aquarium transport water with *B. pseudomallei* used for freshwater tropical fish originating from Singapore has been reported in France (17). Furthermore, freshwater fishing practices have been identified as risk factors in countries to which melioidosis is endemic (7,18,19).

With freshwater aquariums as a newly recognized source of possible transmission of *B. pseudomallei* to humans, further investigations are underway to determine the extent of *B. pseudomallei* contamination at the pet store retailer where the patient purchased the pet fish with accompanying aquarium water and at the vendors that imported and supplied freshwater ornamental fish, aquatic plants, and associated aquarium water to this retail location. Because these vendors might distribute freshwater animals and aquatic plants to pet store retailers throughout the United States, identifying possible source(s) of introduction with *B. pseudomallei* in the supply chain is essential to public health.

Federal regulators classify *B. pseudomallei* as a Tier 1 Select Agent because of its heightened risk for deliberate misuse and major potential for mass casualties, economic disruption, critical infrastructure effects, or damaging public confidence (20). The United States is the largest importer of ornamental fish, most of which are freshwater and originate in Southeast Asia (21,22), where *B. pseudomallei* is widespread in the environment. An estimated 11.5 million US households have pet fish; ≈139 million freshwater fish are owned (23,24). Determining where in the supply chain introduction of the bacteria might occur can lead to development of enhanced surveillance and mitigation procedures at the critical control points, which might prevent further introductions and spread of the bacteria to retailers and homes of consumers.

To prevent or reduce risk of exposure, particularly among persons who have major risk factors, simple precautions can be taken when handling freshwater fish, snails, aquatic plants, aquariums, or other materials in contact with aquarium
water, such as gravel, substrate, decorations, filters, and other equipment. CDC recommends thorough handwashing with soap and water before and after handling or cleaning aquariums and feeding fish, wearing gloves to cover any cuts or wounds in the hand while handling fish or aquariums or allowing wounds to fully heal first, avoiding cleaning fish aquariums if immunocompromised or in areas where immunocompromised persons might be present, and not allowing children <5 years of age to clean fish aquariums (25).

This report highlights the essential role of molecular epidemiology in public health investigations of melioidosis cases, which identified the likely geographic origin of the bacteria and prompted a public health response that characterized a novel route of exposure. There is growing evidence that US melioidosis cases are not limited to international travelers, including a 2021 multistate cluster involving an organism that is not clonal to the isolates described here, as determined by WGS (14,26–29).

We urge clinicians in the United States to consider melioidosis in patients who have clinically compatible symptoms and exposure to tropical ornamental fish and freshwater aquariums, particularly if patients are immunocompromised, even though such exposure events might be exceedingly rare and few persons show development of melioidosis after exposure to B. pseudomallei (5). This organism might be difficult for hospital laboratories to diagnose, and automated identification systems in clinical laboratories can misidentify B. pseudomallei, highlighting the need for LRN confirmation (28).

Clinicians treating melioidosis should consult established treatment guidelines, which were updated in 2020 (30,31). Likewise, public health investigators should consider inquiring about pet freshwater fish exposure in patients given a diagnosis of melioidosis who have not traveled to a disease-endemic area or have only traveled to locations inconsistent with the geographic profile of the genome of their isolate.

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**About the Author**

Dr. Dawson is an epidemiologist in the Office of Science, Centers for Disease Control and Prevention, Atlanta, GA. His primary research interests are emerging infectious diseases, zoonotic diseases, and health equity.

**References**


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Trends in Incidence and Clinical Outcomes of Clostridioides difficile Infection, Hong Kong


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Release date: November 18, 2021; Expiration date: November 18, 2022

Learning Objectives

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

- Describe disease burden, incidence, and clinical outcomes of CDI among hospitalized patients in Hong Kong, according to an updated territory-wide survey study
- Determine antibiotic usage and other CDI-associated risk factors and clinical outcomes among hospitalized patients in Hong Kong, according to an updated territory-wide survey study
- Identify clinical and public health implications of the epidemiologic pattern of CDI, CDI-associated risk factors, and clinical outcomes among hospitalized patients in Hong Kong, according to an updated territory-wide survey study.

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2These authors were co–principal investigators.
We conducted a territorywide survey to investigate the epidemiology, risk factors, and clinical outcomes of *Clostridioides difficile* infection (CDI) among hospitalized patients in Hong Kong. A total of 17,105 cases of CDI were identified, of which 15,717 (91.9%) were healthcare-associated and 1,025 (6.0%) were community-associated. Although CDI incidence increased substantially from 2006 to 2017, it plateaued in 2018 and 2019. The 30-day mortality rates decreased from 20.1% in 2015 to 16.8% in 2019, whereas the 60-day recurrence rates remained constant at 11% during the study period. Cross-correlation statistic showed significant correlations between incidence trend and overall antimicrobial drug use ($r = 0.865, p < 0.001$), which has decreased as a result of an antibiotic stewardship program initiated in 2017. Our data suggest a turning point in *C. difficile* epidemiology that could be related to the changing pattern of antimicrobial drug use.

*Clostridioides difficile* infection (CDI) is a common nosocomial disease; symptoms range from mild diarrhea to life-threatening colitis and toxic megacolon. CDI is associated with a high mortality rate, particularly for patients >75 years of age (1). Epidemiologic studies have identified its substantial incidence especially in the United States and in many countries in Europe (2,3). Recent data suggested that its overall incidence in some of these countries have reached a plateau. For instance, the US Centers for Disease Control and Prevention (CDC) reported a decrease in CDI incidence from 2014 to 2017 (2), whereas the overall CDI incidence in Sweden has decreased by 22% from 2012 to 2016 (4). These declines are often attributed to the implementation of antibiotic stewardship programs. Nonetheless, community-acquired CDI (CA-CDI) represents a growing threat; incidence of CA-CDI doubled from 2011–2015 (5).

The epidemiologic patterns in different geographic regions are highly dynamic. Outbreaks of CDI in North America and Europe were once predominantly caused by the *C. difficile* ribotype 027 (6), which was rarely reported about in Asia (7). Instead, *C. difficile* ribotype 017 has been the predominant strain in Asia (8). Other toxigenic strains, such as *C. difficile* ribotype 369, which was associated with multiple epidemics in Japan, have been reported in various Southeast Asia countries (9), whereas *C. difficile* ribotype 002 was reported to be common in Hong Kong, China, and was associated with increased virulence (10,11). Continuous surveillance, therefore, is important to prevent outbreaks of CDI. However, epidemiologic data of CDI in Asia remain sparse. We have previously characterized the molecular and antimicrobial susceptibility patterns of prevalent *C. difficile* ribotypes in Hong Kong (12). We also conducted an observational study to investigate CDI disease burden and clinical outcomes among hospitalized patients in Hong Kong, which showed a rapidly increasing incidence until 2014 (13). In this study, we continued to update the epidemiologic pattern of CDI among hospitalized patients in Hong Kong and characterize CDI-associated risk factors and clinical outcomes.

We conducted this study in accordance with the Declaration of Helsinki (2013 version). The Joint Clinical Research Ethics Committee of the Chinese University of Hong Kong and Hospital Authority New Territory East Cluster approved the study. All clinical data were anonymized by the Clinical Data Analysis and Reporting System (CDARS), and all potential identifiers were removed upon return of database searches.

**Methods**

**Study Population and Data Extraction**

We identified digital records of all patients hospitalized in public hospitals with a laboratory-confirmed diagnosis of CDI in Hong Kong during January 1, 2015–December 31, 2019, from CDARS, a database of public hospital patient records managed by the Hong Kong Hospital Authority. We obtained clinical data including the patient demographics, laboratory results, drug prescription records, clinical outcomes, and diagnoses of underlying conditions. Patient demographic data include age and gender. We identified relevant diagnoses using codes from the International Classification of Diseases, 9th Revision, in accordance with the Charlson Comorbidity Index (14). We also obtained data on antimicrobial drug use and other drug use within 8 weeks before CDI diagnosis.

**Case Identification and Definitions**

We defined a CDI case as positive result obtained from culture, toxin, or molecular assay for a diarrheal fecal specimen collected from inpatient residents ≥18 years of age. As described previously (13), patients with samples obtained >48 hours after admission or those who were hospitalized in a healthcare facility within the previous 4 weeks were classified as cases of healthcare-associated CDI (HA-CDI). We defined community-associated CDI (CA-CDI) as patients who had not been hospitalized in a healthcare facility within the previous 12 weeks. We defined patients who had been hospitalized in a healthcare facility within the previous 4–12 weeks as indeterminate. We classified patients with a maximum leukocyte count
>15,000 cells/μL or >50% increase in serum creatinine level as having cases of severe CDI, as defined by the Infectious Diseases Society of America (15). We defined refractory disease as a nonresponding disease requiring >14 days of continued treatment and a treatment period as a period during which records of drug prescription records indicate continuous antimicrobial treatment with ≤3 days of interruption.

**Antimicrobial Drug Use Data**

Because the use of antimicrobial drugs is a major risk factor for CDI, we extracted from the data the overall corporate use of antimicrobial drugs in public hospitals in Hong Kong and analyzed. The data were recorded as daily defined doses (DDDs), which is the assumed average maintenance dose per day for each drug. These can demonstrate the absolute changes in use, as well as DDDs per 1,000 bed-days occupied (DDD/1,000 BDO), which can demonstrate changes in use relative to hospital occupancy. Broad-spectrum antimicrobial drugs include cefepime, cefazidime, ceftaxime, cefoperazone/subactam, piperacillin, piperacillin/tazobactam, carbapenems, and quinolones. We determined the risk for CDI for each drug class on the basis of its association with CDI (1). High-risk drugs were lincosamides, cephalosporins, fluoroquinolones, amoxicillin, and ampicillin. Medium-risk drugs included sulphonamides and macrolides. Low-risk drugs included tetracyclines (1).

**Statistical Analysis**

We analyzed data with R version 3.6.0. (R Foundation for Statistical Computing, https://www.r-project.org) We defined annual crude incidence of CDI as the number of patients given a diagnosis of CDI per 100,000 adult population, using data obtained from the Hong Kong Census and Statistics Department. We analyzed potential predictors for 30-day mortality rate and 60-day recurrence rate using univariate and multivariate forward Wald logistic regression. We used Cox proportional hazard regression to identify factors that decreased the time to recurrence after an episode. We used χ² test of proportion to compare differences in incidences, mortality rates, and recurrence rates. We used cross-correlation to identify correlation between CDI incidence and antimicrobial drug use and p = 0.05 as a measure of statistical significance.

**Results**

**Disease Burden and Incidence**

During 2015–2019, we identified 17,105 cases of CDI among hospitalized patients in Hong Kong (average 3,421 cases/year). Of these cases, 15,717 (91.9%) were HA-CDI and 1,025 (6.0%) were CA-CDI. The remaining 363 cases (2.1%) were indeterminate (Table 1; Appendix Figure 1).

Although a rapid increase of CDI incidence was observed during 2006–2014, the crude incidence of CDI in Hong Kong remained relatively stable and the average annual percentage change (APC) from 2015 to 2019 showed a modest increase of 1.53% (Table 1; Figure, panel A). Among the different age groups, the CDI incidence showed a significant decrease for patients ≥75 years of age (Figure, panel B). We observed a similar downward trend in the overall incidences of HA-CDI (Figure, panel A), the first time since the start of our previous study in 2006 (13). However, this decrease was not evident in the younger patient groups between 2015 and 2019, despite these groups only representing a minor proportion of CDI incidence (Table 1; Figure).

The median age of patients was 77 years (inter-quartile range [IQR] 63–86 years); 51.8% were female and 48.2% male (Table 2). The number of patients from old-age homes has significantly decreased, from 29.2% in 2015 to 22.8% in 2019 (p<0.001) (Table 3), compared with an average of 30.2% in the period 2006–2014. Most patients have taken high-risk antimicrobial drugs (81.4%), broad-spectrum antimicrobial drugs (59.3%), or proton-pump inhibitors (PPI) (62.1%) within 8 weeks before diagnosis of CDI (Table 2). Of note, during the period of 2015–2019, the proportion of severe CDI has decreased from 38.2% to 31.2% of patients (p<0.001) (Table 3).

<table>
<thead>
<tr>
<th>Year</th>
<th>Adult population</th>
<th>No. cases</th>
<th>Incidence†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Overall</td>
<td>HA-CDI</td>
</tr>
<tr>
<td>2015</td>
<td>6,247,460</td>
<td>3,160</td>
<td>2,921</td>
</tr>
<tr>
<td>2016</td>
<td>6,301,560</td>
<td>3,303</td>
<td>3,058</td>
</tr>
<tr>
<td>2017</td>
<td>6,357,420</td>
<td>3,618</td>
<td>3,303</td>
</tr>
<tr>
<td>2018</td>
<td>6,410,080</td>
<td>3,557</td>
<td>3,248</td>
</tr>
<tr>
<td>2019</td>
<td>6,481,000</td>
<td>3,467</td>
<td>3,187</td>
</tr>
</tbody>
</table>

*Adult is defined as a person >18 years of age. CA-CDI, community-associated Clostridioides difficile infection; HA-CDI, healthcare-associated Clostridioides difficile infection.
†No. cases/100,000 adults.
Trends in C. difficile Infection, Hong Kong

Clinical Outcomes and Risk Factors
The 30-day all-cause mortality rates have decreased from 20.1% in 2015 to 16.8% in 2019 (p = 0.002), substantially lower than the previous decrease of 22.5% during the period 2006–2014 (Appendix Table 1) (13). Multivariate logistic regression analysis indicated that the main predictors for death in 30 days were advanced age (>85 years, adjusted OR [aOR] 7.23, 95% CI 5.29–10.12; 75–84 years, aOR 4.30, 95% CI 3.16–5.98) and metastatic tumor (aOR 2.63, 95% CI 2.21–3.12) (Table 3).

The 60-day recurrence rate remained at ≈11% (Appendix Table 1). Cox regression analysis showed that the main predictors of 60-day recurrence were healthcare-associated CDI (adjusted hazard ratio [aHR] 8.15, 95% CI 5.25–12.63) and use of quinolones (aHR 1.59, 95% CI 1.41–1.78) or broad-spectrum antibiotics (aHR 1.37, 95% CI 1.18–1.59) within 8 weeks before diagnosis of CDI (Appendix Table 2). Refractory disease rates decreased from 13.6% in 2015 to 11.3% in 2019, but the change was not statistically significant (p<0.076).

The number of patients who used tetracyclines within 8 weeks before CDI increased from 2.7% in 2015 to 6.2% in 2019 (p<0.001). In contrast, patient exposure to certain known risk factors has decreased during the period, including the use of H2 antagonists (from 35.2% to 25.0%; p<0.001), high-risk antibiotics (from 85.5% to 77.7%; p<0.001), penicillin group of drugs (from 74.2% to 68.5%; p<0.001) and fluoroquinolones (from 27.4% to 20.7%; p<0.001). The risk factor of having cerebrovascular accident as an underlying condition also decreased in these patients (from 21.9% to 15.9%; p<0.001) (Appendix Table 3).

Comparison of HA-CDI and CA-CDI
The proportion of HA-CDI among all patients has remained at ≈90% and of CA-CDI at ≈5% (Appendix Figure). The year-to-year changes for the CA-CDI and HA-CDI incidence rates were not statistically significant, suggesting a static trend during the period (Table 1). Comparisons between the patients showed that HA-CDI patients had a median age of 77 (IQR 63–86) years, versus a median age of 74 (IQR 58–85) years for CA-CDI patients. Significantly more HA-CDI patients had severe CDI (36.5% vs 29.0%; p<0.001) and underlying conditions compared with CA-CDI patients (Table 2). HA-CDI patients were more likely to have exposure to high-risk antimicrobial drugs (86.0% vs. 26.5%; p<0.001) and broad-spectrum antimicrobials (63.5% vs 9.2%; p<0.001) within 8 weeks before CDI. The 30-day mortality rate was 19.7% for HA-CDI but 8.5% for CA-CDI patients (p<0.001), although both rates have decreased compared with the earlier period of 2006–2014 (13). Although the 30-day mortality rate for HA-CDI decreased from 2015 to 2019, we did not observe an obvious trend in mortality rate for CA-CDI patients (p = 0.354) (Appendix Table 1). The 60-day recurrence rate was 12.4% for HA-CDI, whereas none of the CA-CDI patients had a recurrence (Table 2).
Data on Antimicrobial Drug Use

The overall use of antimicrobial drugs per year, measured as DDD/1,000 BDO, increased from 1,206 in 2006 to 4,747 in 2018 but then decreased to 3,968 in 2019 (Appendix Table 4). Annual use shared a significant correlation \( r = 0.865; p<0.001 \) with the CDI incidence. In terms of DDD/1,000 BDO, we observed the highest levels of correlation for lincosamides \( r = 0.907; p<0.001 \), carbapenems \( r = 0.893; p<0.001 \) and sulphonamides \( r = 0.872; p<0.001 \).

### Table 2. Characteristics, outcomes, and procedures of patients with Clostridioides difficile infections, Hong Kong, China, 2015–2019*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall</th>
<th>CA-CIDI</th>
<th>HA-CIDI</th>
<th>Indeterminable</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>17,105</td>
<td>1,025 (6.0)</td>
<td>15,717 (91.9)</td>
<td>363 (2.1)</td>
</tr>
<tr>
<td><strong>Age, y</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Median (IQR)</td>
<td>77 (63–86)</td>
<td>74 (58–85)</td>
<td>77 (63–86)</td>
<td>78 (62–86)</td>
</tr>
<tr>
<td>&lt;44</td>
<td>1,056 (6.2)</td>
<td>138 (13.5)</td>
<td>901 (5.7)</td>
<td>17 (4.7)</td>
</tr>
<tr>
<td>45–64</td>
<td>3,679 (21.5)</td>
<td>221 (21.6)</td>
<td>3,375 (21.5)</td>
<td>83 (22.9)</td>
</tr>
<tr>
<td>65–74</td>
<td>3,027 (17.7)</td>
<td>173 (16.9)</td>
<td>2,793 (17.8)</td>
<td>61 (16.8)</td>
</tr>
<tr>
<td>75–84</td>
<td>4,340 (25.4)</td>
<td>207 (20.2)</td>
<td>4,044 (25.7)</td>
<td>89 (24.5)</td>
</tr>
<tr>
<td>&gt;85</td>
<td>5,003 (29.2)</td>
<td>286 (27.9)</td>
<td>4,604 (29.3)</td>
<td>113 (31.1)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>8,252 (48.2)</td>
<td>442 (43.1)</td>
<td>7,642 (48.6)</td>
<td>168 (46.3)</td>
</tr>
<tr>
<td>F</td>
<td>8,853 (51.8)</td>
<td>583 (56.9)</td>
<td>8,075 (51.4)</td>
<td>195 (53.7)</td>
</tr>
<tr>
<td><strong>Admission from OAH</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>13,932 (81.4)</td>
<td>272 (26.5)</td>
<td>13,519 (86.0)</td>
<td>141 (38.8)</td>
</tr>
<tr>
<td>CDI</td>
<td>1,968 (11.5)</td>
<td>4,738 (27.7)</td>
<td>3,220 (21.7)</td>
<td>18 (0.1)</td>
</tr>
<tr>
<td>healthcare CDI</td>
<td>4,747 (27.7)</td>
<td>4,303 (25.5)</td>
<td>4,950 (28.9)</td>
<td>109 (30.0)</td>
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<tr>
<td><strong>IDSA-defined severe disease</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>5,571 (35.8)</td>
<td>295 (29.0)</td>
<td>5,482 (36.5)</td>
<td>94 (26.2)</td>
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<td><strong>Diagnostic test</strong></td>
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<tr>
<td>Bacterial culture</td>
<td>8,191 (40.9)</td>
<td>468 (39.4)</td>
<td>7,560 (41.0)</td>
<td>163 (38.6)</td>
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<td>Nucleic acid amplification test</td>
<td>8,994 (44.2)</td>
<td>547 (46.0)</td>
<td>8,261 (44.8)</td>
<td>186 (44.1)</td>
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<td>Toxin detection</td>
<td>2,855 (14.5)</td>
<td>173 (14.6)</td>
<td>2,609 (14.2)</td>
<td>73 (17.3)</td>
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<td><strong>Antimicrobial drug use</strong></td>
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<tr>
<td>High-risk drugs</td>
<td>13,932 (81.4)</td>
<td>272 (26.5)</td>
<td>13,519 (86.0)</td>
<td>141 (38.8)</td>
</tr>
<tr>
<td>Low-risk drugs</td>
<td>1,968 (11.5)</td>
<td>4,738 (27.7)</td>
<td>3,220 (21.7)</td>
<td>18 (0.1)</td>
</tr>
<tr>
<td>Broad-spectrum drugs</td>
<td>4,747 (27.7)</td>
<td>4,303 (25.5)</td>
<td>4,950 (28.9)</td>
<td>109 (30.0)</td>
</tr>
<tr>
<td><strong>Use of other drugs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proton pump inhibitor</td>
<td>10,614 (62.1)</td>
<td>201 (19.6)</td>
<td>10,255 (65.2)</td>
<td>158 (43.5)</td>
</tr>
<tr>
<td>H2 antagonist</td>
<td>4,550 (28.9)</td>
<td>158 (15.4)</td>
<td>4,722 (30.0)</td>
<td>70 (19.3)</td>
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<td>Corticosteroid</td>
<td>4,477 (26.2)</td>
<td>81 (7.9)</td>
<td>4,350 (27.7)</td>
<td>46 (12.7)</td>
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<tr>
<td><strong>Underlying conditions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Myocardial infarction</td>
<td>1,212 (7.1)</td>
<td>37 (3.6)</td>
<td>1,144 (7.3)</td>
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<td>Congestive heart failure</td>
<td>2,407 (14.1)</td>
<td>64 (6.2)</td>
<td>2,292 (14.6)</td>
<td>51 (14.0)</td>
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<td>Peripheral vascular disease</td>
<td>556 (3.3)</td>
<td>10 (1.0)</td>
<td>536 (3.4)</td>
<td>10 (2.8)</td>
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<tr>
<td>Cerebrovascular disease</td>
<td>3,051 (17.8)</td>
<td>91 (8.9)</td>
<td>2,887 (18.4)</td>
<td>73 (20.1)</td>
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<td>Chronic pulmonary disease</td>
<td>1,937 (11.3)</td>
<td>84 (8.2)</td>
<td>1,806 (11.5)</td>
<td>47 (12.9)</td>
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<tr>
<td>Mild liver disease</td>
<td>338 (2.0)</td>
<td>18 (1.8)</td>
<td>301 (1.9)</td>
<td>19 (5.2)</td>
</tr>
<tr>
<td>Severe liver disease</td>
<td>243 (1.4)</td>
<td>11 (1.1)</td>
<td>221 (1.4)</td>
<td>11 (3.0)</td>
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<td>Diabetes mellitus</td>
<td>3,624 (21.2)</td>
<td>131 (12.8)</td>
<td>3,414 (21.7)</td>
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<td>Diabetes mellitus with complications</td>
<td>1,492 (8.7)</td>
<td>44 (4.3)</td>
<td>1,406 (8.9)</td>
<td>42 (11.6)</td>
</tr>
<tr>
<td>Moderate/severe kidney disease</td>
<td>3,363 (19.7)</td>
<td>94 (9.2)</td>
<td>3,178 (20.2)</td>
<td>91 (25.1)</td>
</tr>
<tr>
<td>Nonmetastatic cancer</td>
<td>3,403 (19.9)</td>
<td>75 (7.3)</td>
<td>3,268 (20.8)</td>
<td>60 (16.5)</td>
</tr>
<tr>
<td>Metastatic cancer</td>
<td>970 (5.7)</td>
<td>21 (2.0)</td>
<td>932 (5.9)</td>
<td>17 (4.7)</td>
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<tr>
<td>HIV</td>
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<td>1 (0.1)</td>
<td>15 (0.1)</td>
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<tr>
<td>Paraplegia</td>
<td>356 (2.1)</td>
<td>12 (1.2)</td>
<td>336 (2.1)</td>
<td>8 (2.2)</td>
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<tr>
<td>Connective tissue disease</td>
<td>174 (1.0)</td>
<td>15 (1.5)</td>
<td>152 (1.0)</td>
<td>7 (1.9)</td>
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<tr>
<td>Dementia</td>
<td>863 (5.0)</td>
<td>47 (4.6)</td>
<td>793 (5.0)</td>
<td>23 (6.3)</td>
</tr>
<tr>
<td>Peptic ulcer</td>
<td>867 (5.1)</td>
<td>24 (2.3)</td>
<td>826 (5.3)</td>
<td>17 (4.7)</td>
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<tr>
<td><strong>Outcomes</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Episode death</td>
<td>3,220 (18.8)</td>
<td>73 (7.1)</td>
<td>3,117 (19.8)</td>
<td>30 (8.3)</td>
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<td>30-day mortality</td>
<td>3,225 (18.9)</td>
<td>87 (8.5)</td>
<td>3,100 (19.7)</td>
<td>38 (10.5)</td>
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<tr>
<td>60-day mortality</td>
<td>4,738 (27.7)</td>
<td>117 (11.4)</td>
<td>4,562 (29.0)</td>
<td>59 (16.3)</td>
</tr>
<tr>
<td>30-day recurrence</td>
<td>1,968 (11.5)</td>
<td>0</td>
<td>1,947 (12.4)</td>
<td>21 (5.8)</td>
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<td>Refractory disease</td>
<td>2,155 (12.6)</td>
<td>59 (5.8)</td>
<td>2,064 (13.1)</td>
<td>32 (8.8)</td>
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<td><strong>Procedures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Partial colectomy</td>
<td>3 (0.0)</td>
<td>1 (0.1)</td>
<td>2 (0.0)</td>
<td>0</td>
</tr>
<tr>
<td>Left colectomy</td>
<td>3 (0.0)</td>
<td>0</td>
<td>3 (0.0)</td>
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<tr>
<td>Right colectomy</td>
<td>6 (0.0)</td>
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<td>6 (0.0)</td>
<td>0</td>
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<tr>
<td>Sigmoid colectomy</td>
<td>1 (0.0)</td>
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<td>1 (0.0)</td>
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<tr>
<td>Total colectomy</td>
<td>4 (0.0)</td>
<td>1 (0.1)</td>
<td>3 (0.0)</td>
<td>0</td>
</tr>
<tr>
<td>Fecal microbiota transplant</td>
<td>3 (0.0)</td>
<td>0</td>
<td>3 (0.0)</td>
<td>0</td>
</tr>
</tbody>
</table>

*Values are no. (%) patients except as indicated; CA-CIDI, community-associated Clostridioides difficile infection; HA-CIDI, healthcare-associated CDI; IDSA, Infectious Diseases Society of America; IQR, interquartile range; OAH, old age home.

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Use of other drugs

- Proton pump inhibitor: 1.614 (1.486–1.755)
- H2 antagonist: 1.237 (1.136–1.346)
- Corticosteroid: 0.954 (0.876–0.938)

Underlying conditions

- Myocardial infarction: 1.268 (1.100–1.457)
- Congestive heart failure: 1.632 (1.475–1.804)
- Peripheral vascular disease: 1.218 (0.989–1.489)
- Cerebrovascular disease: 1.204 (1.092–1.325)
- Nonmetastatic cancer: 1.498 (1.368–1.638)
- Metastatic cancer: 2.827 (2.466–3.237)
- Diabetes mellitus: 1.267 (1.157–1.386)
- Diabetes mellitus with complications: 1.226 (1.076–1.394)
- Mild liver disease: 1.005 (0.758–1.314)
- Severe liver disease: 1.204 (0.878–1.623)
- Peptic ulcer: 1.283 (1.087–1.508)
- Chronic pulmonary disease: 1.302 (1.161–1.459)
- Moderate/severe kidney disease: 1.203 (1.095–1.320)
- Connective tissue disease: 0.566 (0.338–0.865)
- Paraplegia: 1.152 (0.885–1.482)
- Dementia: 1.364 (1.158–1.600)
- HIV: 0.287 (0.016–4.145)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate OR (95% CI)</th>
<th>p value</th>
<th>Multivariate OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;44</td>
<td>Referent</td>
<td>NA</td>
<td>Referent</td>
<td>NA</td>
</tr>
<tr>
<td>45–64</td>
<td>3.115 (2.361–4.322)</td>
<td>&lt;0.001</td>
<td>2.458 (1.794–3.449)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>65–74</td>
<td>4.203 (3.105–5.829)</td>
<td>&lt;0.001</td>
<td>3.203 (2.334–4.502)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>75–84</td>
<td>6.237 (4.643–8.595)</td>
<td>&lt;0.001</td>
<td>5.384 (3.944–7.531)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&gt;85</td>
<td>7.986 (5.959–10.986)</td>
<td>&lt;0.001</td>
<td>7.633 (5.583–10.70)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.124 (1.041–1.213)</td>
<td>0.0029</td>
<td>1.221 (1.121–1.330)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IDSA-defined severe disease</td>
<td>2.296 (2.121–2.486)</td>
<td>&lt;0.001</td>
<td>2.159 (1.986–2.347)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Healthcare-associated disease</td>
<td>2.483 (2.066–3.010)</td>
<td>&lt;0.001</td>
<td>1.378 (1.119–1.708)</td>
<td>0.003</td>
</tr>
<tr>
<td>Admission from OAH</td>
<td>1.716 (1.579–1.884)</td>
<td>&lt;0.001</td>
<td>1.327 (1.203–1.463)</td>
<td>&lt;0.001</td>
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<tr>
<td>Diagnostic test Bacterial culture</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Referent</td>
<td>NA</td>
<td>Referent</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Nucleic acid amplification test</td>
<td>1.070 (0.991–1.157)</td>
<td>0.0845</td>
<td>1.048 (0.962–1.138)</td>
</tr>
<tr>
<td></td>
<td>Toxin detection</td>
<td>0.995 (0.669–1.438)</td>
<td>0.9799</td>
<td>0.904 (0.592–1.343)</td>
</tr>
<tr>
<td>Antimicrobial drug use</td>
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</tr>
<tr>
<td>High-risk drugs</td>
<td>2.243 (1.990–2.534)</td>
<td>&lt;0.001</td>
<td>0.753 (0.603–0.939)</td>
<td>0.12</td>
</tr>
<tr>
<td>Medium-risk drugs</td>
<td>1.021 (0.893–1.164)</td>
<td>0.7602</td>
<td>1.988 (0.653–6.870)</td>
<td>0.281</td>
</tr>
<tr>
<td>Low-risk drugs</td>
<td>1.193 (1.094–1.300)</td>
<td>0.0001</td>
<td>0.841 (0.647–0.902)</td>
<td>0.193</td>
</tr>
<tr>
<td>Broad-spectrum drugs</td>
<td>1.673 (1.542–1.817)</td>
<td>&lt;0.001</td>
<td>1.402 (1.244–1.581)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>0.907 (0.773–1.059)</td>
<td>0.2223</td>
<td>1.026 (0.825–1.272)</td>
<td>0.815</td>
</tr>
<tr>
<td>Beta-lactamase inhibitor</td>
<td>2.276 (2.063–2.516)</td>
<td>&lt;0.001</td>
<td>1.465 (1.047–2.052)</td>
<td>0.026</td>
</tr>
<tr>
<td>Carbapenem</td>
<td>1.229 (1.118–1.349)</td>
<td>&lt;0.001</td>
<td>1.233 (0.967–1.571)</td>
<td>0.091</td>
</tr>
<tr>
<td>Cephalosporin</td>
<td>1.180 (1.087–1.279)</td>
<td>0.0001</td>
<td>0.936 (0.848–1.033)</td>
<td>0.187</td>
</tr>
<tr>
<td>Lincosamides</td>
<td>0.901 (0.554–1.400)</td>
<td>0.6580</td>
<td>1.126 (0.674–1.800)</td>
<td>0.634</td>
</tr>
<tr>
<td>Macrolides</td>
<td>1.112 (1.201–1.650)</td>
<td>&lt;0.001</td>
<td>0.604 (0.410–0.812)</td>
<td>0.042</td>
</tr>
<tr>
<td>Penicillin</td>
<td>2.208 (2.003–2.438)</td>
<td>&lt;0.001</td>
<td>1.165 (0.840–1.624)</td>
<td>0.362</td>
</tr>
<tr>
<td>Quinolones</td>
<td>1.115 (1.020–1.218)</td>
<td>0.0161</td>
<td>0.939 (0.843–1.045)</td>
<td>0.251</td>
</tr>
<tr>
<td>Sulphonamides</td>
<td>0.588 (0.465–0.734)</td>
<td>&lt;0.001</td>
<td>0.395 (0.092–1.167)</td>
<td>0.138</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>1.291 (1.081–1.534)</td>
<td>0.0043</td>
<td>1.219 (0.955–1.548)</td>
<td>0.108</td>
</tr>
</tbody>
</table>

**Table 3.** Logistic regression analysis of potential independent variables associated with 30-day mortality for *Clostridoides difficile* infection, Hong Kong, China, 2015–2019*.

*IDSA, Infectious Diseases Society of America; NA, not applicable; OAH, old age home.

p<0.001), followed by penicillin (r = 0.847; p<0.001) and quinolones (r = 0.825; p<0.001). Similar to the trend in overall antimicrobial drug use, all of these drugs had a decrease in use in 2019, after consistent increases from 2006–2018. We grouped ampicillin and amoxicillin together with other penicillin group drugs because their combined use attributed to 80% of all penicillin use from 2006–2019. In the same period, we observed increased use of tetracyclines, from 9.22 DDD/1,000 BDO in 2006 to 19.44 DDD/1,000 BDO in 2019.

**Discussion**

In this study, we investigated the latest disease burden of CDI in Hong Kong to provide a complete picture of continual disease surveillance since 2006. Because the public hospitals provide >90% of inpatient medical service in Hong Kong, this study provides a
comprehensive and near-complete data on the disease epidemiology among hospitalized patients in the territory. Our main finding was a decrease in the incidence of CDI in 2018 and 2019, in contrast with the distinctive increasing trend in 2006–2017 (13). The average APC during 2015–2019 showed a 1.53% increase, in contrast with the 13.76% increase for the average APC from 2006–2014. Year-to-year changes of crude CDI incidence in 2015–2019, except for 2016–2017, were statistically insignificant, suggesting that the incidence might have reached a plateau. Our reported incidence in Hong Kong (56.9 cases/100,000 population in 2017) was higher than incidence in the United Kingdom (24 cases/100,000 population in 2017), where CDI incidence has seen a decrease that was mostly attributed to a successful antimicrobial drug stewardship program (16,17). In comparison, Guh et al. reported an estimated crude incidence of 143.6 cases/100,000 population in the United States for 2017 (2). Despite the decrease in CDI incidence since 2011, it is still more than double the incidence in Hong Kong (18). In contrast, Liao et al. reported an overall crude CDI incidence in China during 2009–2016 was 34 cases/100,000 population, a relatively low value compared with our observations (19).

The trends in CDI incidence from 2006–2019 may partially be explained by changes in antimicrobial drug use. Usage rates of many drugs, such as penicillin, lincosamides (including clindamycin), quinolones, sulphonamides, and carbapenems have demonstrated significant correlations with CDI incidence. Penicillin, lincosamides, and quinolones are known to be high-risk for CDI, whereas sulphonamides and carbapenems carry a medium to low risk (1). The changes in penicillin use are likely the most relevant, because they were the most prescribed class of drugs during this period. We saw in the same period increased use of tetracyclines, which have been repeatedly demonstrated to have a relatively low risk for CDI (20). The changes in antimicrobial drug use may be attributable to Hong Kong’s antibiotic stewardship program, which was updated in 2017 (21). These changes are consistent with our observation on the decreased use of antimicrobial drugs, including the penicillin group, fluoroquinolones, and other high-risk drugs, in CDI patients from 2015–2019. By extrapolating the CDI incidence from 2006–2017 to predict incidence in 2018–2019, we observed that the predicted incidence in 2019 would be 20% higher than the actual incidence, showing that the potential effect antibiotic stewardship had on the status quo.

In addition to a decrease in incidence, we observed a decrease in 30-day mortality rates, for which there are myriad plausible explanations. The decrease may be attributed to improved effectiveness of treatment and management efforts for CDI, successful antibiotic stewardship programs, or increased use of fecal microbiota transplant as a treatment (22). Alternatively, a decrease in deaths may be attributable to a change in prevalence of ribotypes or their virulence, such as ribotype 002, which is common in Hong Kong. This difference may also explain the decrease in the relative proportion of severe CDI. Furthermore, ribotypes 002 and 017 are both virulent strains with high antimicrobial resistance (8,10,12), which may have been positively selected in the past because of excessive antimicrobial drug use. Now that the use of antimicrobial drugs has been declining, these strains may have seen a decline in prevalence, which lowered mortality rates. However, more data, such as those gathered through molecular typing and antibiotic resistance analysis, are required to validate this hypothesis.

Despite changes in CDI incidence, the proportion of CA-CDI cases of all CDI cases annually has remained steady from 2006–2019, at ≈4–5%. Risk factors for CA-CDI are unclear, although our data suggest that gastric acid suppression, antimicrobial drug use, and old age may be potential factors. Nonetheless, CA-CDI patients tend to be relatively younger. Previous studies have indicated that there is an increase in CA-CDI incidence and severe outcomes (5,23). Our study, however, indicates the proportion of CA-CDI has remained relatively constant and that their clinical outcomes are generally more favorable, including lower rates of mortality and recurrence, than outcomes for HA-CDI patients. Furthermore, we did not observe any significant trend of increase in CA-CDI cases. One potential explanation is that Hong Kong still has lower rates of inflammatory bowel disease compared with Western countries, despite an increasing trend in these illnesses over the past few decades (22,24).

The main strength of this study is that it is territorywide, driven by data extracted from all public hospitals in Hong Kong. All data, including demographics, laboratory results, drug prescription data, and procedures, were extracted from the public hospital database, thus reducing the possibility of recall bias. Furthermore, this study followed on our previous study (13) that investigated the epidemiology of CDI in Hong Kong during 2006–2014, providing a comprehensive epidemiologic pattern and comparisons for CDI in Hong Kong. Nonetheless, we acknowledge several epidemiologic pattern and comparisons for CDI in Hong Kong. Nonetheless, we acknowledge several limitations in our study. First, the data in this study were based only on
inpatient data; we may have missed diagnoses made in outpatient clinics. Patients with mild symptoms could be sufficiently treated in outpatient clinics, which may result in an underestimation of the actual CDI incidence. Second, patient exposure to antimicrobial drugs and other drugs within 8 weeks of CDI was indicated as a logical indicator (i.e., true or false), regardless of dose, frequency, and prescription time, which may overestimate or underestimate the extent of the exposure. Third, there was a lack of data regarding changes in CDI diagnostic tests used from before 2015, making comparisons of CDI incidence unable to account for any shifts toward the use of the nucleic acid amplification test (NAAT), which is known to be a more sensitive test for CDI (2). Fortunately, the use of NAAT from 2015–2019 has remained within 40%–50%, which may mean a smaller impact on the trend of CDI incidence. Last, this is a retrospective study and there are unforeseeable covariates that were not adjustable or measurable, which may affect the analyses and results. Nevertheless, the epidemiology of CDI is dynamic, and changes can occur rapidly. We recommend continued surveillance of this infection in healthcare settings.

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Rapid detection and reporting of novel influenza A virus (IAV) strains are critical to prompt evaluation of a pandemic threat (1). For example, in 2009, health officials in Mexico reported a variant influenza A(H1N1) virus of swine origin; that variant quickly caused a pandemic (2). Although uncommon, transmission of swine variant IAV strains from pigs to humans has been documented on several occasions (3). Pig farming is structured such that the animals move to different types of farms as they grow. Usually, piglets are born in farrowing farms, transferred to nurseries upon weaning, and then sent to finisher barns at 10–12 weeks of age. These farms usually span >2 geographically separate sites. Collectively, the combination of farrowing, nursery, and finisher farms form a chain where later farms are referred to as downstream from prior ones in the chain.

In 2005, updates to the International Health Regulations instituted mandatory reporting of pathogens such as novel influenza variants in all member states of the World Health Organization (2). Since then, 29 cases of swine influenza A(H1N2) variant (H1N2v) strains have been reported to the World Health Organization, including 25 cases in the United States during 2011–2018, 1 case in Brazil in 2015, and 2 cases in Brazil in 2020 (4–7). In addition, 2 cases of H1N2v infection were detected in Canada: 1 in Alberta in October 2020 and 1 in Manitoba in April 2021 (8). Since the 2010–11 influenza season, the US Centers for Disease Control and Prevention has reported >465 cases of swine IAV variants, including H1N1v, H1N2v, and H3N2v, in humans (9). We report the detection and genetic characterization of an H1N2v IAV isolated from a patient in Alberta. We also describe the public health response and relevant investigations regarding the case.

Methods

Case Description
During the second week of October 2020, a child <18 years of age was brought to a local emergency department with a 4-day history of cough, fever, pharyngitis, and rhinorrhea. At admission, the patient was afebrile, had mild tachycardia and tachypnea, and had...
an oxygen saturation of 93% on ambient air. We did not find signs of respiratory distress and discharged the patient after collecting specimens for respiratory virus testing. The patient was born in Canada and up-to-date with all routine immunizations and influenza vaccinations until 2016.

Consent was provided for the patient and the patient’s household members to be included in this report provided that no identifiable information was published. Presentation of the data contained in this report has been approved by the Human Research Ethics Board at the University of Alberta (Edmonton, Alberta, Canada; study no. Pro00105933).

Diagnostic Evaluation
The patient’s nasopharyngeal swab sample tested negative for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by real-time reverse transcription PCR (rRT-PCR) (10). Multiplex molecular respiratory virus testing (NxTAG Respiratory Pathogen Panel; Luminex Corporation, https://www.luminexcorp.com) detected the presence of IAV; however, the virus could not be subtyped. A second rRT-PCR confirmed the presence of IAV but not influenza A(H1N1)pdm09 virus (11,12). Partial Sanger sequencing of the hemagglutinin (HA) and neuraminidase (NA) genes using universal primers (13) yielded sequences that closely resembled isolates of swine H1N2 IAVs available in GenBank.

Epidemiologic Inquiry
The patient had no history of travel, contact with persons from outside the county, or contact with persons who had respiratory illness. One of the patient’s household contacts worked with animals at a local pig farm. No household contacts reported symptoms of influenza-like illness or coronavirus disease (COVID-19) before the onset of illness in the index patient. However, 1 household contact reported influenza-like illness symptoms 2 days after symptom onset in the index patient. All household contacts remained at home for 10 days after symptom onset in the household contact. Both symptomatic persons recovered. Two asymptomatic household members, including one who worked at the farm of interest, consented to serologic testing by hemagglutination inhibition (HI) assay; samples were collected 35 days after identification of the index patient. The index patient and symptomatic household member declined serologic testing.

Public Health Response
Upon confirmation of swine IAV, which occurred 3 weeks after collection of the original nasopharyngeal swab sample, provincial public health teams, in collaboration with the Alberta Precision Laboratories (Edmonton, Alberta, Canada), undertook heightened influenza surveillance measures in the geographic zone of Alberta where the case was identified. All respiratory specimens collected for community and hospital-based SARS-CoV-2 testing in that region during October 5–November 4, 2020, were retrieved from storage and tested for IAV (12). We conducted prospective testing for IAV on all respiratory specimens submitted for SARS-CoV-2 testing from that area during November 4–10, 2020. Patients being tested for SARS-CoV-2 were informed they would also be tested for IAV.

Upon notification of the case, the Office of the Chief Provincial Veterinarian (Edmonton, Alberta, Canada) began a veterinary investigation in collaboration with industry veterinarians and university partners to explore potential links to local pig herds. Staff of the Chief Provincial Veterinarian investigated the health, history and biosecurity practices of the farm where the household contact worked. Past samples collected from local pig herds showed IAVs of multiple subtypes in the farms supplying piglets to the herd of interest. In December 2017, a closely related H1N2 virus had been isolated from the nursery supplying the farm. In October 2019, a virus from the influenza A(H1N1)pdm09 clade was isolated from the nursery; this strain was most recently detected at the nursery in February 2020.

Characterization of H1N2v Strain
We forwarded the patient’s sample to the National Microbiology Laboratory (Winnipeg, Manitoba, Canada) for isolation and further characterization. The virus was cultured on Madin-Darby canine kidney cells in 1 passage using standard techniques (14). We conducted HI assays with 0.5% vol/vol turkey red blood cells and 4 HA units of A/Alberta/01/2020 (H1N2)v. We treated each serum with receptor-destroying enzyme (Denka Seiken, https://www.denka.co.jp) at a 1:4 dilution for 18 hrs at 37°C and 45 min at 56°C, then performed adsorption with packed turkey red blood cells (15). We defined the HI titer as the highest dilution of the serum capable of inhibiting hemagglutination.

We determined phenotypic susceptibility for oseltamivir and zanamivir by using a chemiluminescent NA inhibition assay (NA-Star Influenza Neuraminidase Inhibitor Resistance Detection Kit; Thermo Fisher Scientific, https://www.thermofisher.com) at the National Microbiology Laboratory. The assay used viruses standardized to equivalent
NA enzyme activity and incubated with 0.0316–1,000 nmol of oseltamivir or zanamivir. We calculated the 50% inhibitory concentration \((IC_{50})\) by plotting the percentage inhibition of NA activity against the inhibitor concentration, using PRISM version 4 (GraphPad Software, https://www.graphpad.com) for curve fitting.

We conducted whole-genome sequencing of the H1N2v isolate on the MinION (Oxford Nanopore Technologies, https://nanoporetech.com) and MiSeq (Illumina, https://www.illumina.com) platforms. We generated sequence data and prepared and sequenced libraries using the DNA Library Prep Kit and iSeq 100 (Illumina; Appendix, https://www.ncbi.nlm.nih.gov/BLAST (https://www.illumina.com)) and MiSeq (Illumina, https://www.illumina.com) platforms. We conducted phylogenetic characterization of the H1N2v isolate by comparing human H1N2v and swine H1N2 HA (segment 4) sequences available on GenBank. We also aligned sequences and visualized the phylogenetic trees (Appendix).

### Sampling

We used the rope technique to collect samples from several farms, including the farm of interest (16). We also collected 56 deep nasal swab and 11 pen-based oral fluid samples from farms downstream of the farm of interest, as well as 12 nasal swab and 6 oral fluid samples from the farm of interest. We placed individual nasal swab samples in 1.5 mL of Dulbecco’s modified Eagle medium (Thermo Fisher Scientific) and vortexed them before extracting 500 µL pooled samples from 3–4 swabs for PCR (17). We subtyped the RNA from the strongest positive pooled nasal swab or oral fluid sample from each farm. Samples were analyzed at the University of Saskatchewan (Regina, Saskatchewan, Canada) by using the VetMAX-Gold Swine Influenza Virus Detection rRT-PCR kit and the VetMAX-Gold Swine Influenza Virus Subtyping rRT-PCR kit (Thermo Fisher Scientific) (18).

### Results

BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) analysis of historical swine H1N2 and H1N2 isolates from western Canada yielded a close match to a virus strain found on only a few farms in central Alberta, including a farm in the pig supply chain of the herd of interest. Phylogenetic analysis showed that the human A/Alberta/01/2020 H1N2v isolate belonged to the evolutionary branch found at the nursery that supplied the farm where the household contact worked (A/swine/Alberta/SD0237/2017 and A/swine/Alberta/SD0267/2017); the most similar sequence was collected from the source farm in 2017 (Figure; Appendix Table). The A/Alberta/01/2020 H1N2v sequence shared high genetic similarity (98%–99%) with 8 genes from multiple swine H1N2v and H3N2 strains from western Canada and the United States (Table). The H1 gene sequence most resembled sequences found in the H1α-3a subclade.

Two members of patient’s household had HI titers of 20. Seasonal vaccine serum for A/Hawaii/70/2019 H1N1 elicited no HI titer against A/Alberta/01/2020 H1N2v. We found that A/Alberta/01/2020/H1N2v was susceptible to oseltamivir \((IC_{50} 0.41 \text{ nmol})\) and zanamivir \((IC_{50} 2.16 \text{ nmol})\). The control strain, A/Brisbane/10/07 H3N2, was sensitive to oseltamivir \((IC_{50} 0.61 \text{ nmol})\) and zanamivir \((IC_{50} 3.19 \text{ nmol})\).

### Surveillance for Additional H1N2v Cases

This case occurred during the response to the COVID-19 pandemic, when there was no circulating seasonal influenza in the province (19). We did not identify additional cases of IAV from retrospective testing of 1,276 archived respiratory specimens nor prospective testing of 256 specimens submitted for respiratory virus testing.

### IAV Testing

In total, 3/13 pooled nasal swab and 2/6 oral fluid samples from the farm of interest tested positive for IAV. Cycle threshold \((C_t)\) values were 34.7–36.6; we considered values <38 to be positive. We found that all positive pooled nasal swab and oral fluid samples from the 2 sites of interest with the lowest \(C_t\) value (34.7) had the H1 gene; however, we could not identify the NA type. The sample with the second lowest \(C_t\) value (35.4) from the same downstream site was subtyped as N2, as was the only positive \((C_t 36.0)\) sample from the other downstream farm tested.

At the nursery, all 3 pooled nasal swab and all 6 oral fluid samples tested positive for IAV (\(C_t 29.8–34.7)\). We identified H1, H3, N1, and N2 genes in the pooled nasal swab sample with the lowest \(C_t\) value (29.8) from the nursery.

### Discussion

We report pig-to-human transmission of H1N2v virus in Alberta, Canada. The source of H1N2v infection in the index patient is unclear, but one of the patient’s household contacts worked at a pig farm where a similar H1N2 virus was found in 2017.

IAV is transmitted through close contact and contaminated objects (5). Transmission of IAV from swine to humans is usually the result of close contact or self-inoculation from contaminated farm surfaces (3,20,21). In Canada, pig farms must adhere to strict
farm biosecurity measures, such as policies requiring workers to shower before leaving, use of facility-specific uniforms and boots, and designation of clean and dirty zones (22–24). Thus, the likelihood of the index patient acquiring infection through fomite-related transmission is very low. Because the index patient never visited the farm of interest, the virus was probably transmitted through respiratory droplets from the household contact who worked at the farm. Humans have poor seroconversion to H1N2 viruses (20), so it is possible that the household contact had partial protection from previous exposure, despite having an HI titer of 20. Partial protection might also explain the limited forward transmission observed in this study, especially because H1N2v virus is associated with mild illness and limited transmission (5).

Figure. Phylogenetic trees of A/Alberta/01/2020 H1N2v and related strains, United States and Canada, 2016–2020. A) The H1α-3a subclade. B) The H1α-3a subclade with ≈2 years of changes (2017–2018) of A/swine/Alberta/SD0237/2017 and A/swine/Alberta/SD0267/2017. The trees were built with IQ-TREE version 2.0.3 (http://www.iqtree.org) on the basis of hemagglutinin sequences. Numbers at nodes indicate bootstrap values based on 1,000 replicates. Red bars identifies clades; all clades presented have % age bootstrap values >70. Diamond indicates A/Alberta/01/2020 (H1N2)v. Scale bar indicates number of nucleotide substitutions per site.
Although not an exact predictor of protection, HI titers of 40 are considered the minimum protective level for humans (25). Therefore, titers of 20 against the isolated strain would not indicate a recent stimulation of the antibodies or a protective cross-reaction. However, a very mild or asymptomatic infection might not result in a high titer immune response (26). Because testing with A/Hawaii/70/2019 (H1N1) antisera elicited no titer, seasonal vaccination would probably not provide protection against A/Alberta/01/2020/H1N2v.

Extensive surveillance testing in the geographic area of the index patient did not detect additional cases, indicating minimal spread. After detection of a variant influenza, active case-finding is critical because of the potential pandemic threat posed by emerging strains. A comparative evaluation of H1N1v and H1N2v viruses isolated during 2011–2016 found that many swine H1 strains capable of infecting humans possess adaptations for efficient replication and enhanced transmission through respiratory droplets (27). Furthermore, the H1 antigens in most isolates were distinct from those of vaccine strains; thus, routine influenza vaccine is unlikely to provide adequate protection against variant strains. Even 1 zoonotic event could enable adaptations for human infection and transmission (28).

We found low levels of circulating virus among pigs at the farm where the index patient’s household member worked and those downstream from it, possibly because the 2 sites were finisher barns where medium-sized grower pigs are raised until they reach market weight. Sows who have antibodies to IAV from vaccination or natural exposure can pass maternally derived antibodies to their offspring through colostrum. The amount of maternally derived antibodies...
detected in newborn piglets varies within litters, not only because antibodies vary among sows, but also because the firstborn piglets consume the most colostrum. In total, 60%–100% of nursing piglets born to a vaccinated sow have HI titers >120 at 5 days old. On average, 75% of the nursing piglets of vaccinated sows have a strong titer (>120) that is homologous between the vaccine and circulating strains (i.e., autogenous vaccines) (S. Detmer, University of Saskatchewan, pers. comm., 2021 Jun 5). As such, there is limited detection of virus in farrowing barns. In contrast, IAVs are often detected in and isolated from nursing piglets 14–24 days of age on farms that do not vaccinate sows, further demonstrating that maternally derived antibodies from vaccinated sows limit infection in nursing piglets until these antibodies wane at 6–8 weeks of age (29). By the time pigs reach the finisher barns, they often have experienced infection in the nursery, farrowing barn, or both and have acquired their own immune response to endemic strains of IAV. In finisher barns, acquired immunity among pigs causes many IAV infections to be subclinical and undetected (30).

In 2016, analysis of whole influenza genomes isolated from pigs in Canada and the United States demonstrated the splitting of the H1α clade into 3 distinct subclades: H1α-1, H1α-2, and H1α-3 (31). We found that A/Alberta/01/2020/H1N2v most closely resembles strains belonging to the H1α-3 virus clade; however, the variant strain does not contain the amino acid deletions at sites 129 and 130 in the HA gene. Therefore, A/Alberta/01/2020/H1N2v probably belongs to the H1α-3a virus clade. Further study is required to assess if the H1α-3a virus cluster is antigenically in addition to genetically distinct from the H1α-3 virus cluster.

A zoonotic H1α-3 H1N2v virus strain (A/Ohio/24/2017/H1N2) was isolated from a human in the United States in 2017 (32). The A/Ohio/24/2017/H1N2 and A/Alberta/01/2020/H1N2v shares high nucleotide identity (91.3%–96.6%) within 8 major genes; however, this proportion is lower than that of other H1N2 strains (Table). As of July 2021, 9 distinct H1α-3a viruses have been detected in the United States. These 9 viruses were probably associated with animal movements from Alberta to the US states of Iowa and South Dakota. These animal movements stopped in 2018 and there is no evidence of continued transmission or geographic spread of these strains in the United States (31).

A major limitation of this study is the 3-week delay in confirming the patient’s H1N2v infection. Therefore, active surveillance testing of persons and pigs in the patient’s geographic region was delayed, possibly diminishing our ability to detect additional active cases. However, we mitigated this limitation in human sampling by testing banked samples collected for SARS-CoV-2 testing beginning ≈1 week before the collection of the patient’s nasopharyngeal swab sample. The delay might have contributed to the higher Ct values for IAV testing of samples from pigs at the farm of interest, because the active phase of infection probably occurred several weeks earlier in most pigs. The higher Ct values of >30 also decreased our ability to amplify certain gene segments for strain identification.

As of September 2021, no further H1N2v virus cases have been identified in humans in that area of Alberta. This case occurred when IAV incidence among humans was uncharacteristically low, probably because of nonpharmaceutical interventions implemented in response to the COVID-19 pandemic (33). These interventions simplified IAV screening. Our results highlight the importance of expanding collaborations between the human and veterinary sectors to enable timely identification, reporting, and investigation of emerging zoonotic pathogens of pandemic potential.

About the Author
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Middle East respiratory syndrome coronavirus (MERS-CoV) infects humans and dromedary camels and is responsible for an ongoing outbreak of severe respiratory illness in humans in the Middle East. Although some mutations found in camel-derived MERS-CoV strains have been characterized, most natural variation found across MERS-CoV isolates remains unstudied. We report on the environmental stability, replication kinetics, and pathogenicity of several diverse isolates of MERS-CoV, as well as isolates of severe acute respiratory syndrome coronavirus 2, to serve as a basis of comparison with other stability studies. Although most MERS-CoV isolates had similar stability and pathogenicity in our experiments, the camel-derived isolate C/KSA/13 had reduced surface stability, and another camel isolate, C/BF/15, had reduced pathogenicity in a small animal model. These results suggest that although betacoronaviruses might have similar environmental stability profiles, individual variation can influence this phenotype, underscoring the need for continual global viral surveillance.

**Surface–Aerosol Stability and Pathogenicity of Diverse Middle East Respiratory Syndrome Coronavirus Strains, 2012–2018**


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pathology in an animal model (18). These findings underscore the need for characterizing how MERS-CoV genetic variation alters viral replication, pathogenicity, and stability.

We tested a broad panel of viral isolates collected from humans and camels, representing every major geographic region that has had MERS-CoV outbreaks and spanning from early to contemporary outbreaks. Because MERS-CoV spreads within households and hospitals, we characterized viral phenotypes with immediate implications for public health. We focused on environmental stability in aerosols as well as surface stability on common materials found in hospitals, replication kinetics in immortalized human cell lines and primary human airway epithelial cultures, and pathogenicity in a transgenic mouse model our laboratory developed to test vaccine efficacy (20). For environmental stability studies, we included severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to enable better comparison of these findings with those of previously published stability studies (21).

Methods

Ethics

Animal experiment approval was obtained by the Institutional Animal Care and Use Committee at Rocky Mountain Laboratories, National Institutes of Health (Hamilton, MT, USA). All animal experiments were executed in an Association for Assessment and Accreditation of Laboratory Animal Care–approved facility, following the guidelines in National Institutes of Health Guide for the Care and Use of Laboratory Animals, Animal Welfare Act, US Department of Agriculture, and United States Public Health Service Policy on Humane Care and Use of Laboratory Animals. The Institutional Biosafety Committee approved work with MERS-CoV strains under Biosafety Level 3 conditions.

Viral Stock Propagation

We provide strain-specific details for the viruses used in this study (Table). Viruses were isolated by others and provided for this study. SARS-CoV-2/Washington was isolated by the Centers for Disease Control and Prevention (Atlanta, GA, USA).

We obtained MERS-CoV strains from the following sources: EMC12 from Erasmus Medical Center (Rotterdam, the Netherlands); U/14, KSA/15, and KSA/18 from the Centers for Disease Control and Prevention; SK/15 from Chungbuk National University (Cheongju, South Korea); and C/KSA/13, C/E/13, and C/BF/15 from Hong Kong University (Hong Kong, China). We passaged MERS-CoV and SARS-CoV-2 strains once in Vero E6 cells in Dulbecco’s modified Eagle medium (DMEM; Sigma Aldrich, https://www.sigmaaldrich.com) supplemented with 2% fetal bovine serum (Thermo Fisher Scientific, https://www.thermofisher.com), 50 U/mL of penicillin (Thermo Fisher), and 50 µg/mL of streptomycin (Thermo Fisher).

We maintained Vero E6 cells in DMEM supplemented with 10% fetal bovine serum, 1 mmol/L of L-glutamine, 50 U/mL of penicillin (Thermo Fisher), and 50 µg/mL of streptomycin. We clarified virus stocks by

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<th>Table. Characteristics of Middle East respiratory syndrome coronaviruses tested*</th>
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*NSP, nonstructural protein; ORF, open reading frame; SNP, single-nucleotide polymorphism.
†Present in other sequences.
centrifugation and froze them at −80°C. We performed virus titrations by using endpoint titration in Vero E6 cells inoculated with 10-fold serial dilutions of virus. We scored cytopathic effect at day 5 (for MERS-CoV) or day 6 (for SARS-CoV-2) and calculated median tissue culture infectious dose (TCID50) from 4 replicates by using the Spearman–Karber method (22).

Sequencing Stocks

We visualized final libraries on a Bioanalyzer DNA1000 Chip (Agilent Technologies), and quantified them by using a KAPA Library Quant Kit (Illumina) and a universal qPCR Mix (Kapa Biosystems, https://www.roche.com) on a CFX96 Real-Time System (Bio-Rad Laboratories, https://www.bio-rad.com). We pooled libraries together in equimolar concentrations and sequenced them using MiSeq (Illumina) with on-board cluster generation and 2 × 250 paired-end sequencing. The cluster density was at 454 k/mm²/lane, resulting in 8.7 million reads passing filter/run and an average 85% greater than the Q30 score.

Phylogenetics
We downloaded all available MERS-CoV genome sequences from GenBank and curated them to remove sequences that were not independently sampled. We aligned sequences with the consensus sequences for MERS-CoV isolates used in this study by using MAFFT version 7.388 plugin (23) in Geneious Prime (https://www.geneious.com). We inferred a phylogenetic tree by using the maximum-likelihood method under the general time reversible plus gamma model of nucleotide substitution with 1,000 bootstrap replicates implemented with PhyML version 3.3.20190321 (https://www.atgc-montpellier.fr).

Stability of MERS-CoV on Surfaces and in Aerosols
We sterilized 15-mm polypropylene discs (ePlastics, https://www.eplastics.com), AISI 304 alloy stainless steel discs (Metal Remnants, https://metalremnants.com), copper discs (99.9%; Metal Remnants), and silver discs (99.9%) (Sigma-Aldrich, https://www.sigmaaldrich.com), placed them in 24-well plates, and added 50 µL of MERS-CoV (10^5 TCID50/mL). For timepoints taken at 0, 1, 24, 48, and 72 h, we added 1 mL of DMEM to wells, aliquoted, and stored at −80°C. We titrated samples on Vero E6 cells and maintained the temperature (21°C–22°C) and humidity (45%–55%).

We determined virus stability in aerosols as described (24). In brief, we loaded a collision nebulizer with 10^5 TCID50/mL of MERS-CoV in DMEM containing 2% fetal bovine serum. Aerosols were maintained in a Goldberg drum and samples collected at 0, 30, 60, 120, and 180 min after aerosolization by passing air at a volume of 6 L/min for 30 s from the drum through a 47-mm gelatin filter (Sartorius, https://www.sartorius.com). Filters were dissolved in 10 mL of DMEM containing 10% fetal bovine serum and stored at −80°C. All samples were titrated on Vero E6 cells.

Replication of MERS-CoV Strains In Vitro
We inoculated Vero E6 cells with virus (multiplicity of infection = 0.01) and collected supernatants at 8, 24, 48 and 72 hours postinfection (hpi). Human airway epithelium (HAE) inserts (Epithelix, https://www.epithelix.com) were maintained as specified by the manufacturer. We washed HAEs with 200 µL of phosphate-buffered saline for 30 min, followed by inoculation with MERS-CoV at a multiplicity of infection of 0.1. We obtained samples at 8, 24, 48, 72, and 96 hpi.

Animal Experiments
We inoculated intranasally transgenic BALB/c mice expressing human DPP4 with 10^3 TCID50 MERS-CoV. Mice were weighed and swabbed daily. At day 3, we euthanized 4 mice and harvested lung tissue. We monitored the remaining 6 mice for survival. We euthanized mice if there were signs of severe disease signs based on quantitative assessment (e.g., hunched posture, lack of movement) or >20% weight loss.

RNA Extraction and Quantitative Reverse Transcription PCR
We homogenized lung tissues and extracted RNA by using the RNeasy method (QIAGEN, https://www.qiagen.com) according to the manufacturer’s instructions. We added swab specimens to 1 mL of DMEM, vortexed them, and used 140 µL for RNA extraction by using the QiaAmp Viral RNA Kit and a QIAxtractor (QIAGEN).
We detected MERS-CoV viral RNA by using the UpE MERS-CoV assay (25) and the Rotor-GeneTM Probe Kit (QIAGEN). Primers in this assay target a highly conserved region upstream of MERS-CoV envelope gene. Sequences of MERS-CoV strains used in this study are identical in this region. MERS-CoV dilutions with known genome copies were run in parallel to enable calculation of genome copies in samples.

Histologic and Immunohistochemical Analysis
We fixed harvested tissues for ≥7 days in 10% neutral-buffered formalin, processed them by using a VIP-6 Tissue Tek Tissue Processor (Sakura Finetek, https://www.sakuraus.com), and embedded them in Ultraflin Paraffin Polymer (Cancer Diagnostics, https://www.cancerdiagnostics.com). We stained 5-µm sections with hematoxylin and eosin and detected coronavirus immunoreactivity by using MERS-CoV nucleocapsid protein rabbit antibody (diluted 1:4,000; Sino Biological Inc, https://www.sinobiological.com).

We processed tissues for immunohistochemical analysis by using the Discovery ULTRA Automated IHC/ISH Staining Instrument (and a Discovery ChromoMap DAB Kit (both from Ventana Medical Systems, https://diagnostics.roche.com). For morphometric analysis, we scanned slides by using the Aperio ScanScope AT2 (Aperio Technologies, Inc., https://www.aperio.com) and analyzed the entire section by using ImageScope Positive Pixel Count Algorithm version 9.1 (Aperio Technologies, Inc.). All tissue slides were evaluated by a board-certified veterinary anatomic pathologist.

Statistical Analyses
We performed analyses by using GraphPad Prism version 7.05 for Windows (https://www.graphpad.com). All strains were compared with EMC/12. For aerosol stability data analysis, we determined linear regression for the mean value of 3 runs/virus. We determined statistical significance in deviation from MERS-CoV/EMC12 results by using 1-way analysis of variance, followed by the Bonferroni multiple comparisons test or a 2-way unpaired Student’s t-test. We used simple linear regression to evaluate slopes of decay. Survival of mice compared with mice inoculated with EMC/12 was performed by using the log-rank (Mantel-Cox) test. To calculate the amount of virus shedding per mouse in vivo comparisons, we calculated the area under the curve for a plot of the viral load measured in oropharyngeal swab specimens.

Results

Stability of MERS-CoV Strains in Aerosols or as Fomites Compared with SARS-CoV-2
We selected 8 MERS-CoV strains and 1 SARS-CoV-2 strain (SARS-CoV-2/WA1-2020) to be used in this study (Table; Figure 1). Five MERS-CoV strains were isolated from human cases and 3 strains were isolated from dromedary camels. Strains were isolated during 2012–2018 and originated from the Middle East (5), Africa (2) or South Korea (1) (Table). All originally obtained viruses were passaged once in Vero E6 cells, and virus stocks were deep sequenced (Table). We used MERS-CoV sequences to construct a phylogenetic maximum-likelihood tree, which showed a wide distribution of MERS-CoV strains selected. Thus, our panel represents a broad sample of known genetic variation within currently circulating MERS-CoV strains.

We first investigated the stability of MERS-CoV as fomites on polypropylene, stainless-steel, copper, and silver surfaces, which we selected because they represent commonly encountered surfaces in hospital environments or have virocidal properties (23). For comparison with a pandemic human coronavirus, we also included SARS-CoV-2. Back-titrations of all virus strains showed comparable starting virus titers. Stability of MERS-CoV on polypropylene and stainless-steel surfaces, maintained at 21°C–22°C and a relative humidity of 45%–55% under standard laboratory light conditions, was similar to that reported for MERS-CoV and SARS-CoV-2 stability on surfaces (21,26). We found major differences in decay rates when comparing EMC/12 to SK/15, KSA/18, C/KSA/13, and C/BF/15 on polypropylene. These differences were not found for the other surfaces (Figure 2; Appendix Figure, https://wwwnc.cdc.gov/EID/article/27/12/21-0344-App1.pdf). Infectious virus titers were low for all strains on copper and silver surfaces at 24 hours. We analyzed data by using linear regression for the first 24 hours for each surface and each virus. Decay, averaged between all virus strains, was higher for copper (−0.11576 log₁₀ TCID₅₀/h) and stainless-steel (−0.08744 log₁₀ TCID₅₀/h) and polypropylene (−0.0529 log₁₀ TCID₅₀/h) surfaces for MERS-CoV and SARS-CoV-2 stability on surfaces (21,26).

We aerosolized all MERS-CoV strains in a Goldberg drum at 21°C and a relative humidity of 60%–70% in the dark. We then tested samples at 0, 30, 60, 120 and 180 min after aerosolization, titrated them, and compared results with those for SARS-CoV-2. We detected no major differences in linear regression of loss of infectious virus in aerosols between strains. For all
MERS-CoV strains, infectious virus could still be detected at 180 min after aerosolization (Figure 2, panel B).

**In Vitro Replication of MERS-CoV Strains**

To investigate any in vitro growth differences, we grew strains in 2 cell systems, Vero E6 cells and HAE cultures, in comparison to the reference strain EMC/12. At 48 hpi, C/KSA/13 and KSA/15 showed higher titers than EMC/12 in Vero E6 cells. At 72 hpi, C/KSA/13 and C/BF/15 showed lower titers than EMC/12 in HAE cultures. We observed no other major differences in either culture type. Although differences were not always statistically significant, all camel-derived viruses had reduced replication kinetics compared with those for EMC/12 in HAE cells at 24–72 hpi (Figure 3).

**Disease Progression for MERS-CoV Strains in hDPP4 Transgenic Mice**

MERS-CoV enters cells expressing the receptor human dipeptidyl peptidase IV (hDPP4). Our laboratory developed hDPP4 transgenic mice to test MERS-CoV vaccine efficacy (20). We intranasally inoculated 10 mice/group with $10^3$ TCID$_{50}$ MERS-CoV/mouse. Mice started to lose weight on days 2–5 postchallenge; weight continued to decrease for all groups, except for mice inoculated with C/BF/15, in which only 1 mouse continued to lose weight (Figure 4, panel B).
Stability and Pathogenicity of MERS-CoV Strains

panel A). For all groups, including C/BF/15, weight loss was also associated with other signs: ruffled coat, increased breathing rate, reluctance to move, and hunched posture. Only animals in the groups inoculated with SK/15 (1/6) and the group inoculated with C/BF/15 (5/6) survived. Average time to death was similar for all groups, excluding C/BF/15: EMC/12, 7.33 days; U/14, 6.5 days; KSA/15, 7 days; SK/15, 7.6 days; KSA/18, 7.67 days; C/KSA/13, 7.5 days; and C/E/13, 8 days (Figure 4, panel B).

We measured viral RNA in oral swab specimens obtained during days 1–7 postchallenge and found no

Figure 2. Stability of MERS-CoV strains on surfaces and in aerosols compared with those for SARS-CoV-2. Simple linear regression of virus was used for different surfaces and in aerosols. For surface stability, 50 µL of MERS-CoV or SARS-CoV-2 was spread on a surface, either polypropylene, stainless steel, copper, or silver; 1 mL of Dulbecco’s modified Eagle medium was added at times 0, 1, 24, 48, or 72 hours, and samples were titrated. For aerosol stability, MERS-CoV– or SARS-CoV-2–containing aerosols were sprayed into a Goldberg drum; samples were taken at times 0, 30, 60, 120, and 180 min and then titrated. Linear regression was calculated per virus and indicated as lines. Dotted lines indicate limits of detection. Strain sources are listed in the legend for Figure 1. MERS-CoV, Middle East respiratory syndrome coronavirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TCID<sub>50</sub>, median tissue culture infectious dose.
major differences in the amount of shedding between different groups (Figure 4, panels C, D). Viral genome RNA was lower in lung tissue collected on day 3 from mice inoculated with SK/15, C/E/15, and C/BF/15. Subgenomic RNA was lower to a major degree only in lung tissue of mice inoculated with C/BF/15 (Figure 4, panels E, F).

We observed no differences in pathology between different groups. Animals rarely showed pulmonary pathology at day 3. However, animals that had lesions showed only a minimal and random lymphocytic infiltrate. Immunohistochemical analysis showed that MERS-CoV antigen was expressed rarely or randomly in type I and II pneumocytes and not located in areas of inflammation. Morphometric analysis of pulmonary tissue that had immunoreactivity showed no major differences between groups (Figure 4, panel G).

Discussion
The ongoing MERS-CoV endemic in the Middle East and subsequent discovery of the virus in camel herds across Africa has resulted in a wealth of publicly available genetic data for various viral strains and isolates. In this study, we assessed several of these isolates for viral phenotypes related to public health in an attempt to better inform public health policy making with regards to MERS-CoV and other human coronaviruses that cause respiratory diseases, such as SARS-CoV-2.

Because nosocomial spread is at the center of MERS-CoV outbreaks, we assessed the stability of the virus on various surface material types commonly found in hospitals (polypropylene plastic and stainless steel), as well as materials that had potential antiviral and known antimicrobial properties (silver and copper) (27,28). Our experiments were performed at environmental conditions similar to those in hospitals, in which there is high risk for human-to-human, nosocomial transmission. Regardless of the surface material tested, strain C/KSA/13 was the least stable over time and was below detectable levels by 24 hours (Figures 2, 4). This strain had the lowest starting titer in these experiments, which might explain this difference in stability. In addition, our C/KSA/13 stock contains 2 nonsynonymous mutations in the viral structural proteins, spike and matrix, not found in our other strains, which might also play a role in this difference, either directly or indirectly (Table).

These findings warrant further studies on how specific MERS-CoV polymorphisms in structural proteins affect viral growth.

As shown by Doremalen et al. (21), all virus strains tested had notably reduced stability on copper and silver surfaces (Figure 2, panel A). Copper has been shown to also have antiviral properties against influenza A(H1N1) virus and SARS-CoV-2 (29–31). The exact antiviral mechanism for copper is still unclear, but might be related to formation of hydroxyl radicals by copper ions when in aqueous solution (31). Silver-based nanoparticles have been shown to be antiviral for HIV-1 (32), herpes simplex virus 2 (33), hepatitis B virus (34), respiratory syncytial virus (35), and...
monkeypox virus (36). Taking advantage of the antiviral properties of copper and silver might help decrease nosocomial transmission. Both silver and copper can be used for coating medical tools (37) and commonly touched items, such as bed rails, door handles, and intravenous poles (38). These findings appear to be more broadly applicable for other coronaviruses because we observed similar results for SARS-CoV-2 (Figure 2) (21). Further research should be invested in determining coronavirus susceptibility to metal ion inactivation.

MERS-CoV transmission might occur through aerosols and fomites (39), although the role of each route is not known. Transmission often occurs in hospitals; thus, aerosol-generating medical procedures might play a major role (40). MERS-CoV transmission has occurred over distances of >6 feet (41), and evidence of MERS-CoV on surfaces and in air in hospitals has been found (39). Studies have suggested that a hospital air-handling system might have contributed to nosocomial spread during the 2015 MERS-CoV outbreak in South Korea (14,39), and our group has shown that the virus can remain viable suspended in air for ≤10 min (26). We tested aerosol stability of viral isolates and observed that all viruses remained viable for a minimum of 180 min with an ≈10-fold

![Figure 4. In vivo replication of different Middle East respiratory syndrome coronavirus (MERS-CoV) strains. hDPP4 mice were inoculated intranasally with 10^3 TCID_50 of MERS-CoV. Four mice were euthanized on day 3, and the remaining 6 mice were monitored for survival. A) Relative weight loss of hDPP4 mice. B) Survival of hDPP4 mice. C) Oropharyngeal shedding of MERS-CoV as measured by using an UpE quantitative reverse transcription PCR. D) Amount of shedding per experiment per mouse calculated by using area under the curve (AUC) analysis of viral load in oropharyngeal swab specimens. Results are displayed per mouse per virus strain. E) Viral load in lung tissue obtained from mice euthanized at day 3. F) Viral mRNA load in lung tissue obtained from mice euthanized at day 3. G) Percentage of positive pixels quantified from lung tissues stained for MERS-CoV antigen. Colors in panels D–F match those for strains in panels A–C; strain sources are listed in the legend for Figure 1. Statistical significance was compared by using 1-way analysis of variance, followed by a Bonferroni multiple comparisons test. Dotted lines indicate limits of detection. TCID_50, median tissue culture infectious dose. *p<0.05.](image-url)
reduction in viral titer observed on average within the collected aerosols (Figure 2, panel B).

Although we did not observe major differences in this study, strain stability is an useful phenotype to continue monitoring because mutations in viral capsid proteins have been shown to enhance environmental stability of bacteriophages, dengue virus, and transmissible gastroenteritis virus (42–44). Because MERS-CoV isolates contain polymorphisms throughout the entire viral genome, including the structural proteins that form virions, mutations might arise that influence overall virus particle stability. C/KSA/13, which showed reduced stability on surfaces in our experiments, contains polymorphisms in open reading frame 1b, the spike glycoprotein, and the virion matrix protein in comparison to the other strains tested. Recent studies have further demonstrated the influence of various external factors on environmental stability for SARS-CoV-2, including experimental ambient conditions and matrix in which the virus is suspended (45,46). Our experiments were performed in standard, indoor laboratory settings and with virus suspended in culture media, which enabled us to observe intrinsic differences determined solely at the viral level. Tracking and assessing the stability of coronavirus strains will improve our understanding of coronavirus variant spread.

We tested viral replication kinetics in Vero E6 cells and primary HAE cells (Figure 3). All viruses replicated to similar titers on Vero E6 cells by 72 hours. However, KSA/15 and C/KSA/13 had higher titers than EMC/12 by 48 hpi. Although the difference is not significant, C/BF/15 has a lower viral titer than EMC/12 at 48 hpi and 72 hpi. These results are consistent with those of a previous study, which showed that C/BF/15 has impaired replication (18). In primary HAE cultures, all camel-derived viral isolates had reduced replication kinetics compared with that for EMC/12 (Figure 3, panel B). More studies are needed with these camel-derived isolates to determine whether their differences in replication kinetics results from a comparison with EMC12, which has well-described tissue culture adaptations, or to see if MERS-CoV might adapt in humans after transmission from camels. Sequence analysis of the viral variants did not identify any obvious mutation patterns in any single viral protein that would explain the differences in replication kinetics. Thus, we speculate that these differences are the result of cumulative effects across >1 types of genetic variation.

We have shown that MERS-CoV replicates in type I and II pneumocytes in the lower respiratory tract of an animal model (20). Although disease progression after infection with this virus does not involve the central nervous system in humans, this small animal model is suitable for vaccine candidate testing, using animal survival or viral-induced death as a binary readout for vaccine efficacy. MERS-CoV C/BF/15 contains a deletion in open reading frame 4b, which has been shown in a similar mouse model to result in impaired suppression of the host interferon response and increased type I and type III interferon signaling (18). Taken together, these results pave the way for testing MERS-CoV vaccine candidates for broadly neutralizing potential in this animal model (20,47).

Our results with MERS-CoV C/KSA/13 suggest there might be a potential tradeoff between environmental surface stability and replication kinetics. This tradeoff was observed for a camel-derived isolate, and we did not observe similar phenotypic relationships for the other strains tested (Figures 2, 3). Future research efforts with camel-derived viruses and more closely related human-derived viruses could show whether adaptations are likely to occur after zoonosis. Our previous viral stability results with SARS-CoV-2 and the findings of this study with MERS-CoV suggest copper should be incorporated more in hospital settings, particularly in materials in areas of high contact between hospital workers and MERS patients, such as door handles, bed rails, and medical tools (21). Overall, we observed a range of stability, replication, and pathogenesis phenotypes between different MERS-CoV isolates, underscoring the need for continued surveillance of this virus and other coronaviruses, including SARS-CoV-2.

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References


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Contact tracing is the process by which persons who are believed to have come into contact with a confirmed case-patient with an infectious disease during the infectious period are located and checked for the presence of the infection or disease. Under traditional approaches, contact tracing involves 3 distinct steps: contact identification, in which potential contacts are identified through interview with the primary case-patient; contact listing, in which those identified contacts are listed and communication established with them; and contact follow-up, in which those listed contacts are monitored for presence of infection or onset of disease over a predefined period (1).

Because of its important role in case detection to monitor and curtail chains of transmission, contact tracing often forms part of the public health response to directly transmitted infectious diseases (2). Recently, contact tracing has received widespread attention because of its critical role in the response to outbreaks of diphtheria (3), Ebola virus disease (EVD) (4–6), and the ongoing coronavirus disease (COVID-19) pandemic (7,8).

During 2018–2020, the Democratic Republic of the Congo (DRC) experienced its 10th and largest EVD outbreak, the second largest ever experienced globally (9). EVD is a disease caused by viruses of the genus *Ebolavirus*, family *Filoviridae*. Zoonotic spillover events from the animal reservoir have led to large, explosive outbreaks in West and Central Africa in recent years (9–12). Owing to the high pathogenicity and virulence of Ebola virus, an elimination control strategy is always adopted, aiming to ensure that all case-patients are identified, isolated, and treated promptly after disease onset, thereby limiting the opportunity for onward community spread. Although contact tracing is a central pillar of control (13), no standardized methods have been established to assess a critical aspect of performance, its sensitivity...
(i.e., the ability to detect all contacts and secondary infections resulting from case-patients).

One approach to quantifying this metric is to employ capture-recapture (CRC) methods (14,15). Broadly, this family of methodologic approaches enables researchers to quantify any unit of interest missing from lists and subsequently estimate the sensitivity of the surveillance effort and the probability of detection. Although CRC has previously been used to estimate the number of unobserved cases of disease (16,17), such approaches typically rely on comparison of multiple lists, which are generally not available for contact lists. Therefore, we describe the application of a unilist capture-recapture approach (15) to quantifying the number of unobserved case-patients and contacts and describe their sociodemographic profile, helping to identify plausible risk factors that can be used to target limited resources at those unobserved case-patients most likely to generate onward transmission. More precisely, we aimed to address 2 questions, from which we can derive contact tracing sensitivity estimates: how many case-patients with any contacts did contact tracing miss, and how many case-patients with infected contacts did contact tracing miss?

Materials and Methods

Study Participants
We included all confirmed and probable EVD case-patients and contacts (classified according to standardized case definitions [18,19]) identified in Beni Health Zone, DRC, during July 31, 2018–April 26, 2020. Case-patients were principally detected through 3 identification mechanisms: passive detection at healthcare facilities from persons manifesting symptoms consistent with EVD, house-to-house active case-finding by community health workers, and tracing the contacts of EVD case-patients. Contact tracing was coordinated by the DRC Ministry of Public Health, with support from the World Health Organization, and conducted by locally recruited teams of contact-tracers. Upon detection of a case, efforts to identify and list the case-patient’s contacts were undertaken.

For case-patients, our data contain basic information on sociodemographic characteristics (e.g., age, sex, and DRC Health Area of residence) and dates of disease onset and isolation. For contacts, our data contain similar sociodemographic information and information on the daily follow-up and final status of the contact (either “completed the 21 days follow-up,” “confirmed as EVD case-patient,” “lost to follow-up,” “never seen,” or “died during follow-up”). Contacts recorded as “confirmed as EVD case-patients” were those identified by the contact tracing teams during the course of their work. EVD was assumed to be the cause of death for contacts recorded as “died during follow-up” because of the short interval between their contact with an EVD case-patient and their death.

Exploratory Data Analysis
We determined the distribution of case-patients according to age, sex, and timing of disease onset. We used the Wilcoxon test to explore differences in continuous variables and the χ² test for categoric variables to determine the distribution of the number of contacts per case-patient between 2 distinct epidemic waves. Overdispersion (i.e., superspreading) in the offspring distribution of secondary case-patients arising from infectious persons may have profound effects on control strategies in low-resource settings (20,21), and we describe the extent of this phenomenon in 2 ways: first, by assessing the proportion of infectious persons linked to 80% of onward transmission using methods described by Endo et al. (22); and second, by estimating the dispersion parameter (k) using methods described by Althaus (23).

We used a multivariable logistic regression model to explore risk factors associated with loss to follow-up, in which previously successfully traced contacts (i.e., those identified, listed, and among whom follow-up has begun) become untraceable at some point during the 21-day follow-up period. In such instances, contacts unable to be traced for 3 consecutive days are recorded as having been lost to follow-up, and no further attempts at tracing are made. To explore characteristics of case-patients with infected contacts, we calculated the mean number of contacts, mean age, and sex ratio of case-patients with ≥1 listed contact (among whom we can be confident that at least a minimal investigation was conducted), according to 3 categories: those with no infected contacts identified, those with exactly 1 contact, and those with ≥2 contacts.

CRC Modeling
We classified the observed case-patients according to their number of listed contacts (either exactly 0 or ≥1 contact), further classifying this latter category according to the number of infected contacts observed (either exactly 0 or ≥1 contact). For each detected case, the contact tracing process generates a list of persons fitting the definition for a contact (Appendix, https://wwwnc.cdc.gov/EID/article/27/12/20-4958-App1.pdf), some of whom may themselves have been infected and will eventually become secondary...
case-patients. From this list, frequency distributions of case-patients with any listed contacts, and of case-patients with infected contacts, can be generated by first excluding (truncating) those case-patients with 0 contacts. For example, the data can be binned into the number of case-patients with exactly 1 contact (f_1), 2 contacts (f_2), and so on, to the number of case-patients with the maximum number of contacts (f_m). Statistically, this process leads to a 0-truncated observed count distribution of case-patients with \( \geq 1 \) contact. By applying a unilist CRC approach designed to estimate unobserved population sizes using the distribution of count data within single lists (15), we can infer \( f_m \) the number of unobserved case-patients with \( \geq 1 \) contact. Associated with the observed frequencies \( f_1, f_2, \ldots, f_m \) and unobserved \( f_0 \) are probabilities \( p_1, p_2, \ldots, p_m \) and \( p_0 \) that inform the probability of identifying a case-patient with exactly 1, 2, …, \( m \) and 0 contacts, respectively. A conventional approach assumes that the frequencies arise from a discrete distribution such as the Poisson, where

\[
p_0 = e^{-\lambda}, \quad p_1 = \frac{e^{-\lambda} \lambda}{1!}, \quad p_2 = \frac{e^{-\lambda} \lambda^2}{2!}, \ldots, \quad p_m = \frac{e^{-\lambda} \lambda^m}{m!}.
\]

Other common distributions are the negative binomial and the geometric distribution. The geometric distribution has probabilities \( p_0 = p, \quad p_1 = p(1 - p), \quad p_2 = p(1 - p)^2, \ldots, \quad p_m = p(1 - p)^m \), where \( p \) is a probability parameter. Poisson and geometric are special cases of the negative binomial distribution, which provides a flexible model family (Appendix). Because the observed distribution contains only positive numbers of contacts, we need to consider the associated zero-truncated distribution \( p_1/(1 - p), \quad p_2/(1 - p)^2, \ldots, \quad p_m/(1 - p)^m \). In other words, we assume that the number of observed contacts among case-patients who actually had contacts follows a parametric distribution (although nonparametric approaches are possible [15,24,25]), find the best-fitting zero-truncated distribution of case-patients with \( \geq 1 \) observed contact (we explore the zero-truncated Poisson, negative binomial, and geometric distributions [Appendix]), and use the estimated probability \( p_0 \) of not observing a case-patient with contacts (calculated from the best-fitting distribution) to inform standard population estimators. We use the Horvitz–Thompson estimator to estimate \( f_0 \) the unobserved number of case-patients:

\[
\hat{f}_0 = \frac{n}{1 - p_0} \cdot \frac{p_0}{1 - p_0}
\]

where \( n \) is the number of observed case-patients with \( \geq 1 \) observed contact and \( p_0 \) is as previously defined. The Horvitz–Thompson estimator provides an unbiased estimate of \( f_0 \) provided that \( p_0 \) is correctly specified; hence, using a correctly-specified distribution for the number of observed contacts is important. We use maximum likelihood for model fitting, selecting the model with the smallest Akaike information criteria (AIC) and Bayesian information criteria (BIC) (Appendix).

To estimate 95% CIs, we use a parametric bootstrap, described as follows. Suppose that \( N \) is the estimated size of the (observed and unobserved) population under a fitted model. We generate \( B \) samples of size \( N_b \) using the fitted model and its estimated parameter or parameters. For each sample, all zeros are truncated and the size estimate \( N_b \) computed, for each of the samples \( b = 1, \ldots, B \). We chose \( B = 10,000 \) to minimize bootstrap simulation random error. We constructed 95% CIs by using the 2.5th percentile of the distribution of \( N_b \) as the lower end and the 97.5th percentile as the upper end.

Results

Exploratory Data Analysis

We identified 913 confirmed and 10 probable EVD case-patients in Beni Health Zone. The contact tracing process listed 80,556 contacts, of whom 6,375 were duplicates, having been listed as the contact of >1 case-patient, resulting in 74,181 contacts to trace. In discussion with contact tracing teams, duplicates were identified by matching name and residential location; for operational reasons, these persons were recorded as a contact of only the earliest-identified primary case-patient with whom they were associated. More than half of case-patients for whom sex and age information were available were women and girls (n = 515 [55.8%]); median age for all case-patients was 25 years (interquartile range [IQR] 13–38 years). Most contacts (64,545 [87.0%]) were successfully traced, leading to the detection of 396 secondary case-patients. The median delay between last contact with the primary case-patient and first contact by the contact tracing teams was 4 days (IQR 3–6 days).

Disease onset dates spanned the period from July 31, 2018, to April 26, 2020, and was bimodally distributed, showing 2 waves that peaked in October 2018 and June 2019 (Figure 1, panel A). The second wave followed a period of insecurity in this conflict-affected area that severely hampered response activities (26).

The median number of contacts among all case-patients was 61 (IQR 18–120), but this number was significantly lower during the first wave than the second (34 vs. 80; \( p<0.001 \)). Case-patients infected in the first wave were more likely to have 0 listed contacts than those in the second wave (31.3% vs. 9.6%; \( p<0.001 \))
by χ² test), and second-wave case-patients were more likely to have a large number (>100) of contacts (Figure 1, panel B). A total of 792 case-patients (85.8%) reported >1 contact (Figure 2, 3), among whom the median number of contacts was 74 (IQR 36–134) and the mean number of contacts was 102.

A total of 64,545 contacts (87.0%) were successfully traced, of whom 308 were confirmed as EVD case-patients and 88 died during follow-up. Therefore, the inferred total number of infected contacts was 396 (308 + 88), or 0.7% of the contacts successfully traced to completion of the follow-up period. Precise detail on the mechanism of identification of confirmed case-patients among contacts is not available; although we assume these infected contacts were identified by contact tracers during follow-up, the role of other surveillance activities cannot be excluded.

We observed substantial overdispersion in the offspring distribution of secondary case-patients; 80% of onward transmission was linked to only 13.9% (95% CI 11.4%–16.2%) of primary case-patients, and all secondary case-patients concentrated among the contacts of 207 (22.4%) primary case-patients. Furthermore, only 99 (10.7%) primary case-patients led to >1 secondary case-patient (Figure 2, 4). We estimated k as 0.27 (95% CI 0.20–0.33).

Male contacts had slightly (but statistically significantly) greater odds of being lost to follow-up (odds ratio [OR] 1.06, 95% CI 1.01–1.11) (Table 1). Contacts in older age groups had significantly greater odds of being lost to follow-up compared with contacts in the youngest age group (0–15 years). We observed the greatest effect among contacts ≥60 years of age (OR 1.65, 95% CI 1.47–1.86) and a marginally smaller effect among contacts 45–59 years of age (OR 1.55, 95% CI 1.43–1.69). Conversely, contacts traced during the second wave had lower odds of being lost to follow-up (OR 0.83, 95% CI 0.79–0.88).

CRC Modeling

Completeness of Contract Tracing for Case-Patients with >1 Listed Contact

Among case-patients with >1 contact listed, the best-fitting distribution of the count of case-patients with any contacts was given by the zero-truncated geometrical distribution.
ric model, which produced the lowest AIC and BIC (Appendix Table 1). This distribution was very long-tailed (Figure 5), indicating that most case-patients with contacts were successfully detected, given that with an increasing mean of any count distribution, the probability for a zero count becomes smaller. This pattern is observed from the expression of the geometric distribution, described previously, where for $x = 0$ (i.e., the zero count), its estimated probability $p_0$ resolves the equation

$$
\frac{1}{1+\mu} \left( \frac{\mu}{1+\mu} \right)^x
$$

where $\mu$ is the mean of the geometric model; the larger the mean, the smaller the probability of $x = 0$.

We estimated $f_0$ (the unobserved number of case-patients with any contacts) = 8 (95% CI = 8–10), where sample size ($n$) was 792 and $p_0$ was found as 0.01. The sensitivity of contact tracing to detect case-patients with any contacts was therefore $792/(792 + 8) = 0.99\%$ (95% CI 0.99%–0.99%). We observed no difference in sensitivity by epidemic wave (wave 1 = 0.99% [95% CI 0.99%–0.99%]; wave 2 = 0.99% [95% CI 0.99–0.99%]).

**Completeness of Contact Tracing for Case-Patients with Infected Contacts**

Among case-patients with infected contacts, the best-fitting distribution of the count of case-patients with infected contacts was again given by the zero-truncated geometric model, which produced the lowest AIC and BIC (Appendix Table 1). This distribution is concentrated on the lower counts from 1 to 4 (Figure 6), indicating that a substantial proportion of case-patients with infected contacts may not have been detected.

We estimated $f_0$ (the unobserved number of case-patients with infected contacts) = 227 (95% CI 171–241), where sample size ($n$) was 207 and $p_0$ was found as 0.52. The sensitivity of contact tracing to detect case-patients with infected contacts was therefore $207/(207 + 227) = 0.49\%$ (95% CI 0.43%–0.55%). We observed a statistically significant difference in sensitivity by epidemic wave, with lower sensitivity.
during wave 1 (0.24% [95% CI = 0.11%–0.38%]) than during wave 2 (0.48% [95% CI = 0.40%–0.56%]). Among the 792 case-patients with ≥1 listed contact, those with 0 infected contacts had fewer contacts overall, were slightly older, and were slightly more likely to be women or girls compared with the other groups (Table 2).

**Discussion**

Our findings suggest that contract tracing efforts were very successful at identifying case-patients with ≥1 contact but much less successful at identifying case-patients with contacts who later had EVD symptoms. This finding is unsurprising, given that the investigation component (typically by interview with case-patients under treatment, their caregivers, or both) is easier to conduct than the tracing component (typically requiring daily visits to a large number of difficult-to-locate and mobile persons). This difference has important implications, because infected contacts contribute to ongoing chains of transmission when case investigation and contract tracing is inadequate; to prioritize scarce resources, control efforts should target those case-patients among whose contacts secondary infections arise (20,21,27). A high proportion of case-patients listed ≥1 contact (≈85%), compared with 27% during an EVD outbreak in Liberia (28) and 44% during an EVD outbreak in Sierra Leone (27), suggesting that lessons about enhancing the quality of contract tracing were learned from previous EVD outbreaks (4,5,27,28).

Case-patients with infected contacts had more contacts on average, which may result from 3 possible explanations. First, case-patients with more contacts are more likely to have ≥1 infected contact among these. Second, fewer overall listed contacts may be the result of poorly conducted case investigations. We found some evidence in support of this; the mean number of contacts increased as the

**Figure 4.** Frequency distribution of Ebola virus disease case-patients, by number of infected contacts, Beni Health Zone, Democratic Republic of the Congo, July 31, 2018–April 26, 2020.

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**Table 1.** Multivariable logistic regression for predictors of loss to follow-up of contacts of Ebola virus disease case-patients, Beni Health Zone, Democratic Republic of the Congo, July 31, 2018–April 26, 2020*

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>No. contacts</th>
<th>Unadjusted</th>
<th>Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td>OR (95% CI)</td>
<td>p value</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>1.07 (1.02–1.12)</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Referent</td>
<td>1.06 (1.01–1.11)</td>
<td>0.013</td>
</tr>
<tr>
<td>Age group, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–14</td>
<td>20,616</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>15–29</td>
<td>26,142</td>
<td>1.18 (1.11–1.25)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Referent</td>
<td>1.19 (1.12–1.27)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>30–44</td>
<td>17,665</td>
<td>1.16 (1.09–1.24)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Referent</td>
<td>1.18 (1.10–1.26)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>45–59</td>
<td>6,157</td>
<td>1.56 (1.43–1.70)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Referent</td>
<td>1.55 (1.43–1.69)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&gt;60</td>
<td>2,599</td>
<td>1.64 (1.46–1.84)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Referent</td>
<td>1.65 (1.47–1.86)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Epidemic wave†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First wave</td>
<td>14,374</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Second wave</td>
<td>66,182</td>
<td>0.85 (0.81–0.90)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Referent</td>
<td>0.83 (0.79–0.88)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*OR, odds ratio.
†Contacts were divided into 2 epidemic waves according to the date of symptom onset of their associated primary case-patient (first wave, July 31, 2018–February 28, 2019; second wave, March 1, 2019–April 26, 2020).
epidemic progressed, indicating possible improvements in case investigation quality over time as staff became more accustomed to the procedure and community trust and engagement in the response improved (29). Third, case-patients with infected contacts may differ from other case-patients; in this study, such case-patients were younger and more likely to be men or boys, which are demographic factors previously shown to affect transmission of EVD and other diseases (30–32). Case-patients with more contacts have been shown to play a greater role in disease transmission and are more likely to have infected contacts (33,34). This tendency is particularly true of diseases that demonstrate heterogeneous transmission, including EVD and COVID-19, and our results suggest a high degree of overdispersion and superspreading, consistent with what has previously been reported during large EVD outbreaks (23). Overdispersion can lead to rapid expansion, particularly among hidden chains of transmission, and a promising area of research is to identify correlates of superspreading to better target limited resources for greatest impact. Previous research suggests that if highly infectious persons can be predictively identified and targeted, the efficiency of control can be greatly enhanced, such that focusing half of all control effort on the most infectious 20% of case-patients can improve effectiveness up to 3-fold (20,21).

Although estimating the number of unobserved case-patients with (infected) contacts is possible, identifying whether these case-patients have been misclassified as having 0 (infected) contacts or if they were undetected by the surveillance system in general is not possible. However, the greater probability of having 0 contacts listed during the first epidemic wave suggests substantial misclassification and suboptimal performance in the period during which surveillance activities were being established, as reported during previous EVD outbreaks (4,27,28). The sensitivity of contact tracing to detect case-patients with infected contacts was lower, and loss to follow-up greater, during the first epidemic wave, indicating quality improvements of this activity over time, either because the ability to conduct contact follow-up was hampered by the insecurity experienced during the first wave or because of greater familiarity with, and acceptance of, the process among contact tracing staff and the local population during later efforts.

Although the method we describe proposes a robust framework to assess the sensitivity of contact tracing, limitations include that no standard list of contacts against which to validate this method exists. However, the method itself has been validated to estimate actual population size in various other settings (25). The dataset does not permit the distinction between case-patients who were confirmed to have no contacts after a thorough case investigation and case-

<table>
<thead>
<tr>
<th>No. infected contacts</th>
<th>No. case-patients</th>
<th>Median age of case-patients, y</th>
<th>% Women and girls among case-patients</th>
<th>Mean (95% CI) total number of contacts</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>585</td>
<td>28.2</td>
<td>59.5</td>
<td>85.7 (79.1–92.4)</td>
</tr>
<tr>
<td>1</td>
<td>108</td>
<td>23.6</td>
<td>56.7</td>
<td>122 (102.0–141.0)</td>
</tr>
<tr>
<td>&gt;2</td>
<td>99</td>
<td>25.6</td>
<td>54.6</td>
<td>174 (144.0–204.0)</td>
</tr>
</tbody>
</table>
patients having no listed contacts because of no (or inadequate) case investigation. However, our method may help to identify the magnitude of the misclassification arising from this limitation. The inferences made are exclusively informed by the definition of case-patients as defined by contact tracing protocols; for example, our results would not inform the sensitivity of contact tracing as applied to asymptomatic EVD case-patients if these persons are not part of the testing strategy.

Differences in performance between contact tracers could result in strong heterogeneity in the count distribution, which might be detectable. For this reason, we applied Chao’s estimator (which allows for heterogeneity), and only if this was significantly different from the model-based estimate would we consider that an issue exists. In our results, we did not observe such a difference (Appendix Tables 2, 3). Finally, we have not adjusted for observed heterogeneity, such as age, sex, profession, geographic location of the case-patients, and delays in the contact tracing process. Further work is planned to incorporate such considerations.

In conclusion, contact tracing is crucial to containing certain disease outbreaks. However, as with many surveillance activities, contact tracing has the potential to suffer reduced effectiveness from underreporting and poor sensitivity (4,27,28). The consequences of poor ascertainment and misclassification can be disastrous, potentially creating explosive expansion among hidden chains of transmission, particularly during containment and de-escalation phases.

We have described a novel application of CRC models to estimate a crucial yet elusive performance indicator of a key component of the public health response to epidemics, namely the sensitivity of contact tracing, as applied to a recent outbreak of EVD. The method demonstrated that most case-patients with any contacts were observed, suggesting that the case investigation component of contact tracing performed well, whereas less than half of case-patients with infected contacts were observed, suggesting that the contact follow-up component of contact tracing performed poorly in this setting. The approach described can be used to assess the sensitivity of contact tracing for any disease, including COVID-19, for which contact tracing has been identified as a crucial component of response activities.

Acknowledgments
We thank the people of the DRC’s Beni Health Zone for their participation in conducting this study. We acknowledge the enormous dedication of the various organizations and persons that responded and provided healthcare to this population and supported the public health response during this outbreak.

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December 1 is World AIDS Day, an opportunity for people to work actively and collaboratively with partners around the world to raise awareness about HIV and help us move closer to the goal of an AIDS-free generation. The U.S. Government’s theme for World AIDS Day 2021 is Ending the HIV Epidemic: Equitable Access, Everyone’s Voice.

http://wwwnc.cdc.gov/eid/page/world-aids
Zika virus (ZIKV) is a member of the Flaviviridae family, which includes other clinically notable viruses such as the 4 dengue virus serotypes (DENV-1–4). Distinguishing DENVs from ZIKV using the established serologic assays widely used for monitoring DENV transmission is difficult because of antibody cross-reactivity between these closely related flaviviruses. We describe a modified and improved recombinant envelope domain III-based serologic assay for detecting ZIKV type-specific antibodies in regions with endemic DENV transmission. When the assay was used to measure ZIKV seroprevalence in 2017 among children 9–14 years of age living in a region of the Philippines with endemic DENV transmission, we observed a ZIKV seroprevalence of 18%. Investigators should consider using the ZIKV envelope domain III-based assay, which is simple and readily adaptable for use in standard clinical and public health laboratories, to assess ZIKV seroprevalence in areas with endemic DENV transmission.

Although sporadic transmission of Asian lineages of ZIKV in Southeast Asia and Pacific islands is well-documented (8,9), its prevalence in the region has been difficult to estimate using current serologic assays because of intense transmission of multiple DENV serotypes and antibody cross-reactivity between DENV and ZIKV. Most serologic assays for flaviviruses measure antibodies binding to viral-envelope glycoprotein (E protein) because this antigen is a major target of human antibodies. The Flavivirus E protein contains immunodominant antibody epitopes that are conserved (cross-reactive) between different flaviviruses or unique to each virus (type-specific) (10–12).

Traditional Flavivirus serologic assays exhibit poor specificity in distinguishing DENV from ZIKV infections because these assays use whole virions or E proteins containing conserved epitopes as antigens (13–15). More recently, the ZIKV epidemic in the Americas spurred the development of recombinant viral antigens and serologic assays for distinguishing ZIKV from DENV (16–18). We previously described a serologic assay using domain III of the ZIKV E protein.
protein (EDIII) to detect ZIKV type-specific antibodies among persons in areas with DENV and ZIKV co-circulation (19). Here we describe development of a second-generation ZIKV EDIII-based serologic assay and its use to measure the seroprevalence of ZIKV among children 9–14 years of age in the Cebu Province of the Philippines.

Materials and Methods

ZIKV EDIII Antigen Production

We expressed a codon-optimized gene encoding for EDIII from ZIKV strain H/PF/2013 in Expi293 cells as a fusion protein containing a human serum albumin signal peptide for secretion, a polyhistidine tag (his-tag) for affinity purification, and a HaloTag (Promega, https://www.promega.com) for biotinylation (20). We deposited the nucleotide sequence of the construct into Genbank (accession no. MZ592925). HaloTag enables single, site-specific biotinylation distant from the folded EDIII protein. We purified recombinant EDIII antigen from the culture supernatant using nickel-nitrilotriacetic acid agarose (QIAGEN, https://www.qiagen.com) and biotinylated it using HaloTag PEG biotin ligand (Promega), according to manufacturer protocol. We analyzed the identity and purity of the biotinylated EDIII antigen using SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) mobility-shift analysis.

ZIKV EDIII ELISA

We coated a 96-well high binding microtiter plate (Greiner Bio-One, https://www.gbo.com) with 50 µL of streptavidin at 4 µg/mL in tris-buffered saline (TBS, pH 7.4) for 1 h at 37°C. We captured the biotinylated EDIII at 2 µg/mL in TBS, washed the plate 3 times with wash buffer (TBS containing 0.2% Tween 20), and then blocked it with 100 µL of blocking solution (3% milk in TBS containing 0.05% Tween 20) for 1 h at 37°C. After removing the blocking solution, we added 50 µL of heat-inactivated (56°C for 30 min) serum sample at 1:20 or indicated dilutions in blocking buffer and incubated for 1 hour at 37°C. After washing the plate in the wash buffer, we added 50 µL of alkaline phosphatase-conjugated secondary goat anti-human secondary IgG (Sigma) at 1:2,500 dilution for 1 hour at 37°C. We washed the plate, added 50 µL of p-nitrophenyl phosphate substrate (Sigma, https://www.sigmaaldrich.com), and measured absorbance at 405 nm using an Epoch plate reader (Biotek, https://www.biotek.com). For analyzing the 547 serum samples from the Cebu cohort using ELISA assays performed over several days, we used human monoclonal antibody ZKA190 (21), which binds to highly accessible regions of ZIKV EDIII as a control to standardize EDIII ELISA optical density (OD) values across assays. Each plate was developed until wells with ZKA190 generated an OD value within a 1.0–1.3 range. If the signal was outside this range, we considered the assay invalid and repeated the process. We divided all OD values for human samples by the ZKA190 OD value on the same plate before determining the ZIKV-immune status of each participant (>0.34 cutoff).

Human Serum Panel Used to Validate the EDIII Assay

To validate the EDIII assay as described elsewhere (19), we used a panel of 142 archived convalescent serum samples from 15 participants who received a licensed Flavivirus (yellow fever/Japanese encephalitis virus) vaccine, 27 serum samples from Flavivirus-naive participants, 33 from participants with immunity to ZIKV (including some with both ZIKV and DENV immunity), and 67 from participants with DENV immunity but no immunity to ZIKV. The DENV- and ZIKV-immunity status of convalescent specimens in the panel was based on participants in febrile illness study cohorts in Nicaragua and Sri Lanka with laboratory-confirmed acute DENV or ZIKV infections or tests for the presence of neutralizing DENV or ZIKV antibodies in single serum samples from healthy persons. We designated samples with no detectable neutralizing DENV and ZIKV antibodies or 50% plaque reduction neutralization test (PRNT50) results <10 as naive. Collection, storage, and use of these convalescent serum samples for research was approved by the Institutional Review Board of the University of North Carolina at Chapel Hill (protocol 08–0895).

Centers for Disease Control and Prevention ZIKV Persistence Samples

The Centers for Disease Control and Prevention (CDC) and the Ponce Medical School Foundation monitored DENV and ZIKV patients seeking treatment at 2 hospitals in southern Puerto Rico. During triage, we identified case-patients with ≥1 signs or symptoms: fever (temperature ≥38.0°C or ≥100.5°F) or reporting fever lasting ≥7 days, rash, arthralgia or arthritis, or conjunctivitis and offered them participation in the study. We identified ZIKV cases through test results from the CDC Dengue Branch laboratory in San Juan, Puerto Rico, where reverse transcription (RT) PCR testing for DENV, ZIKV, CHIKV, and other respiratory infectious diseases is performed and
followed ZIKV cases longitudinally as described elsewhere (22). For our study, we selected and prospectively followed 27 ZIKV RT-PCR-positive cases for up to 2.5 years. Specimen collection was approved by institutional review boards at CDC and Ponce Medical School Foundation.

**Pediatric Samples from Cebu Province**

We published the Cebu study protocol and approvals elsewhere (23). In brief, our study used baseline serum samples that were collected from 2,996 children 9–14 years of age enrolled in a postlicensure DENV vaccine study. Cohort residence was equally split between Bogo and Balamban, both semiurban areas. We collected samples and demographic information before participants were vaccinated at the same visit.

**DENV and ZIKV Focus Reduction Neutralization Test**

We determined neutralization titers against DENV and ZIKV by focus-reduction neutralization test (FRNT) in a 96-well format described elsewhere (24). Serially diluted serum was mixed with 50–100 foci-forming units of the virus in Dulbecco modified Eagle medium with 2% fetal bovine serum. We incubated the antibody and virus complexes (1 h, 37°C), then transferred them to a monolayer of Vero-81 cells for infection. After 1 additional hour of incubating antibody and virus complex on Vero-81 monolayer, we overlaid cells with GIBCO Opti-MEM (https://www.thermofisher.com), a modified Eagle medium containing 2% fetal bovine serum and 1% carboxymethylcellulose. After allowing the predetermined time required to form viral foci, we fixed Vero-81 cells and immunostained them with Flavivirus-specific antibody 1M7 to Tosyl-activated Dynabeads (Thermo Fisher) magnetic beads and incubated purified DENV-1–4 viral antigens (1 h at 37°C) with the beads. To deplete DENV-specific antibodies, we incubated serum samples (3 h at 37°C) with DENV-conjugated Dynabeads and incubated serum samples with Dynabeads conjugated to an equivalent bovine serum albumin concentration. We confirmed cross-reactive and DENV antibody depletion using whole virion capture ELISA against DENV-1–4. We measured serotype-specific ZIKV antibodies in serum samples using ZIKV whole-virion capture ELISA after depletion, as described elsewhere (24).

**Receiver Operator Characteristic Analysis**

We used SPSS Statistics for Macintosh version 27.0 (https://www.ibm.com) to report the performance of the EDIII ELISA based on the receiver operating characteristic (ROC) curve, which presents test performance as true-positive (sensitivity) versus false-positive (1 − specificity). We calculated the optimal assay cutoff value, which maximizes sensitivity and specificity, from the ROC curve using the same software. According to the test, sensitivity is the fraction of total confirmed positive samples with true positives, and specificity is total confirmed negative samples with true negatives.

**Results**

**Development of Immunobios assay for Detecting ZIKV Antibodies in Patient Serum**

We previously demonstrated that a serologic assay using the ZIKV EDIII fused to maltose-binding protein (MBP) reliably differentiated persons with past ZIKV infections from those with DENV infections (19). However, we observed a high background signal in some specimens, originating from human antibodies binding to MBP or the mouse antigen capture antibody used in the assay. To overcome this problem in the earlier version of the assay, we produced the ZIKV EDIII antigen fused to a HaloTag (Figure 1, panel A), derived from the haloalkane dehalogenase enzyme from Rhodococcus rhodochrous bacteria (26). By using a biotin HaloTag ligand (27), we added a single biotin molecule for each protein molecule at a site distant from the EDIII antigen (Figure 1, panel B). Using streptavidin-coated ELISA plates to capture the antigen, we established an assay for detecting ZIKV EDIII–binding antibodies in human clinical samples (Figure 1, panel C).

**Performance of the Second-Generation ZIKV EDIII ELISA**

We performed an ROC analysis to determine the diagnostic performance of the new EDIII-capture
ELISA in a panel of serum samples collected >12 weeks after symptom onset among persons with laboratory-confirmed ZIKV or DENV infections or who received a licensed Flavivirus vaccine. The panel included serum samples from DENV-naive and DENV-immune participants who received positive ZIKV RT-PCR test results, archived serum samples from persons who had primary (only 1 serotype) or secondary (>2 serotypes) DENV infections, control samples from persons who had not experienced DENV or ZIKV infections, and samples from travelers who had received Japanese encephalitis virus vaccine or yellow fever vaccine virus or both. The EDIII assay was positive for 32 of 33 ZIKV-immune, 7 of 67 DENV-immune, and 2 of 42 DENV- and ZIKV-naive or yellow fever/Japanese encephalitis virus vaccine recipients (Figure 2, panel A). The ROC analysis demonstrated an area under the curve of 0.97 (95% CI of 0.94–0.99) (Figure 2, panel B). At a cutoff value of OD 0.34, the sensitivity of the EDIII-capture ELISA was 97% and the specificity was 92%.

**Durability of ZIKV EDIII Antibodies in Persons Exposed to ZIKV Infections**

Next, we assessed the durability of EDIII antibodies using a panel of 98 longitudinal samples collected 1–600 days after symptom onset from 27 residents of Puerto Rico with PCR-confirmed ZIKV infections. By testing the acute specimens for DENV-specific antibodies, we stratified the DENV-immune status at the time of ZIKV infection as 12 DENV-naive and 15 DENV-immune cases. We measured endpoint titers at each time point to follow the kinetics of serum ZIKV EDIII antibodies (Figure 3). The ZIKV EDIII antibody titers reached peak levels by 1 month after initial infection and stayed well above detection level for ≥20 months in DENV-naive and DENV-immune participants.

**Seroprevalence of ZIKV in Cebu Province**

Having established the performance of the ZIKV EDIII ELISA among participants exposed to multiple DENV serotypes, we used the assay to estimate the seroprevalence of ZIKV among children living in Balamban and Bogo City in Cebu Province. In 2017, we collected baseline blood samples from a DENV vaccine study of 2,996 children 9–14 years of age. Elsewhere we reported that 89.3% of the children were DENV-immune at baseline, demonstrating the high endemicity of the virus in this population (23). We selected a representative sample of 547 children on the basis of DENV-immune status from the baseline cohort to determine the seroprevalence of ZIKV (Table 1). The children selected for the study

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**Figure 1. ZIKV Biotinylated-EDIII antigen capture ELISA in study of novel assay to measure ZIKV seroprevalence in the Philippines.** A) Schematic of the ZIKV EDIII construct with an N-terminal human albumin secretion signal, a HaloTag (Promega, https://www.promega.com) for site-specific biotinylation and a C-terminal 6-histidine residue tag for affinity purification. The EDIII construct design also included a TEV protease cleavage site between HaloTag and EDIII. B) Biotinylated ZIKV EDIII displays an electrophoretic mobility shift with streptavidin. A site-specific biotinylated ZIKV EDIII was prepared using HaloTag biotin ligand. Electrophoretic gel shift analysis was performed in sodium dodecyl sulphate-polyacrylamide gel electrophoresis with biotinylated EDIII antigen in the presence and absence of streptavidin. C) Schematic of second-generation ZIKV EDIII ELISA using streptavidin-biotin interaction. Biotinylated EDIII antigen is captured by plate immobilized streptavidin. The antibody bound to EDIII is detected by a secondary anti-human IgG conjugated to alkaline phosphatase. EDIII, E protein domain III; IgG-AP, secondary IgG antibody conjugated to alkaline phosphatase; SA, streptavidin; SP, N terminal human albumin secretion signal; TEV, tobacco etch virus protease cleavage site; ZIKV, Zika virus.
Seroprevalence of Zika Virus, the Philippines consisted of 60 (11%) with no immunity to DENV, 43 (8%) with immunity to 1 DENV serotype, and 444 (81%) with immunity to ≥2 serotypes (Table 1; Figure 4, panel B) (23). We observed that 98/547 (18%) children were ZIKV antibody positive in the ZIKV EDIII assay (Figure 4, panel A). The number of children who tested positive or negative for ZIKV antibodies did not differ by sex, location, or prior DENV-immune status (Table 1).

Comparison of ZIKV EDIII ELISA and Virus Neutralizing Antibody Assays

The current standard for estimating the seroprevalence of a particular flavivirus is based on measuring virus neutralizing antibodies in cell culture systems. Although neutralizing antibody assays are more specific than assays that measure antibody binding to flaviviruses or whole recombinant proteins, persons exposed to multiple DENV serotypes can develop ZIKV cross-neutralizing antibodies as reported elsewhere (10,24,28,29). Given the large number of children in our cohort with a history of ≥2 DENV serotype infections, we compared the performance for estimating ZIKV seroprevalence of the EDIII ELISA using FRNT.

From the 547 baseline samples tested by ZIKV EDIII assay, we tested 495 samples in a single dilution (1:40) ZIKV neutralization assay. We have demonstrated elsewhere that at 1:40 dilution, neutralization of ≥70% of the input virus is a reliable screening criterion for previous DENV infection (23). When we used the same criterion for the single-dilution ZIKV neutralization assay, we observed a much higher seroprevalence (39%) compared with the estimate (18%) based on the EDIII ELISA (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/27/12/21-1150-App1.pdf). Most of the discordant samples (positive in the ZIKV neutralization assay and negative in the ZIKV EDIII assay) were from children with preexisting multitypic immunity to DENVs (105/110 participants) (Appendix Table 1), raising the possibility that high levels of DENV antibodies cross-neutralize ZIKV.

To evaluate whether the discordant results were because of the poor specificity of the ZIKV neutralization assay or poor sensitivity of the ZIKV EDIII ELISA, we selected 24 samples that had been classified by full curve neutralization testing against the 4 DENV serotypes and ZIKV as primary DENV-immune only (n = 3), primary ZIKV-immune only (n = 4), both primary DENV- and primary ZIKV-immune (n = 6), multitypic DENV-immune only (n = 2), or both multitypic DENV- and ZIKV-immune (n = 9) participants (Appendix Table 2, Figure 2) for further study. By selectively removing all antibodies to DENVs in a sample before testing for ZIKV binding antibodies, it is possible to detect ZIKV type-specific antibodies indicative of a past ZIKV infection (11). We incubated all 24 samples with magnetic beads coated with the 4 DENV serotypes to remove all DENV-binding antibodies. After

Figure 2. Performance evaluation of ZIKV EDIII assay in study of novel assay to measure ZIKV seroprevalence in the Philippines. Shown are the binding (A) and ROC (B) curve analysis of ZIKV EDIII ELISA using human convalescent serum samples. A panel of convalescent serum samples collected >12 weeks after onset of symptoms from primary and secondary ZIKV infections (n = 33), primary and multitypic DENV infections (n = 67), and serum samples collected >12 weeks after vaccination with a licensed Flavivirus vaccine or serum samples from Flavivirus-naïve participants (n = 42) were tested by ZIKV EDIII ELISA. ROC demonstrated 0.966 (95% CI 0.94–0.99) area under the curve. The sensitivity of the EDIII capture ELISA was 97% (32/33) and the specificity 92% (100/109) at a cutoff value of 0.34. Red line indicates a random classifier and represents data points with equal true-positive rate and false-positive rate. The blue line is the ROC curve showing high performance of the ZIKV EDIII assay because the blue line is above and further away from a random classifier. DENV, dengue virus; EDIII, E protein domain III; OD_{405}, optical density at 405 nm; ROC, receiver operating characteristic; ZIKV, Zika virus.
confirming removal of all DENV-binding antibodies, we tested the samples for ZIKV-binding antibodies. After depleting DENV-binding antibodies, we observed ZIKV-binding antibodies for the participants designated as immune to primary Zika only and primary DENV and primary ZIKV (Appendix Table 2, Figure 1, panel A). In contrast, 6/9 participants designated by neutralization testing as multitypic DENV- and ZIKV-immune showed no ZIKV-binding antibodies (Appendix Figure 1, panel A). All participants that retained ZIKV-binding antibodies after DENV-binding antibody depletion also tested positive in the ZIKV EDIII ELISA and all participants with no detectable ZIKV-specific antibodies tested negative in the EDIII assay (Appendix Table 2, Figure 1, panel B). These results suggest that the ZIKV EDIII ELISA is more reliable than neutralization testing for estimating ZIKV seroprevalence independent of DENV status.

Discussion
The 2015 ZIKV pandemic in the Americas mostly affected communities with high DENV endemicity because these viruses share the same mosquito vector. Efforts to monitor the spread of ZIKV and the effect of cross-reactive immunity on viral pathogenesis were severely hampered by the inability of conventional serologic assays to accurately distinguish DENV from ZIKV seropositivity. Several groups developed ZIKV recombinant antigen–based assays or antigen-antibody competition assays to improve the specificity of serologic assays (16–18).

In a previous study, we documented development of an ELISA to detect antibodies in persons who had recovered from ZIKV infections, using ZIKV EDIII fused to Escherichia coli MBP as an antigen (19). We observed in some persons high background levels of antibodies to the MBP fusion protein alone, most likely from natural exposure to bacterial proteins, highlighting the need for modifying the fusion
protein used for antigen production. We describe a ZIKV EDIII antigen improved by replacing the MBP fusion tag with a HaloTag amenable to site-specific biotinylation. Using streptavidin-biotin chemistry to capture the antigen, we developed an ELISA with 97% sensitivity and 92% specificity for detecting past ZIKV infections.

A strength of our study was the use of serum samples from participants living in Flavivirus-endemic regions with well-defined exposures to DENVs or ZIKV or both to determine the performance of the assay when testing specimens with high levels of cross-reactive antibodies. A potential problem with using modular domains such as EDIII instead of the full-length envelope protein is a loss in assay sensitivity over time. However, our analysis of the longitudinal samples from patients with documented ZIKV infection demonstrated that ZIKV EDIII antibodies robustly developed and remained at high levels even after 2.7 years. This finding suggests that serologic assays based on EDIII will have the necessary sensitivity to characterize past ZIKV circulation at individual and population levels.

The severity of the epidemic in the Americas renewed interest in the epidemiology and pathogenesis of ZIKV in Asia. Asian lineages of the virus have been circulating for decades if not longer. The virus has been detected by molecular methods or virus isolation by cell culture in many countries, including Thailand, the Philippines, Vietnam, Malaysia, Cambodia, and India (30–35). One study of 600 migrant workers in Taiwan (predominantly from Indonesia, the Philippines, Thailand, and Vietnam) reported 37% seroprevalence using a ZIKV IgG assay (36,37). A similar study of Taiwan residents (n = 212) identified 4.2% of all participants with ZIKV IgG but confirmed only 1 participant by neutralization test (36). Investigators tested a cohort of children 1–4 years of age (n = 662) in Indonesia and estimated 9.1% seroprevalence using 90% neutralization as a cutoff value (38). A 2017 study of healthy adults (n = 801) in Vietnam estimated 1.1% seroprevalence using a ZIKV neutralization assay (39). An additional study in Guangxi Province, China, in March 2019 found 6% of 273 participants bound to ZIKV NS1 using IgG ELISA testing and neutralized ZIKV in cell cultures (40). Those past studies, especially those relying on tests of whole Zika virions and full-length antigens, had poor specificity, mainly because of antibodies induced by DENV infections cross-reacting with ZIKV antigens. In our study, we used a recombinant ZIKV antigen with a high specificity for distinguishing ZIKV-induced from DENV-induced antibodies; results showed that 18% of children 9–14 years of age living in Cebu Province had previously experienced a ZIKV infection. Although ZIKV is currently not considered by public health agencies to be a common infection in the Philippines, our results indicate otherwise.

Our results also highlight a problem with using accepted standards for neutralizing antibody testing to identify ZIKV infections in populations heavily exposed to DENVs. We observed ZIKV neutralizing antibodies in 39% of the children in this study, which was more than double the estimate based on the EDIII assay results. By depleting all DENV-binding antibodies from a subset of samples and then measuring ZIKV antibodies, we demonstrated that some children exposed to ≥2 DENV serotypes have low levels of ZIKV cross-neutralizing antibodies that can lead to false-positive results. In contrast, the ZIKV EDIII assay was not impacted by DENV-immune status because, even after removing all DENV-binding antibodies, the children maintained ZIKV EDIII–binding antibodies.

The novel assay we describe, which uses a biotinylated EDIII antigen to detect ZIKV type-specific antibodies, is simple and readily adaptable for use in standard clinical and public health laboratories to monitor ZIKV transmission at both the individual and population levels. Given that the 2015 ZIKV epidemic spread to >40 countries and infected more than a million people, many in regions with endemic transmission of DENVs, and that DENVs demonstrate cross-reactive immunity with ZIKV, the improved specificity of this assay provides a necessary resource for accurately monitoring ZIKV transmission in regions with high DENV endemicity.

<table>
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<tr>
<th>Table. Demographics of study cohort and number of participants with ZIKV EDIII type-specific antibody in study of novel assay to measure ZIKV seroprevalence in the Philippines</th>
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*Association between ZIKV serostatus and sex or location measured by Fisher exact test. Association between ZIKV serostatus and DENV-type serostatus measured by χ² test.
Acknowledgments
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About the Author
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References


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A quaculture is the fastest growing sector in food production worldwide (1); innovative intensive production technologies, such as recirculating aquaculture systems (RAS), are becoming increasingly valuable for commercial fish production (2). Attempts are continually made to introduce new types of fish into aquaculture, to reduce overfishing of wild fish populations, and to satisfy the progressing consumer demand for diverse supply (3). One example is the adaptation of European perch (Perca fluviatilis; order Perciformes, family Percidae) to farming and the growing consumer interest in this fish (4). European perch are actinopterygiids that naturally inhabit slow-flowing rivers, lakes, or ponds in Europe and northern Asia. During the 19th century, they were introduced into Australia as angling fish, where they are now considered invasive, competing with native fish for food and space, preying on other fish, and breeding to overpopulation (5).

Although little is known about diseases affecting European perch in the wild, infectious diseases lead to high mortality among these fish in aquaculture, making farming of these fish economically challenging. One of the main threats is infection with perch rhabdovirus (PRV; family Rhabdoviridae, genus Perhabdovirus), leading to the central nervous system (CNS) signs of loss of equilibrium and aberrant swimming behavior and to higher mortality (2,6–10). Lack of investigation of the occurrence and diversity of other pathogenic virus infections of European perch that result in disease impairs the treatment, control, and prevention of disease outbreaks in farm populations. To address this knowledge gap, we applied metatranscriptomics, including metatranscriptomics (virus discovery by high-throughput RNA sequencing and bioinformatics ), to a set of samples collected from sick juvenile European perch in a perch farm in Switzerland in 2017. Although we did not find PRV in these fish, our investigation led to the discovery of 5 novel negative-sense RNA viruses, belonging to the negarnaviricot families Filoviridae, Hantaviridae, and Rhabdoviridae, that could possibly contribute to disease development.

Methods

European Perch Origin

The European perch used in this study were raised in a private pond in Saxony, Germany, and were exported to a farm that uses RAS in Bernese Oberland,
Switzerland, at 16 g (≈11,200 fish) and 33 g (≈4,800 fish). Eleven live juvenile European perch were sent to the Centre for Fish and Wildlife Health (FIWI), University of Bern (Bern, Switzerland), where they were euthanized and subjected to microbiological (including parasitologic, bacteriologic, mycologic, and virologic) and pathologic (including histopathologic) examination. Because the remaining fish at the farm exhibited clinical signs of disease, they were kept in quarantine for an additional 2 months and subsequently euthanized.

**Cell Culture**

We used bluegill (*Lepomis macrochirus*) fry cells (BF-2) and fathead minnow (*Pimephales promelas*) epithelioma papulosum cyprini (EPC) cells. These cells were originally obtained from the Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health (Greifswald-Insel Riems, Germany; Collection of Cell Lines in Veterinary Medicine, catalog nos. CCLV-RIE 290 and CCLV-RIE 173 are maintained at FIWI).

We inoculated the cells with small pieces of pooled CNS or spleen, kidney, heart, and pyloric ceca tissue from 5 of the 11 euthanized European perch (in total <5 g) and incubated at 15°C. We monitored the cell cultures for CPE daily for 7 days by using light microscopy and then monitored subcultures for another 7 days. We harvested supernatants from cell cultures showing CPE and tested by reverse transcription PCR (RT-PCR) and subjected the amplicons to Sanger sequencing (Appendix). We performed 3′ and 5′ rapid amplification of cDNA ends (RACE), as described previously, on RNA extracted from pooled organs and CNS tissue as well as cell culture supernatants (13) (Appendix).

**Results**

During a complete necropsy of the 11 fish, we used 3 whole perch for histologic examination. We fixed these fish in 10% buffered formalin for 24 hours and embedded cut sections of the gills, longitudinal head sections, and longitudinal cut sections of the body cavity in paraffin. We prepared 3-µm sections and stained them with hematoxylin and eosin according to standard protocols. We conducted chromogenic in situ hybridization on all formalin-fixed paraffin-embedded (FFPE) tissues used for histopathology. We performed staining with the RNAscope system (Advanced Cell Diagnostics, https://acdbio.com) (Appendix).
anorexia, lethargy, skin ulcerations, multifocal hemorrhages, and eroded tail fins. Culture and PCR indicated that skin ulcerations were caused by oomycete (Saprolegnia parasitica) infection (21). Histopathology revealed mild to moderate gill epithelial proliferation and epithelial cell hypertrophy. Additional findings included necrotizing dermatitis with hemorrhage and intralymphatic oomycete hyphi and bacterial colonies. The newly arrived fish were treated with flubendazole, formalin, and peracetic acid, but death increased to 1.8%–4.2% per day. Two months after importation, deaths for the quarantined fish reached 22%–27% in total. All remaining fish were euthanized and discarded, thereby preventing these fish from entering the food chain.

Virus Isolation

For routine virus investigation, we exposed standard fish cell cultures (BF-2 and EPC) to suspensions of pooled perch CNS and pooled visceral organs. We selected these cell lines because of their high susceptibility to diverse fish viruses (22). CPE developed 10 days after inoculation of CNS suspension into BF-2 cells and 13 days after inoculation into EPC cells. Affected cell supernatants were harvested and tested preliminarily positive for PRV infection by RT-PCR. The sequence of the detected amplicon was, however, only 78% identical to the sequence of the perhabdovirus lake trout rhabdovirus (LTRV; GenBank accession no. AF434991), indicating the presence of a perhabdovirus distinct from PRV/LTRV.

Novel Perhabdovirus

To further characterize the putative novel perhabdovirus, we performed metatranscriptomics by using HTS and bioinformatic analysis of pooled CNS tissue and visceral organ RNA extracts of 5 fish. We found 16 sequence scaffolds 247–1,454-nt long with mean k-mer coverages of 1.0–5.9 and nucleotide sequence identities of 62%–98% to perhabdovirus genomes (Appendix Table 1). We mapped these scaffolds to LTRV (GenBank accession no. AF434991) and PRV (GenBank accession no. JX679246) as references and closed sequence gaps by RT-PCR and RACE followed by Sanger sequencing to bridge sequence gaps. This effort resulted in 3 long scaffolds of 13,764 nt (k-mer coverage 15), 14,593 nt (k-mer coverage 15), and 13,066 nt (k-mer coverage 5), corresponding to 3 novel viruses.

Four Novel Filoviruses

In addition to scaffolds that ultimately led us to identify EGLV, we found 41 scaffolds 240–4,726-nt long with low k-mer coverage (0.8–4.8×). The deduced amino acid sequences were 28%–30% identical to proteins of Huángjiāo virus (HUJV), a recently identified virus of marine greenfin horse–faced filefish (Thamnaconus septentrionalis) captured in the East China Sea (23) (Appendix Table 1). Alignment of these scaffolds to the HUJV genome (GenBank accession no. MG599981) and the deduced amino acid sequences to those of HUJV-encoded proteins revealed a complex scenario suggesting the presence of several distinct thannonoviral genomes, however with numerous gaps between the scaffolds. To obtain complete or coding-complete viral genome sequences, we resequenced the HTS library to generate ≥10 times more paired-end reads (2,051,046,671) than during the initial HTS run, reassambled the sequences, and performed RT-PCR and Sanger sequencing to bridge sequence gaps. This effort resulted in 3 long scaffolds of 13,764 nt (k-mer coverage 24×), 14,593 nt (k-mer coverage 15×), and 13,066 nt (k-mer coverage 5×), corresponding to 3 novel viruses.

We named these viruses Fiwi virus (FIWIV; GenBank accession no. MNS10772), after FIWI; Oberland virus (OBLV; GenBank accession no. MNS10773), after Bernese Oberland; and Kander virus (KNDV;
Viruses in Freshwater Fish, Switzerland

GenBank accession no. MW093492), after the Kander River, which flows through Bernese Oberland. Whereas the FIWIV genome appears to be coding complete, the sequences of OBLV are coding incomplete at the 5′ terminus and of KNDV at both the 5′ and the 3′ termini. All attempts to determine the authentic 3′ and 5′ termini by RACE were unsuccessful, most likely because of low viral RNA loads. However, all 3 sequences have the genomic features of HUJV, encoding the filovirus-typical proteins nucleoprotein (NP), polymerase cofactor (VP35), glycoprotein (GP 1,2), transcriptional activator (VP30), and large protein (L) containing an RNA-directed RNA polymerase (RdRp) domain, as well as 1–2 novel proteins (14,23,24) (Figure 2, panel A). Phylogenetic comparison of the FIWIV, OBLV, and KNDV genomic sequences (Figure 2, panel B) and L gene sequences with those of representative classified viruses of the family Filoviridae (Figure 2, panel C) confirmed the genetic relationship of all 3 viruses to HUJV. The current demarcation criteria for filovirus sequence-based genus and species are ≥55% and ≥23% sequence divergence over complete genome sequences determined by using pairwise sequence comparison (14,15). We found a pairwise divergence of 49% compared with HUJV by using the FIWIV genome sequence (Table), indicating that FIWIV is a member of a new thamnovirus species (“Thamnovirus percae”). The available KNDV genome sequence is 49% divergent from HUJV and 37% divergent from FIWIV (Table), suggesting that KNDV represents yet another novel thamnovirus species (“Thamnovirus kanderense”). In contrast, OBLV was >62% divergent from HUJV, FIWIV, and KNDV viruses and thus represents a new species (“Oblavirus percae”) within a new genus (“Oblavirus”). All attempts to detect FIWIV or OBLV viruses in 3 European perch FFPE tissue sections were unsuccessful.

In addition to FIWIV, OBLV, and KNDV, we found 4 shorter scaffolds (573–3,259 nt; Appendix Figure 2) similar to various HUJV genes, but attempts to demonstrate a physical linkage of these sequences by RT-PCR were not successful. Still, the
presence of these scaffolds is indicative of at least 1 additional novel filovirus, which we were not able to further characterize.

**Novel Hantavirus**

In the same RNA extract of pooled organs, we found 3 scaffolds, of which the deduced amino acid sequences
were 25%–35% similar to those of the large (L), medium (M), and small (S) segments of Wēnling minipizza batfish virus (WEMBV) and Wēnling red spikefish virus (WERSV). WEMBV was recently identified in apparently healthy minipizza batfish (*Halietuana stellata*) and WERSV in red spikefish (*Triacanthodes anomalus*) captured in the East China Sea (Appendix Table 1) (23). The mean k-mer coverage of these scaffolds ranged from 3 × 10^3 to 1.5 × 10^4, indicating a high viral RNA load. Using Sanger sequencing and RACE, we determined the complete sequences of the genomic L (GenBank accession no. MN510769), M (GenBank accession no. MN510770), and S (GenBank accession no. MN510771) segments of a novel virus, here named Bern perch virus (BPV). Similar to WEMBV and WERSV, the Bern perch virus L segment (6,372 nt) was deduced to encode the L protein including an RdRp domain, the M segment (3,804 nt) was deduced to encode the glycoprotein precursor, and the S segment (2,435 nt) was deduced to encode the nucleocapsid protein (Figure 3, panel A). The S and M segments contain 2 additional ORFs encoding putative proteins not represented in current protein databases in antisense (S segment) and sense (M segment) orientation. Alignment of the 3' and 5' sequences of all 3 segments revealed that the 8 terminal nucleotides are complementary within and conserved among segments (Figure 3, panel B), a known feature of members of the order *Bunyavirales* (25). However, these terminal sequences differ from those of members of the genus *Orthohantavirus* and are similar to those of members of the genus *Orthobunyavirus* (Appendix Table 1). Phylogenetic analysis of the L protein confirmed the close relationship of Bern perch virus to all currently classified actinoviruses (Figure 3, panel C; Appendix Figure 3) but indicated the need for a novel species to accommodate this virus. This need was confirmed by DEmARC (Diversity Partitioning by Hierarchical Clustering) analysis (26); on the basis of this evidence, the ICTV officially established this species as *Perch actinovirus* in 2021 (27,28).

Using in situ hybridization on FFPE tissue sections, we were able to detect BPV genomic RNA in gills with histopathologic lesions of 2 fish (Figure 4, panels A, B; Appendix Figure 4) and in a granuloma in the perivisceral fat tissue of 1 of these animals. Morphologically, we identified the affected cells in the gills and the perivisceral fat tissue as putative macrophages. In addition, putative endothelial cells were labeled positively in the gills (Figure 4, panel B).

**Discussion**

The diversity of fish viruses, in particular that of RNA viruses, remains poorly understood (29). Recent initial studies indicate that this diversity is enormous and that many viral taxa that have been established for pathogens of humans and other mammals need to be redefined (23,30–32). Husbandry conditions in huge tanks used on farms may favor the emergence and rapid intraspecies and interspecies transmission of fish viruses, potentially resulting in high economic loss for the fish industry. Also of concern is the introduction of novel viruses from fish farms into native fauna, which could have disastrous ecologic consequences. In addition, viruses of unknown pathogenicity in food animals may have zoonotic potential of yet unpredictable importance.

In this study, we identified 1 novel rhabdovirus, 4 novel filoviruses (3 confirmed and 1 likely), and 1 novel hantavirus in morbid farmed European perch. The discovery of a novel rhabdovirus was not surprising and adds to the role of rhabdoviruses in fish health; rhabdoviruses, in particular those of the genera *Novirhabdovirus*, *Perhabdovirus*, *Sprivivirus*, and *Vesiculovirus*, are notorious marine and freshwater fish pathogens, causing diseases characterized by high lethality (33,34). Thus, these viruses pose a considerable threat to aquaculture. The European perch examined in this study exhibited signs compatible with rhabdovirus infection (7). Using HTS and exposing cell cultures to CNS tissue suspensions, we discovered a novel perhabdovirus, Egli virus. Although the viral RNA loads in tissues were low and we could not detect RNA by in situ hybridization in tissue sections, we were able to isolate the virus from brain tissue. Rhabdoviruses in perch are usually associated with disease and not known as commensals. The host range of Egli virus is unknown, but genetically it is more closely related to viruses identified in trout than to those infecting perch, suggesting the possibility of cross-species transmission, highlighting a concern for farms that raise fish other than perch and for native fish populations. Discovery of a lake trout rhabdovirus

**Table.** Pairwise distances of complete or coding-complete genome nucleotide sequences between the newly identified Fiwi virus, Huángjiāo virus, and the closest related mammalian filovirus, Bombali virus*

<table>
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<tr>
<th>Virus</th>
<th>Fiwi</th>
<th>Oberland</th>
<th>Kander</th>
<th>Huángjiāo</th>
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<tbody>
<tr>
<td>Oberland</td>
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*Fiwi virus, GenBank accession no. MN510772; Oberland virus, GenBank accession no. MN510773; Kander virus, GenBank accession no. MW093492; Bombali virus, GenBank accession no. MK340750. Oberland and Kander virus sequences are not coding complete and were compared on the basis of the available incomplete sequences.
as a probable cause of disease in European perch in Ireland (7) supports the hypothesis of high potential for interspecies transmission of these viruses.

Unexpectedly, we were able to assemble near-complete viral genomes of 3 novel filoviruses (Fiwi, Oberland, and Kander viruses) and detected a likely fourth filovirus in the diseased perch. Until recently, filoviruses, notorious for causing disease in humans with extremely high lethality (35), were thought to exclusively infect mammals. This view changed with the discovery of Xīlǎng virus (the only member of genus Striavirus) in striated frogfish (Antennarius striatus) and HUJV in greenfin horse-faced filefish captured in the East China Sea (23) as well as apparent thamnoviruses John Dory filovirus in John Dory (Zeus faber) and blue spotted goatfish filovirus in blue spotted goatfish (Upeneichthys lineatus) purchased at a fish market in Sydney, New

Figure 3. Identifying a novel hantavirus in European perch. A) Schematic representation of the 3 genome segments of Bern perch virus; open reading frames are indicated as colored arrows. Coverage plots of high-throughput sequencing reads are shown for each segment, and maximum-read coverages are indicated on the right. B) Alignment of the terminal sequences (11 nt) of the 3 segments. The terminal 8 nucleotides (gray box) are complementary within and conserved among segments. C) Maximum-likelihood phylogenetic tree of the Bern perch virus RNA-directed RNA-polymerase amino-acid sequence (bold blue) with RNA-directed RNA-polymerase amino-acid sequences of representative members of the family Hantaviridae. Numbers near nodes on the trees indicate bootstrap values. Branches are labeled by GenBank accession number, and virus name. Scale bar indicates number of substitutions per site, reflected by branch lengths. GP, glycoprotein gene; L, large; M, medium; NP, nucleocapsid protein gene; S, small.
Viruses in Freshwater Fish, Switzerland

South Wales, Australia (31). However, currently, <300 nt contigs are known from John Dory fish and blue spotted goatfish filoviruses; hence, their true taxonomic affiliation remains to be determined. In contrast to Xīlǎng virus, HUJV, John Dory filovirus, and blue spotted goatfish filovirus (all of which were found in marine fish in China and Australia), FIWIV, OBLV, and KNDV apparently infect freshwater fish in Europe. This geographic and ecologic distribution indicates that filoviruses are broadly dispersed fish commensals or potential pathogens that probably number in the hundreds or thousands. Although on the basis of our data we cannot attribute filovirus infection to individual fish, a possible scenario includes co-infection with FIWIV, OBLV, KNDV, EGLV, or BPV or any combination of these viruses. Thamnovirus abundance was low in the sampled European perch, and detection of thamnoviral genomic RNA proved impossible in FFPE tissues. This low abundance, together with the unsuccessful attempt to demonstrate viral RNA in the tissue, suggests that infection in the investigated fish was subclinical rather than the cause of the observed clinical signs.

We also identified a novel hantavirus, BPV. Hantaviruses are best known as rodent-borne viruses of the mammantavirus genus Orthohantavirus, which cause hemorrhagic fever with renal syndrome or hantavirus pulmonary syndrome in humans (36) but have also been found in bats and eulipotyphla. Reptile hantaviruses (family Repantavirinae, genus Reptiliovirus) and fish hantaviruses (family Actantavirinae, genus Actinovirus, and family Agantavirinae, genus Agnathovirus) (32) have only recently been discovered. The fish viruses include the actinoviruses WEMBV, WERSV (23), and Wenling yellow goosefish virus (detected in yellow goosefish [Lophius litulon] captured in China [23]); the likely actinovirus Aronnax virus, found in pygmy goby (Eviota zebrina) purchased at a fish market in Sydney (31); and the agnathovirus Wenling hagfish virus, detected in inshore hagfish (Eptatretus burgeri) captured in China (23). Similar to our filovirus findings, the discovery of BPV is remarkable because this actinovirus was found in freshwater fish from Europe rather than in marine fish from China or Australia. Actinoviruses have not yet been associated with disease in fish. Using in situ hybridization, we demonstrated, however, high concentrations of BPV RNA in macrophages and endothelial cells in the gills as well as in macrophages in the perivisceral fat tissue of morbid European perch. Other cell types tested negative. Human pathogenic orthohantaviruses predominantly infect macrophages and microvascular endothelial cells of a variety of organs, which leads to increased vascular permeability and severe disease (11). It is therefore tempting to speculate that the pathology observed in the gills of the European perch may have resulted in dyspnea, contributing to elevated mortality. In conclusion, our identification of new rhabdoviruses, filoviruses, and hantaviruses in farmed European perch in Switzerland raises concerns about the global distribution, host spectrum, and risks to human and animal health for these viruses.

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HTS raw data from this study have been deposited into the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) as SRK12586223.

About the Author

Dr. Hierweger works as a postdoctoral researcher at the Division of Neurological Sciences, Vetsuisse Faculty, University of Bern. Her main research interest is the discovery of emerging viruses and their involvement in disease.

References

Viruses in Freshwater Fish, Switzerland


27. International Committee on Taxonomy of Viruses [cited 2021 Jul 10]. https://talk.ictvonline.org/taxonomy


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In response to the emergence of Lassa fever, Marburg virus, and Ebola virus in Africa, a Biosafety Level 4 laboratory was constructed at the National Institute for Communicable Diseases (NICD) in Johannesburg, South Africa, and became operational in 1980 (1). To establish which known viral hemorrhagic fevers in Africa occurred in South Africa and neighboring countries, antibody surveys were conducted on selected human, livestock, and wild animal populations. Findings for Crimean-Congo hemorrhagic fever were reported (2,3), but subsequent engagement of the laboratory in the investigation of a series of hemorrhagic fever outbreaks in Africa led to the suspension of survey publication. We present the results of a survey of 5,363 rodents for evidence of infection with mammarenaviruses and details of the isolation of mammarenaviruses from seropositive species. This project was undertaken with approval of the Ethics Committee of the National Institute for Virology, subsequently incorporated into NICD.

Materials and Methods

Viruses, Antigens, Antiserum, and Antibody Tests

We prepared antigen slides to screen for group-specific antibody activity to mammarenaviruses by using Lassa fever and Mopeia virus antigens on serum specimens of 5,363 rodents of 33 species collected in South Africa and Zimbabwe during 1964–1994. Rodents were collected for unrelated purposes or for this study and stored at −70°C. We found antibody to be widely distributed in the 2 countries; antibody was detected in serum specimens of 1.2%–31.8% of 14 species of myomorph rodents, whereas 19 mammarenavirus isolates were obtained from serum specimens and viscera of 4 seropositive species. Phylogenetic analysis on the basis of partial nucleoprotein sequences indicates that 14 isolates from *Mastomys natalensis*, the Natal multimammathe mouse, were Mopeia virus, whereas Merino Walk virus was characterized as a novel virus in a separate study. The remaining 4 isolates from 3 rodent species potentially constitute novel viruses pending full characterization.

We conducted a survey for group-specific indirect immunofluorescence antibody to mammarenaviruses by using Lassa fever and Mopeia virus antigens on serum specimens of 5,363 rodents of 33 species collected in South Africa and Zimbabwe during 1964–1994. Rodents were collected for unrelated purposes or for this study and stored at −70°C. We found antibody to be widely distributed in the 2 countries; antibody was detected in serum specimens of 1.2%–31.8% of 14 species of myomorph rodents, whereas 19 mammarenavirus isolates were obtained from serum specimens and viscera of 4 seropositive species. Phylogenetic analysis on the basis of partial nucleoprotein sequences indicates that 14 isolates from *Mastomys natalensis*, the Natal multimammate mouse, were Mopeia virus, whereas Merino Walk virus was characterized as a novel virus in a separate study. The remaining 4 isolates from 3 rodent species potentially constitute novel viruses pending full characterization.


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USA) or prepared at NICD as described elsewhere for Crimean-Congo hemorrhagic fever virus (Table 2) (6).

**Rodent Samples and Virus Isolation Studies**

Most samples were opportunistically derived from material collected for unrelated surveys and stored at NICD. The initial 213 samples were collected at NICD during 1964–1981 for arbovirus surveys, 3,542 samples were collected and submitted by the Department of Health of South Africa during 1971–1988 for plague surveillance in the central part of the country, 831 rodents (with an emphasis on *Mastomys natalensis* mice) were collected in northeastern South Africa during 1984–1994 specifically for the investigation of mammarenaviruses, 764 rodent samples collected in Zimbabwe in 1974 were remnants of a study on Rift Valley fever virus (7), and 13 samples were collected in 1982 on a farm in south-central Zimbabwe where there had been a suspected but unconfirmed case of viral hemorrhagic fever in a patient admitted to a hospital in South Africa. Live-trapped rodents were euthanized and exsanguinated; serum samples and visceral organ (lung, heart, liver, spleen, and kidney) samples were conveyed to NICD with ice packs and stored at −70°C. Coordinates of sample collection sites were recorded as quarter-degree grid cells.

We confirmed identities of rodent species yielding virus isolates by determining partial cytochrome b gene sequences for 8 selected samples (8). Skull and skin preparations of rodents from plague surveillance were deposited in the Ditsong National Museum of Natural History (Pretoria, South Africa), and selected materials from other surveys were preserved at NICD.

We attempted isolation of mammarenaviruses for rodent species at locations where antibody was found. We inoculated serum and 10% clarified suspensions of pooled viscera onto Vero 76 monolayer cultures in replicate Lab-Tek 8-chamber slides (ThermoFisher Scientific) and examined after incubation for 7–10 days at 37°C by IF with pooled mouse antiserum to MOPV and LASV. We passed samples 3 times before recording them as negative. The 5 original isolates of MOPV from *M. natalensis* rodents from Mozambique were taken to CDC in 1977 (9); we used duplicate organ samples stored at NICD to reisolate the viruses. We screened antigen cell spots prepared from cultures infected with selected known mammarenaviruses plus isolates from this study by IF against mammarenavirus monoclonal antibodies at doubling dilutions from 1:100. We tested all isolates for intracerebral pathogenicity for 1-day-old mice by inoculation of 2 litters (8 infant mice/litter) for each virus.

**Molecular Characterization and Phylogenetic Analysis of Mammarenavirus Isolates**

We performed phylogenetic analysis on 48 isolates by using an ~912-nt (299–304-aa) fragment of the nucleocapsid protein (NP) gene, consisting of 15 isolates from this study, 5 MOPV isolates from Mozambique (9) that were reisolated during this study, 3 mammarenaviruses received from other laboratories—namely, LASV 331 and MOPV isolates SPB801478 and SPB801480 from Zimbabwe (10)—and 25 viruses for which nucleotide sequences were retrieved from GenBank, including 3 New World arenaviruses as outgroup taxa (Table 1, https://wwwnc.cdc.gov/EID/article/27/12/21-1088-T1.htm). We omitted from analysis 4 isolates from this study with identical sequences to Mopelia virus isolate SPU84/491/40 (Table 1). We excluded potential related viruses for which inadequate information was available, such as Kodoko virus from Guinea (11) and Lemniscomys virus from Tanzania (12).

**Table 2. Pairwise comparison of partial nucleocapsid protein amino acid sequence (299–304 aa) percentage difference between 5 selected southern Africa Old World mammarenavirus isolates from current study and closest relatives, South Africa and Zimbabwe**

<table>
<thead>
<tr>
<th>Isolate name</th>
<th>MOPV</th>
<th>IPPYV</th>
<th>DakAn</th>
<th>LAVS</th>
<th>MOBV</th>
<th>LCMV</th>
<th>LUJO</th>
<th>LUNK</th>
<th>LUNA</th>
<th>NW</th>
<th>MRTV</th>
<th>OKAV</th>
<th>NR27</th>
<th>OKR73</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPU 86/415/2</td>
<td>30</td>
<td>29</td>
<td>29.6</td>
<td>31.3</td>
<td>37.7</td>
<td>43.4</td>
<td>34.7</td>
<td>30</td>
<td>33</td>
<td>15.5</td>
<td>14.2</td>
<td>0</td>
<td>3.7</td>
<td>15.2</td>
</tr>
<tr>
<td>SPU 86/415/4</td>
<td>30</td>
<td>28.3</td>
<td>29</td>
<td>31.3</td>
<td>37</td>
<td>43.3</td>
<td>34.3</td>
<td>29.6</td>
<td>32</td>
<td>15.2</td>
<td>14.2</td>
<td>3.7</td>
<td>0</td>
<td>14.8</td>
</tr>
<tr>
<td>SPU 86/485</td>
<td>27.6</td>
<td>27.6</td>
<td>29.6</td>
<td>29.3</td>
<td>33</td>
<td>39.4</td>
<td>31</td>
<td>28.3</td>
<td>29.3</td>
<td>8.4</td>
<td>9.3</td>
<td>15.2</td>
<td>15</td>
<td>29.3</td>
</tr>
<tr>
<td>SPU 84/491/ 406</td>
<td>24</td>
<td>22</td>
<td>25</td>
<td>25</td>
<td>34</td>
<td>44</td>
<td>35</td>
<td>26</td>
<td>13</td>
<td>30</td>
<td>35.3</td>
<td>32</td>
<td>31.3</td>
<td>29.3</td>
</tr>
<tr>
<td>SPU 85/353</td>
<td>26.6</td>
<td>27.6</td>
<td>28.3</td>
<td>29.6</td>
<td>36</td>
<td>42.8</td>
<td>32</td>
<td>28.3</td>
<td>28.3</td>
<td>20.9</td>
<td>19.4</td>
<td>19.9</td>
<td>19.2</td>
<td>20.5</td>
</tr>
</tbody>
</table>

BITU, Bitu virus; IPPYV, Ippy virus; LAVS, Lassa virus; LCMV, lymphocytic choriomeningitis virus; LUJO, Lujo virus; LUNA, Luna virus; LUNK, Lunk virus; MRTV, Mariental virus; MOBV, Mobala virus; MOPV, Mopeia virus; OKAV, Okahandja virus.
We extracted total RNA from cultures by using the High Pure RNA isolation kit (Roche Diagnostics, https://www.roche.com) and performed reverse transcription PCR with primers 19C (5'-CGCACCAGTGATCTAGGCGC-3') (13) and OWA2 (5'-TTCTTCATAAGGGTTCCTTACC-3') (J.C.S. Clegg, Centre for Applied Microbiology and Research, pers. comm., 1991) by using the Titan One Tube reverse transcription PCR kit (Roche) to amplify an ~1,000-bp fragment of the NP gene. The 19C primer is complementary to a conserved sequence at the 3’ terminus of the S RNA segment and the OWA2 primer corresponds to nucleotide positions 2357–2376 relative to LASV 391 and 2401–2420 relative to MOPV AN20410 (GenBank accession no. NC006575), to amplify the region of interest for isolates SPU94/88/235 and SPU86/485. Cycling conditions were 50°C for 30 min, 94°C for 2 min, 30 cycles of 94°C for 30 s, 47°C for 30 s, and 68°C for 90 s, plus extension at 72°C for 7 min. We purified PCR products with the Wizard SV Gel and PCR clean-up kit (Promega Corporation, https://www.promega.com), sequenced with the BigDye Terminator v3.1 kit (Promega Corporation, https://www.promega.com), and performed reverse transcription PCR with primers 19C (5′-CGCACCAGTGATCTAGGCGC-3′) (J.C.S. Clegg, Centre for Applied Microbiology and Research, pers. comm., 1991) by using the Titan One Tube reverse transcription PCR kit (Roche) to amplify an ~1,000-bp fragment of the NP gene. The 19C primer is complementary to a conserved sequence at the 3’ terminus of the S RNA segment and the OWA2 primer corresponds to nucleotide positions 2357–2376 relative to LASV 391 and 2401–2420 relative to MOPV AN20410 (GenBank accession no. NC006575), to amplify the region of interest for isolates SPU94/88/235 and SPU86/485. Cycling conditions were 50°C for 30 min, 94°C for 2 min, 30 cycles of 94°C for 30 s, 47°C for 30 s, and 68°C for 90 s, plus extension at 72°C for 7 min. We purified PCR products with the Wizard SV Gel and PCR clean-up kit (Promega Corporation, https://www.promega.com), sequenced with the BigDye Terminator v3.1 Cycle Sequencing Kit (ThermoFisher Scientific), purified on Centrisep columns (Princeton Separations Inc., https://www.prinsep.com), and ran testing on an ABI Prism 377 DNA Sequencing Unit. We aligned nucleotide and predicted amino acid

<table>
<thead>
<tr>
<th>Rodent species scientific name</th>
<th>Rodent species common name</th>
<th>Mammarenavirus IF antibody tests</th>
<th>Mammarenavirus isolation attempts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Tested</td>
<td>No. (%) positive</td>
</tr>
<tr>
<td>Aethomys chrysophilus</td>
<td>Red veld rat</td>
<td>135</td>
<td>10 (7.4)</td>
</tr>
<tr>
<td>A. ineptus</td>
<td>Tete veld rat</td>
<td>103</td>
<td>0</td>
</tr>
<tr>
<td>Dasymys incommutus</td>
<td>Water rat</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Dendromys melanotis</td>
<td>Grey climbing mouse</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Desmodillus auriculatus</td>
<td>Short-tailed gerbil</td>
<td>69</td>
<td>0</td>
</tr>
<tr>
<td>Gerbillurus afra</td>
<td>Cape gerbil</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>G. brantsii</td>
<td>Highveld gerbil</td>
<td>529</td>
<td>4 (0.8)</td>
</tr>
<tr>
<td>G. leucogaster</td>
<td>Bushveld gerbil</td>
<td>378</td>
<td>14 (3.7)</td>
</tr>
<tr>
<td>G. paeba</td>
<td>Hairy-footed gerbil</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Lemniscomys rosalia</td>
<td>Single-striped mouse</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>M. natalensis</td>
<td>Cape multimammate mouse</td>
<td>664</td>
<td>11 (1.7)</td>
</tr>
<tr>
<td>M. natalensis</td>
<td>Natal multimammate mouse</td>
<td>1165</td>
<td>370 (31.8)</td>
</tr>
<tr>
<td>Micaelamus namaquaensis</td>
<td>Namaqua rock mouse</td>
<td>273</td>
<td>20 (7.3)</td>
</tr>
<tr>
<td>Mus minutoides</td>
<td>Pygmy mouse</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>M. musculus</td>
<td>House mouse</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Mystromys albicaudatus</td>
<td>White-tailed mouse</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Otomys angoniensis</td>
<td>Angoni vlei rat</td>
<td>30</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td>O. irroratus</td>
<td>Vlei rat</td>
<td>266</td>
<td>67 (25.2)</td>
</tr>
<tr>
<td>O. unisulcatus</td>
<td>Bush vlei rat</td>
<td>178</td>
<td>28 (21.3)</td>
</tr>
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<td>Parotomys brantsii</td>
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<td>10</td>
<td>1 (10.0)</td>
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<tr>
<td>P. liddellae</td>
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<tr>
<td>Rattus norvegicus</td>
<td>Brown rat</td>
<td>125</td>
<td>6 (4.8)</td>
</tr>
<tr>
<td>R. rattus</td>
<td>House rat</td>
<td>211</td>
<td>1 (0.5)</td>
</tr>
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<td>Rhabdomys pumilio s.l.</td>
<td>Four-striped mouse</td>
<td>933</td>
<td>11 (1.2)</td>
</tr>
<tr>
<td>Saccostomus campestris</td>
<td>Pouched mouse</td>
<td>82</td>
<td>6 (7.3)</td>
</tr>
<tr>
<td>Steatomys pratensis</td>
<td>Fat mouse</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Thalomys paedulcus</td>
<td>Tree mouse</td>
<td>31</td>
<td>0</td>
</tr>
<tr>
<td>Zelotomys woosmani</td>
<td>Woosnam’s desert rat</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Cryptomys hottentotus</td>
<td>Common mole rat</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Graphirius murinus</td>
<td>Woodland dormouse</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Paraxerus cepapi</td>
<td>Tree squirrel</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Xerus inauris</td>
<td>Ground squirrel</td>
<td>36</td>
<td>0</td>
</tr>
<tr>
<td>Pedetes capensis</td>
<td>Springhare</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>5,363</td>
<td>560</td>
</tr>
</tbody>
</table>

†Organ pool from 1 O. irroratus rat was positive in mammarenavirus IF on first pass in culture but the potential isolate was lost on subculture.

Table 3. Summary of rodent samples, serologic test results, and virus isolation studies in study of mammarenaviruses, South Africa and Zimbabwe.
sequences using ClustalW (http://www.clustal.org/clustal2) incorporated in MEGA7 (https://www.megasoftware.net), performed phylogenetic analysis by the neighbor-joining method with 1,000 bootstrap iterations, and calculated sequence diversities (p-distances) (14).

**Results**

We tested a total of 5,363 rodents of 33 species from collection sites throughout South Africa and Zimbabwe for antibody to mammarenaviruses (Table 3; Figures 1–4). Antibody was found to be widely distributed in the 2 countries (Figures 1–4) and was detected in serum samples of 1.2%–31.8% of 14 species of myomorph rodents; 19 mammarenavirus isolates were obtained from serum and viscera of 4 seropositive species (Table 3).

Identities of the 4 myomorph species that yielded mammarenavirus isolates—*M. natalensis* mice and *Aethomys chrysophilus*, *Micaelamys namaquensis*, and *Otomys unisulcatus* rats—were confirmed from partial cytochrome b gene sequences (8) (GenBank accession nos. MK531528–35). However, the genus *Micaelamys* has subsequently proved to be polyphyletic and due for revision (16), whereas there is debate about inclusion of *O. unisulcatus* in the genus *Myotomys* (17). Furthermore, *O. unisulcatus* tissue remained available only for the Omdraavlei isolates and not for the Merino Walk isolate. Most of the other myomorph rodents were identified from morphologic features and distribution patterns (18), but new species and subspecies with partially overlapping distributions have since been recognized in the genus *Rhabdomys* (19–21). No organs remained available, and serum specimens failed to yield DNA for phylogenetic studies; thus, the samples are recorded as *R. pumilio* sensu lato (Table 3).

No mammarenavirus antibody or virus was found in 14 of the myomorph rodent species (Table 3), and although these rodents were relatively poorly represented in the collection, they tend to be rare.
species or occur in specialized habitats, such as deserts. A further 9 species of rodents—M. coucha, O. angoniensis, Parotomys brantsii, Rattus norvegicus, R. rattus, R. pumilio s.l., Saccostomus campestris, Gerbilliscus brantsii, and G. leucogaster—had low prevalence (0.5%–7.3%) of IF antibody to mammarenaviruses; no clear tendency to cluster was noted, except that the reactions were detected in locations where antibody was prevalent in other species. Although IF titers were generally low in these species (geometric mean titers [GMT] 19.3–57.9), the few ≥16 tended to be supported by ELISA reactions, but samples cultured yielded no virus. Anomalous high IF titers of 8,192 supported by ELISA reactions were recorded in 2 serum samples from R. norvegicus rats collected from a location where antibody prevalence of 29.1% was recorded in serum specimens from O. irroratus rats, but no isolates were obtained.

The remaining 5 species of myomorph rodents—A. chrysophilus, M. namaquensis, M. natalensis, O. unisulcatus, and O. irroratus—had mammarenavirus IF antibody prevalence of 4.2%–31.8%; positive reactions tended to cluster and reached 30%–50% prevalence at some trapping sites. The IF titers ranged from 8–16,384 (GMT 99.1–219.5), and titers ≥16 were supported by positive ELISA reactions. A total of 19 mammarenavirus isolates were obtained from 4 of these species (Table 3), but a single sample of O. irroratus rat produced an IF reaction on first pass in cell cultures that was lost during subculture and could not be repeated in further attempts to isolate virus. In addition, attempts to reisolate MOPV from 5 sets of M. natalensis organs from Mozambique (9) held in storage at NICD were successful (Table 1). All isolates were pathogenic for day-old mice inoculated intracerebrally.

A total of 6 isolates from this study plus 1 reisolated MOPV from Mozambique demonstrated 4 patterns of reactivity in IF screening tests with monoclonal antibodies and selected mammarenavirus isolates (Table 2). Deduced NP amino acid distances between selected isolates and closest relatives were calculated (Table 4). We determined the phylogenetic relationships of 48 mammarenavirus isolates, including 15/19 isolates from this study and the 5 reisolated MOPV isolates from Mozambique (Table 1), on the basis of neighbor-joining analysis of partial NP sequences (≈912 nt), together with host relationships (Figure 5).
The *M. natalensis* isolates from Mozambique and from this study grouped with 2 earlier isolates from Zimbabwe as Mopeia virus, whereas 5 isolates from this study fell into 4 groups; isolate Bobomene from South Africa grouped with more recent isolates Mariental from Namibia and isolate Witsand from South Africa grouped with Okahandja from Namibia and with isolate Bitu from Angola (Figure 5). We determined phylogenetic relationships on the basis of neighbor-joining analysis of a 136 bp cytochrome b barcode sequence for 8 selected rodents from which mammarenavirus isolates were obtained in this study and reference taxonomic voucher sequences from Gen-Bank (Figure 6).

**Discussion**

The main impetus for this rodent survey came from the isolation of the mammarenavirus MOPV at NICD from *M. natalensis* rodents collected in a village in Mozambique during an arbovirus study in 1972; within months, the same rodent species was identified as the host of LASV in West Africa (9,22,23). As a consequence, work ceased on Mopeia virus at NICD and the isolates were transferred to CDC, where the relationship to LASV was confirmed (9). Although MOPV proved to be non-pathogenic for nonhuman primates (24), investigating the possible occurrence and role of mammarenaviruses as causes of human infection in South Africa was considered necessary.

Our survey detected widespread presence of antibody activity to mammarenaviruses in myomorph rodent serum specimens within the study area. Because *M. natalensis* mice have an eastern distribution in South Africa (18), rodents were trapped along the northeastern border and MOPV was successfully isolated. However, a mammarenavirus isolated from another rodent species, *A. chrysophilus*, within the

![Figure 3](Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 27, No. 12, December 2021 3097)
distribution range of *M. natalensis* mice was found to be distinct from MOPV; 4 isolates obtained from 2 other rodent species further to the west also differed from MOPV (Tables 1–4; Figure 5).

Unpublished serosurveys conducted on humans in South Africa during 1984–1988 in parallel with the rodent survey included a study of 7,665 long-term (>5 years) healthcare workers from 66 secondary...
Mammarenaviruses, South Africa and Zimbabwe

hospitals that receive patient referrals from district hospitals who were tested for evidence of nosocomial infection, plus a study of 2,041 long-term (>5 years) rural residents and workers in the livestock and wildlife industries who were investigated for evidence of exposure to zoonotic viruses (R. Swanepoel, unpub. data). An overall prevalence of 1.0% (93/9,704) of IF antibody to MOPV antigen was recorded at titers of 8–2,048, gmt 33.0; higher prevalences of 10%–15% occurred in a few widely separated locations near the eastern border, but no histories of disease considered indicative of mammarenavirus infection were obtained. To the west, in Free State, Northern Cape, North West, and Gauteng Provinces, no antibody to mammarenaviruses was detected in rural residents and workers in the livestock industry despite the isolation of mammarenaviruses from rodents. Aliquots of human serum samples collected during the original investigations in Mopeia village, Mozambique, in 1972 remained available at NICD, and IF antibody to

Figure 5. Phylogenetic relationships of 48 arenavirus isolates based on neighbor-joining analysis applying a Jukes-Cantor model of partial nucleoprotein sequences (≈912 nt), together with known host relationships and collection dates. Values at nodes indicate the level (%) of bootstrap support from 1,000 replicates. Scale bar indicates base substitutions per site. Bold indicates sequences determined in this study. ANGL, Angola; AR, Argentina; AU, Australia; CAM, Cameroon; CAR, Central Africa Republic; CO, Colombia; CIV, Côte d’Ivoire; ETH, Ethiopia; GHA, Ghana; MOZ, Mozambique; NAMB, Namibia; NIG, Nigeria; RSA, Republic of South Africa; SL, Sierra Leone; TAN, Tanzania; TR, Trinidad; USA, United States; ZAM, Zambia; ZIM, Zimbabwe.
MOPV antigen was detected at a prevalence of 16.1% (32/199) with titers ranging from 8 to 8,192, gmt 229.6, similar to the findings initially recorded when no disease associations were identified (R. Swanepoel, unpub. data). In further checks on the possible occurrence of mammarenavirus-associated disease, 379 patients experiencing febrile illness in 4 district hospitals along the northeastern coast of KwaZulu-Natal Province, South Africa, were monitored for evidence of MOPV infection or seroconversion in 1985 without positive result. No antibody was detected in 100 chronic renal failure patients on dialysis in Gauteng Province, South Africa, in 1993 (R. Swanepoel, unpub. data).

Among routine diagnostic samples submitted to NICD, resting IF titers of 128 and 256 of IgG to MOPV antigen were detected in 2 patients from South Africa, but no etiologic significance could be attached to these findings. A single case of fatal LASV infection was diagnosed in a patient from Nigeria who was evacuated to a hospital in South Africa in 2007 (R. Swanepoel, unpub. data).

The isolates from this study are provisionally named for their locations of origin (Table 1; Figure 5), but the isolates obtained from *M. natalensis* mice represent exemplar isolates of MOPV, and Merino Walk virus is clearly distinct. Although the apparent sharing of rodent hosts mitigates against species recognition within the mammarenaviruses (30), clarifying the interrelationships between the Bobomene, Witsand, and Omdraavlei isolates and their relationship to the Mariental and Okahandja viruses from Namibia (31) and Bati virus from Angola (32) anticipates complete genomic characterization of the isolates.

The phylogenetic relationships between 8 rodents from which mammarenaviruses were isolated in this study and reference taxonomic voucher sequences from GenBank. Tree was constructed based on neighbor-joining analysis of a 136-bp cytochrome b barcode sequence. Values at nodes indicate the level of bootstrap support from 1,000 replicates. Scale bar indicates base substitutions per site. GenBank accession numbers, rodent reference number, and country of collection are indicated. RSA, Republic of South Africa; ZIM, Zimbabwe.
GenBank are compatible with the concept of cospeciation of arenaviruses and their rodent hosts (Figure 6), except that the interrelationships between Witsand, Okahandja, and Bitu isolates await clarification as previously noted. Moreover, the unavailability of rodent host tissue for Merino Walk virus precluded comparison with ostensibly the same host species, the *O. unisulcatus* rat, of the Omdraaivlei isolates. However, *O. unisulcatus* rats reportedly comprise a coastal lowland group that is located where the host of Merino Walk virus was collected and a central interior group that covers the area where the hosts of the Omdraaivlei isolates were obtained, although the low sequence divergences did not warrant recognition of subspecies (33). The observations on rodents from Zimbabwe were limited, and the single isolation of Mopeia virus obtained from *M. natalensis* mice from a farm near Masvingo was not related to the nonfatal illness of a former farm resident who was hospitalized in South Africa.

Further research on mammarenaviruses in rodents in South Africa should include attempts to isolate virus from *O. irroratus* rats and possibly *Lemniscomys ronselaria* mice, which were underrepresented in this survey; the presence of Luna-related and Lunk-related viruses that were identified in Zambia in *M. natalensis* and *M. minutoides* rodents should also be investigated (34). Furthermore, the reservoir host and distribution range of Lujo virus in southern Africa have not been determined. A greater knowledge of the occurrence and diversity of mammarenaviruses in Africa is foundational to understanding the possible health risks associated with these viruses and preparedness for the emergence of such viruses in the future.

Acknowledgments

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About the Author

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References

Institute and Endangered Wildlife Trust; 2017.


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Potential Use for Serosurveillance of Feral Swine to Map Risk for Anthrax Exposure, Texas, USA

Rachel M. Maison, Courtney F. Pierce, Izabela K. Ragan, Vienna R. Brown, Michael J. Bodenchuk, Richard A. Bowen, Angela M. Bosco-Lauth

Anthrax is a disease of concern in many mammals, including humans. Management primarily consists of prevention through vaccination and tracking clinical-level observations because environmental isolation is laborious and bacterial distribution across large geographic areas difficult to confirm. Feral swine (Sus scrofa) are an invasive species with an extensive range in the southern United States that rarely succumbs to anthrax. We present evidence that feral swine might serve as biosentinels based on comparative seroprevalence in swine from historically defined anthrax-endemic and non–anthrax-endemic regions of Texas. Overall seropositivity was 43.7% (n = 478), and logistic regression revealed county endemicity status, age-class, sex, latitude, and longitude were informative for predicting antibody status. However, of these covariates, only latitude was statistically significant (β = −0.153, p = 0.047). These results suggest anthrax exposure in swine, when paired with continuous location data, could serve as a proxy for bacterial presence in specific areas.

Anthrax, caused by Bacillus anthracis, is a zoonotic disease of global importance because of its ecologic effects on wildlife and free-ranging livestock and resulting economic impact on farmers and herders, its worldwide distribution, and its ability to cause disease even after decades of lying dormant in the environment. Known risks of exposure, considered together with unconfirmed environmental distribution in most regions and unidentified or evolving epidemiologic risk factors, make B. anthracis a pathogen of continuing human and animal health concern.

B. anthracis is a gram-positive, endospore-forming bacterium. Anthrax cases have been clinically described since the 1700s, but symptomatic descriptions of the disease have been recorded as early as 1000 BCE (1,2). Genetic studies however, suggest that the geographic origin of B. anthracis was in sub-Saharan Africa; subsequent environmental spread followed the migration of humans and domesticated animals (3,4). Current case report data indicate that enzootic anthrax correlates with warmer climates, although some cases have been documented above the arctic circle, in Canada, and in northern Siberia (5). The true incidence of the disease remains unknown in many countries, although it is assumed that the bacterium resides in most regions (6). Extensive ecologic modeling efforts now offer some ability to predict outbreak risks spatially and temporally in several countries (7–10). Of note, recent modeling efforts have indicated that enzootic anthrax correlates with warmer climates, although some cases have been documented above the arctic circle, in Canada, and in northern Siberia (5).

Thought to affect all mammals to varying degrees, B. anthracis infection generally causes the highest levels of illness and death in herbivorous species (12,13). Exposure most commonly occurs when an animal ingests the dormant spore form of the bacterium, but cutaneous and inhalational infections also occur (14). Once inside a susceptible host, bacteria transform into a vegetative form that secretes a combination of lethal and edema factor proteins as well as the cell receptor–binding protein–protective antigen (PA), which mediates their entry into host cells and activates them to produce lethal factor and edema factor toxins, contributing to the ultimate death of susceptible hosts. Upon host death, exposure of vegetative bacilli to atmospheric oxygen, typically


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though carcass manipulation by scavengers, initiates the sporulation process, in which bacteria return to their dormant form. Sporulated *B. anthracis* is highly resistant to environmental degradation; some environmental isolations have detected viable spores up to 200 years old (4). Humans and other animals that encounter infected carcasses or animal materials are therefore at increased risk of exposure because infected carcasses that are manipulated or opened can initiate sporulation and consequently perpetuate the environmental persistence of infectious *B. anthracis*.

Current preventive management for domestic herbivores is primarily vaccine-based (12), but vaccination is not a requirement for livestock owners, who instead commonly use it reactively to control outbreaks (11,15). Outbreaks of anthrax in wild and domestic animals today are defined by the detection of carcasses, often from otherwise healthy animals. Unlike among domestic populations however, observation of anthrax is extremely difficult among wild or free-ranging herbivores, because detecting carcasses over large landscapes is an imperfect and likely inaccurate method for reporting true incidence, and wildlife usually cannot be observed for clinical signs of disease (16–18).

Humans, suids, and carnivores are considered incidental hosts and considerably less susceptible to lethal infection than herbivores (19). Although the causes of these variations in susceptibility remain largely unknown, it is likely they are a combination of differences in physiology, behavior, dosage, and transmission routes (20). For example, carnivores, omnivores, and scavengers all have lower stomach pH than herbivores, likely killing *B. anthracis* spores or vegetative cells incidentally ingested while foraging (12,21). In addition, some evidence indicates that necrophilic and hemophagic arthropods can contribute to infection (19,22), suggesting that transmission routes might also differ by a region’s competent vector species. In endemic regions such as Africa, there appears to be little evidence of predators and scavengers dying of anthrax; those animals instead exhibit a high prevalence of antibodies against the bacterium (20). On the basis of these observations, it has previously been proposed that anthrax-resistant suid species, such as the Eurasian wild boar (*Sus scrofa*) in Ukraine and feral hog in the United States, might be used as biosentinels for anthrax (23). Of note, although a previous study (23) described serologic evidence of exposure in wild boars Ukraine, no studies to date have formally evaluated exposure in taxonomically identical feral swine (also *S. scrofa*) present in the United States. Introduced initially in the 1500s to states bordering the Gulf of Mexico, populations of feral swine have exploded since the 1980s and have become established throughout most suitable habitats in the southern United States (24).

In addition to known pathways of transmission, the shared presence of *B. anthracis* and anthrax-resistant wildlife species might contribute to anthrax epidemiology under certain conditions by increasing the risk for exposure to humans or more susceptible herbivorous species. Resistant species may also help to disseminate infectious spores to new landscapes through mechanical transmission or bacterial shedding (6,25). Feral swine are known to be opportunistic omnivores that occasionally scavenge carcasses, as well as routinely root in soils for food (26). These behaviors, coupled with their documented resistance to anthrax, suggest that feral swine might be a good indicator of bacterial presence on the landscapes they occupy. We report the potential biosentinel utility of feral swine for measuring anthrax distribution by examining antibody prevalence in confirmed endemic and nonendemic regions of Texas, USA.

**Materials and Methods**

**Study Area**

We conducted our investigation in Texas because anthrax is a reportable disease and is relatively predictable in select regions of the state. Feral swine populations are also present in most counties, offering a unique opportunity to evaluate the species as a biosentinel for *B. anthracis*. In addition, observations by residents of the state’s endemic region have described resurgences in anthrax in areas recently colonized by feral swine, anecdotally suggesting the 2 events might be related.

Outbreaks of anthrax occur regularly in portions of Crockett, Val Verde, Sutton, Edwards, Kinney, Uvalde, and Maverick Counties, colloquially referred to as the Anthrax Triangle, usually in dry summer months following heavy spring rains (27,28). Conversely, eastern Texas does not experience regular outbreaks, despite also being heavily populated with domestic livestock (29). Furthermore, populations of ranched white-tailed deer in areas of Val Verde, Uvalde, and Webb Counties are also regularly affected, suggesting wild herbivores in the same region might become infected at similar rates. We binarily defined areas as either endemic for anthrax for those 7 counties on the western side of the state comprising the historic Anthrax Triangle (Figure 1) or nonendemic if outside of this region, because these counties do not experience regular, seasonal cases.
Feral Swine and Risk for Anthrax Exposure

Field Sampling
Wildlife Services, a branch within the US Department of Agriculture (USDA), routinely removes feral swine from the landscape for damage control and invasive species management, and as part of these efforts, collect serum samples from a subset of swine for disease surveillance. Samples not used for routine surveillance are archived and can be used for select retrospective studies. Through these efforts, we obtained 478 serum samples collected during 2007–2019 from feral swine removed from areas throughout Texas and tested them to determine the prevalence of feral swine exposure to B. anthracis by measuring antibodies against PA. We illustrated spatial data on the geographic origins of the feral swine serum samples (Table 1; Figure 1) at the county level to protect personally identifiable information because many samples were collected on private property. Approximately half (n = 243) of the serum samples originated in the 7 endemic counties within the Anthrax Triangle and the rest (n = 235) from 7 nonendemic counties outside of it. We randomly selected the 7 nonendemic counties from the 246 Texas counties located outside of the Anthrax Triangle; 7 counties were selected so that the sampling effort was equal between endemic and nonendemic regions. Sampling events took place year-round.

Serum samples were taken from male and female feral swine classified as either adult, estimated by Wildlife Services field personnel to be >1 year of age, or subadult, estimated as 2 months–1 year of age (Table 1). We did not collect samples from juveniles (<2 months of age) to avoid confounding serology that could result from the presence of maternal antibodies (30). All blood samples were collected postmortem and serum extracted within 12 hours of clotting and shipped overnight on ice to the National Wildlife Research Center (Fort Collins, CO, USA), where they were stored at –80°C until testing.

Serology
We used an indirect ELISA platform similar to those described elsewhere (31-34), with slight modifications to target antibodies of swine origin. We assayed samples blindly relative to the origin, sex, and age-class of individual animals until all results were finalized. We coated high binding polystyrene 96-well flat-bottom microtiter plates (ThermoFisher Scientific, https://www.thermofisher.com) with recombinant protective antigen (rPA) from B. anthracis (American...
We examined how the probability of an individual animal being positive for anthrax antibodies varied by region (endemic vs. nonendemic), sex, age-class (adult vs. subadult), latitude, and longitude using logistic regression and mixed-effects models implemented in R version 4.0.2 (R Foundation for Statistical Computing, https://www.r-project.org). We examined region, sex, age-class, latitude, and longitude as fixed effects and evaluated sampling year as a random effect to account for temporal variation in anthrax prevalence and sampling. Since most anthrax cases in Texas originate from the Anthrax Triangle (27,28), we included region as a fixed effect to evaluate whether feral swine residing in known contaminated environments are more likely to be antibody positive than those outside. We used county centroids as a proxy for sampling locations and considered latitude and longitude fixed effects to account for spatial trends in anthrax prevalence. Interaction between age-class and sex was also examined to account for potential impacts of age variations by sex.

We evaluated support for including a random effect (sampling year) using Akaike’s information criterion (AIC) and likelihood ratio test (LRT) in R. As recommended elsewhere (38), we first examined whether sample year should be included by comparing AIC and LRT with and without its addition from a fully parameterized fixed effects model. If the random effect was supported (ΔAIC >2 compared with the model excluding the random effect), then it was retained in all models and the fixed effects compared. Using LRT as an additional method of evaluating the inclusion of sampling year, we calculated the difference in the log likelihoods of the 2 nested models (i.e., fully parameterized fixed effect model with or without the addition of the random effect) and if the difference was statistically significant (α = 0.05), we included the random effect in all models.

We compared all combinations of fixed effects covariates using AIC implemented in the R package MuMIn (R Foundation for Statistical Computing); the lowest AIC value represented the most parsimonious model. If model uncertainty existed (i.e., >1 competing model <2 ΔAIC of the top model), we included the random effect (sampling year) using Akaike’s information criterion (AIC) and likelihood ratio test (LRT) in R.

Type Culture Collection, https://www.atcc.org) diluted in carbonate buffer solution at a concentration of 5 μg/mL per well and incubated plates overnight at 4°C. The following day, we discarded the coating buffer and washed the wells 5x with phosphate-buffered saline containing 0.05% Tween 20 washing buffer. We blocked wells by adding 300 µL of 10% skim milk in phosphate-buffered saline and allowed plates to incubate for 1.5 h at room temperature. We again washed wells, then added 100 µL of test serum diluted 1:100 in blocking buffer and incubated plates for 1 h with shaking at room temperature. After additional washing, we added 100 µL/well of protein A/G-horseradish peroxidase (ThermoFisher Scientific) diluted 1:1,000 in blocking buffer, and further incubated plates with shaking for 30 min. After 1 final washing step, we added 150 µL of one-step ABTS (ThermoFisher Scientific), incubated for 15 min, and then added 100 µL of 1% sodium dodecyl sulfate solution to stop the reaction. We measured absorbance at 25°C and 405 nm using a BioTek microplate reader paired with Gen5 version 3.09 microplate reader and imager software (https://www.biotek.com). We considered samples positive for rPA antibodies if their mean absorbance measurements were >3 times the SD above the mean of the negative controls. We ran individual samples in triplicate.

Because of their inherent resistance to anthrax infection, domestic pigs are not as routinely vaccinated as ruminant livestock species. As such, swine serum samples were unavailable for use as antibody-positive and -negative controls for this assay. Instead, we obtained control serum samples included in each assay from one male domestic goat (Capra aegagrus hircus) before and after vaccination with Anthrax Vaccine Adsorbed (BioThrax, https://www.beiresources.org). Protein A/G is known to bind to the constant region of both goat and swine IgG with comparable affinity (35–37).

### Statistics

We examined how the probability of an individual animal being positive for anthrax antibodies varied by region (endemic vs. nonendemic), sex, age-class (adult vs. subadult), latitude, and longitude using logistic regression and mixed-effects models implemented in R.

<table>
<thead>
<tr>
<th>Region type</th>
<th>Adult</th>
<th>Subadult</th>
<th>Adult</th>
<th>Subadult</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endemic</td>
<td>90</td>
<td>16</td>
<td>121</td>
<td>16</td>
<td>243</td>
</tr>
<tr>
<td>Nonendemic</td>
<td>113</td>
<td>12</td>
<td>101</td>
<td>9</td>
<td>235</td>
</tr>
<tr>
<td>Total</td>
<td>203</td>
<td>28</td>
<td>222</td>
<td>25</td>
<td>478</td>
</tr>
</tbody>
</table>

**Table 1.** Sampling distribution of feral swine serum samples collected from endemic and nonendemic regions of Texas, USA.
the ROC curve, we calculated the AUC, an aggregated measure of binary classification model performance, in which the model AUC = 0.5 for no predictive power, >0.5–<0.7 for poor predictive power, ≥0.7–<0.8 for acceptable predictive power, and ≥0.8–<0.9 for excellent predictive power (40).

Results

Serology
Negative control goat serum collected before vaccination exhibited absorbance readings of 0.018–0.11 (mean 0.08, SD 0.022). Pooled positive serum taken 3 and 5 weeks after anthrax vaccination exhibited an absorbance range of 0.26–3.42 (mean 1.62, SD 1.27). We calculated the assay cutoff of +3 SD above the mean of the negative controls at 0.15. Of the 478 samples examined, we identified 209 (43.7%) as positive and 269 (56.3%) as negative for PA antibodies. From the entire sample pool, we recorded a minimum absorbance value of −0.006 and maximum value of 3.9.

Statistics
Basic data structure, including anthrax antibody status stratified by covariate and apparent seroprevalence (Table 2), includes raw data confirming that more swine from the endemic region (49.49%) compared with the nonendemic region (37.45%) were seropositive; we also illustrate individual sample absorbance by region (Figure 2). Seroprevalence was higher among female (48.18%) than male (38.96%) swine and among adult (44.71%) than subadult (35.85%) swine. The fully parametrized model failed to converge, so we excluded longitude and the interaction term (age-class*sex) from the fully parameterized model to evaluate inclusion of sampling year as a random effect. Sampling year did not improve the predictive power of the model (fixed effects model AIC = 649.87 and mixed-effects model AIC = 648.59; LRT p = 0.070); probability of an individual animal being seropositive was therefore best predicted by a fixed effects model. There was uncertainty about the optimal model (7 models were <2 ΔAIC). To determine their relative importance, we examined cumulative covariate weights and found that county endemicity status, age-class, sex, latitude, and longitude were informative for predicting antibody status, and therefore included them in the final model. We calculated odds ratios and 95% CI for each predictor variable (Table 3), but only latitude was statistically significant (β = −0.153; p = 0.047). The final model had poor predictive ability (AUC = 0.613) suggesting the presence of unexplained variance in anthrax antibody status.

Table 2. Distribution of anthrax seroprevalence in feral swine by region, sex, and age group.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>No. tested</th>
<th>No. positive</th>
<th>Apparent seroprevalence, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endemic</td>
<td>243</td>
<td>121</td>
<td>49.49</td>
</tr>
<tr>
<td>Nonendemic</td>
<td>235</td>
<td>88</td>
<td>37.45</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
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<td>M</td>
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<td>F</td>
<td>247</td>
<td>119</td>
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<td>Age group</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Subadult</td>
<td>53</td>
<td>19</td>
<td>35.85</td>
</tr>
<tr>
<td>Adult</td>
<td>425</td>
<td>190</td>
<td>44.71</td>
</tr>
</tbody>
</table>
the geographic extent of this pathogen, while exposure data obtained through serosurveillance might enable acquisition of multidimensional biologic information, such as environmental range and relative time of exposure. Because swine are resistant to anthrax (19) and there is serologic evidence of exposure to taxonomically identical species such as wild boar in Ukraine, feral swine might be good indicators of bacterial presence throughout their range in the United States. Feral swine also exhibit relatively small home ranges, 1–5 km² (41,42), potentially enabling high resolution in estimating the geographic extent of contaminated environments.

Data presented here demonstrate that the overall odds of feral swine in Texas with anthrax antibodies differ between those inhabiting broadly defined endemic and nonendemic regions; animals originating within the Anthrax Triangle exhibit higher odds of being seropositive than those outside. This finding is not surprising given the regularity of outbreaks in domestic herbivores within this region and supports our preliminary hypothesis that feral swine are being exposed in regions experiencing regular occurrences of the disease. However, ≈37% of individual animals from nonendemic counties were also seropositive, so county status alone proved not to be a significant predictor covariate, and the size of that proportion suggests that bacteria might be present and therefore swine exposed beyond the confines of the Anthrax Triangle. This possibility is further supported by latitude but not county status being a statistically significant covariate in our top-performing model.

Although the role that feral swine might play in the overall epidemiology of anthrax is unknown, swine do exhibit close relationships with soil (26) and thus likely experience higher rates of exposure than humans and perhaps some domestic and wild ruminants; therefore, they might contribute to bacterial spread through biologic or mechanical dissemination. However, the level of exposure might simply reflect bacterial presence irrespective of swine involvement in dissemination, because outbreaks outside of the Anthrax Triangle are reported occasionally (28). Although our statistical analysis was unable to distinguish anthropogenically defined endemic and nonendemic regions, the high apparent seroprevalence observed in feral swine across the state of Texas is still useful information, because exposure data are further indicative of bacterial distribution occurring beyond the confines of the Anthrax Triangle, as has been predicted by the ecologic modeling efforts of others (8,11).

Of note, female and adult swine tended to have higher seropositivity than male and subadult swine, although the measures were not statistically significant. Higher odds by sex might be because of the inherent dynamics of swine sounders; groups typically are composed of several females and their offspring, whereas adult and subadult males are often solitary, only associating with females during breeding (26). The likelihood then of observing seropositive female swine in a *B. anthracis*-contaminated region might be higher simply because female swine traveling together are experiencing the same environmental exposures compared with their solitary male counterparts. The potential age-class bias observed could be explained in part by the unequal sample sizes between these covariates; more extensive data might be necessary to confirm this association. Finally, feral swine have been observed to opportunistically feed on carcasses of other animals, as well as prey on some livestock (43–45). Thus, feral swine might be contributing to anthrax epidemiology through a variety of mechanisms, including carrying and depositing spores or vegetative cells acquired from rooting in soil or by feeding on the carcasses of animals who have died from anthrax.

As with any retrospective, opportunistic serosurvey, the data and subsequent findings presented here are not without limitations. First, the fact that we broadly defined regions as endemic and nonendemic solely on the basis of whether a county was located in the Anthrax Triangle likely does not account for the contiguous or disjointed presence of this bacterium predicted in soils throughout the state (11), and counties that were sampled on the border of the Anthrax Triangle, such as Kimble, might have skewed results with some antibody-positive animals originating from this region. Also, in conjunction with the regions we defined, we did not examine any environmental conditions or weather patterns, which likely are substantial factors influencing bacterial distribution and infectivity rates between the sampling years examined and could be the source of the unexplained variance suggested during model evaluation.

In conclusion, feral swine are a fecund invasive species that often encounter people and domestic animals, as well as other wildlife species. Past investigations have identified myriad pathogens that can
be transmitted or carried by these animals (46), and national programs supported by the USDA regularly survey populations for diseases of national concern to humans or related to agriculturally important species (24). Despite the amount of attention feral swine receive for harboring some pathogens, future investigations are needed to fully define the role feral swine play in anthrax epidemiology, particularly whether they are contributing to bacterial dissemination. However, our investigation suggests that levels of anthrax exposure in feral swine, when paired with continuous location data, could serve as a proxy for identifying \textit{B. anthracis} presence in a specific area.

**Acknowledgments**

We thank the staff and field personnel of USDA’s National Feral Swine Damage Management Program for collecting and providing the field samples and corresponding animal data for this study. We also thank Terry Nett and Eric Weber for their guidance in developing and troubleshooting ELISAs and the American Type Culture Collection for providing the recombinant protective antigen used for our assays. Finally, we thank Amy Davis, Ryan Miller, and Tim Smyser for their helpful suggestions on statistical analysis and assistance during manuscript revision.

This research was supported by the US Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services. The findings and conclusions in this manuscript are those of the authors and should not be interpreted to represent any official USDA or US Government determination or policy, nor should the materials interpreted to represent any official USDA or US Government position.

**References**


20. Bagamian KH, Alexander KA, Hadfield TL, Blackburn JK. Ante- and postmortem diagnostic techniques for anthrax:


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Detection of SARS-CoV-2 in Wastewater at Residential College, Maine, USA, August–November 2020

Yolanda M. Brooks, Bailey Gryskwicz, Shawn Sheehan, Sheri Piers, Parag Mahale, Susan McNeil, Jenna Chase, Doreen Webber, David Borys, Michael Hilton, Dion Robinson, Stephen Sears, Emer Smith, Emily K. Lesher, Robert Wilson, Matthew Goodwin,1 Michael Pardales

We used wastewater surveillance to identify 2 coronavirus disease outbreaks at a college in Maine, USA. Cumulative increases of >1 log_{10} of severe acute respiratory syndrome coronavirus 2 RNA in consecutive 24-hour composite samples preceded the outbreaks. For 76% of cases, RNA was identified in grab samples from residence halls ≤7 days before case discovery.

Wastewater surveillance can indicate the presence and temporal trends of coronavirus disease (COVID-19) cases in a sewershed (1, 2). Large universities have used wastewater surveillance to identify residence halls at high risk for transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19 (3, 4). We demonstrate that wastewater surveillance using grab samples collected from residential halls and 24-hour composite samples from lift stations can detect COVID-19 outbreaks at a small residential college.

The Study
During August 21–November 20, 2020 (days 0–92), we collected weekly grab samples of flowing untreated wastewater from 6 residence halls at a residential college in Maine, USA. The residence halls served 605 students; hall A housed 64 students, hall B housed 127 students, hall C housed 80 students, hall D housed 109 students, hall E housed 87 students, and hall F housed 138 students. During days 13–92 we also collected 24-hour composite samples approximately twice a week from 2 lift stations (i.e., L1 and L3) where wastewater from various buildings on campus was consolidated in holding tanks and pumped to septic tanks; these composite samples represented the total population in the residence halls. L1 contained effluent from halls E–F and L3 contained effluent from halls A–D. Both lift stations also contained effluent from other campus buildings. The wastewater was collected and stored at 4°C for <72 hours before we assayed 105-mL samples using the Water SARS-CoV-2 RT-PCR test (IDEXX Laboratories, Inc., https://www.idexx.com) according to the manufacturer’s instructions. The 2019-nCoV_N_Positive Control plasmid (Integrated DNA Technologies, Inc., https://www.idtdna.com) had a limit of quantification of 2 (average cycle threshold [Ct] 39.42) copies per reaction, and the purified 2019-nCoV_N_Positive Control plasmid cloned into Escherichia coli had a limit of quantification of 20 (average Ct 35.95) copies per reaction. The theoretical limit of detection in all samples was 1 copy per reaction. We included a negative extraction control, no-template control, and a step from the standard curve (2 × 103 or 2 × 104 copies/reaction) in each run. We calculated SARS-CoV-2 RNA concentrations as copies per day per person for 24-hour composite samples and copies per liter per person for grab samples. Nondetectable samples were reported at one half the theoretical limit of detection (5).

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Each week, ≈100 students were randomly selected for individual surveillance testing by reverse transcription PCR. We instituted expanded surveillance testing for students living in residence halls with detectable RNA in grab samples or served by lift stations that had increased RNA concentrations. Affected students were isolated or quarantined according to guidelines from the Centers for Disease Control and Prevention (6,7). COVID-19 outbreak investigations by the Maine Center for Disease Control and Prevention (Augusta, Maine, USA) ended 28 days (2 infectious periods) after the specimen collection date of the last identified case. All residential students were tested for SARS-CoV-2 upon outbreak identification, beginning on day 25 for the first outbreak and day 81 for the second outbreak. Widespread testing among students continued weekly until all students had negative test results for 2 successive rounds during the outbreak period.

On day 18, we detected SARS-CoV-2 RNA from the wastewater discharge of hall F and both lift stations, preceding an outbreak on days 21–58 (Figure, panels A, B). By day 22, RNA concentrations in both lift stations increased by >1 log10, subsequently, concentrations decreased by >1 log10 by day 33 for L3 and day 40 for L1 (Figure, panel B). On day 21, expanded individual surveillance testing of half of the students living in hall F identified 2 COVID-19 cases (Figure, panels C, D). Widespread surveillance testing beginning on day 22 identified 6 additional cases in hall F, 1 case in hall D, and 1 case in hall E (Figure, panel C). In response, the college implemented remote learning during days 22–36.

Students returned to their residence halls from quarantine or isolation on days 21–45; we observed an increase of SARS-CoV-2 RNA in L3 on days 40–46 (Figure, panel B). We detected RNA in grab samples from hall D on days 45 and 53 and hall F on days 50 and 53 (Figure, panel A); however, concentrations were undetectable in L3 on days 49–70 (Figure, panel B). On day 53, individual surveillance testing of all students in hall F did not identify any COVID-19 cases (Figure, panels C, D).

From days 70 to 82, SARS-CoV-2 RNA concentrations in L3 increased by >1 log10. A second outbreak occurred during days 78–92. Expanded surveillance testing of all students in hall B identified 3 COVID-19 cases, prompting an outbreak investigation. During days 83–89, widespread testing of all students detected 7 additional COVID-19 cases in hall B, 1 case in hall C, and 1 case in hall E (Figure, panel C). The first COVID-19 case in hall E was discovered on day

Figure. Detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in wastewater samples at a residential college, Maine, USA, August–November 2020. A) Grab samples from 6 residence halls (i.e., A–F). B) 24-hour composite samples from 2 lift stations: L1 for halls E–F and L3 for halls A–D. Dashed lines indicate theoretical limit of detection; dotted lines indicate limit of quantification. These limits were dependent on flow rate (B only) and population served for each sample. Data points between dashed and dotted lines indicate undetectable concentrations of SARS-CoV-2 RNA and are recorded at one-half the limit of quantification (5). Data points between dashed and dotted lines indicate detectable but nonquantifiable concentrations of SARS-CoV-2 RNA and are recorded at one-half the limit of quantification. C) Daily positivity rate of COVID-19 tests. The number above the bar indicates the number of positive cases. Positive results were typically received within 24 hours after administration of the diagnostic test. D) Total COVID-19 diagnostic tests, including surveillance and nonsurveillance tests. Letter and symbol indicate resident hall of affected student. Shaded areas indicate the days during which the college declared an active outbreak. COVID-19, coronavirus disease.
We found that a >1 log₁₀ increase of SARS-CoV-2 RNA in L1 on day 40–56 (Figure, panel B) were caused by residual shedding among recovering students who were no longer infectious, as described in clinical data (8,9). In addition, a study in Utah, USA, documented evidence of residual shedding in municipal wastewater systems after a decrease in reported cases (2). In L1, SARS-CoV-2 RNA was undetectable 26 days in the wastewater system after the last known case was identified. The decrease of RNA to undetectable levels was consistent with the previously reported median fecal shedding time of 25 days among hospitalized COVID-19 patients (9).

In conclusion, wastewater surveillance can identify changes in SARS-CoV-2 transmission in the student population at a small residential college. Wastewater surveillance can quickly identify outbreaks, localize the detection of COVID-19 cases, and inform the management of resources for clinical surveillance testing.

Acknowledgments
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About the Author
Dr. Brooks is an assistant professor at Saint Joseph’s College of Maine, Standish, Maine, USA. Her research interests include the sources and persistence of fecal pollution in drinking water, recreational waters, and wastewater, as well as its potential effects on human health.

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January 2021

Waterborne Infections

- Impact of Human Papillomavirus Vaccination, Rwanda and Bhutan
- Aspergillosis Complicating Severe Coronavirus Disease
- Rising Ethnic Inequalities in Acute Rheumatic Fever and Rheumatic Heart Disease, New Zealand, 2000–2018
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- Hospitalization for Invasive Pneumococcal Diseases in Young Children Before Use of 13-Valent Pneumococcal Conjugate
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- Estimating the Force of Infection for Dengue Virus Using Repeated Serosurveys, Ouagadougou, Burkina Faso
- Attribution of Illnesses Transmitted by Food and Water to Comprehensive Transmission Pathways Using Structured Expert Judgment, United States
- Invasive Fusariosis in Nonneutropenic Patients, Spain, 2000–2015
- Nosocomial Coronavirus Disease Outbreak Containment, Hanoi, Vietnam, March–April 2020
- Intrafamilial Exposure to SARS-CoV-2 Associated with Cellular Immune Response without Seroconversion, France
- Estimate of Burden and Direct Healthcare Cost of Infectious Waterborne Disease in the United States
- Post–13-Valent Pneumococcal Conjugate Vaccine Dynamics in Young Children of Serotypes Included in Candidate Extended-Spectrum Conjugate Vaccines
- Precise Species Identification by Whole-Genome Sequencing of Enterobacter Bloodstream Infection
- Delineating and Analyzing Locality-Level Determinants of Cholera, Haiti
- Territorywide Study of Early Coronavirus Disease Outbreak, Hong Kong, China
- Viral Metagenomic Analysis of Cerebrospinal Fluid from Patients with Acute Central Nervous System Infections of Unknown Origin, Vietnam
- Prevalence of SARS-CoV-2, Verona, Italy, April–May 2020
- Recency-Weighted Statistical Modeling Approach to Attribute Illnesses Caused by 4 Pathogens to Food Sources Using Outbreak Data, United States

To revisit the January 2021 issue, go to: https://wwwnc.cdc.gov/eid/articles/issue/27/1/table-of-contents
SARS-CoV-2–Specific Antibodies in Domestic Cats during First COVID-19 Wave, Europe

Claudia Schulz,1 Byron Martina,1 Monica Mirolo, Elisabeth Müller, Ruth Klein, Holger Volk, Herman Egberink, Mariana Gonzalez-Hernandez, Franziska Kaiser, Maren von Köckritz-Blickwede, Albert Osterhaus

We conducted a severe acute respiratory syndrome coronavirus 2 antibody seroprevalence study among >2,000 domestic cats from 4 countries during the first coronavirus disease wave in Europe. We found 4.4% seroprevalence using a virus neutralization test and 4.3% using a receptor-binding domain ELISA, demonstrating probable human-to-cat transmission.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of the ongoing coronavirus disease (COVID-19) pandemic, causes high rates of illness and death among humans. SARS-CoV-2 is a newly recognized member of the genus Betacoronavirus, family Coronaviridae, that infects humans. An early serosurvey among domestic cats in Wuhan, China, during January–March 2020 reported 14.7% seropositivity (1). Experimental infections demonstrated susceptibility to SARS-CoV-2 infection in cats and other carnivore species, such as ferrets (Mustela putorius furo), minks (Neovison vison), and to a lesser extent domestic dogs (2,3), and confirmed anecdotal observations of naturally occurring human-to-animal transmissions (4,5). Respiratory and gastrointestinal signs were observed in SARS-CoV-2–infected cats (6–8). We conducted a seroprevalence study for SARS-CoV-2–specific antibodies among domestic cats in Europe during and after the first COVID-19 pandemic wave, using a plaque-reduction virus neutralization test (VNT) and a SARS-CoV-2 receptor-binding domain–specific ELISA (RBD-ELISA).

The Study
We analyzed serum samples collected from 2,160 domestic cats during April–June 2020. Samples had been sent to a veterinary diagnostic laboratory (LABOklin; Kissingen, Germany) for diagnostic purposes unrelated to suspicion of SARS-CoV-2 infection (9). Samples were from 1,136 cats in Germany, 331 in the United Kingdom, 333 in Italy, and 360 in Spain. Among 1,799 samples with demographic data, cats ranged from 0.1–23 years of age (median and mean age 11 years). We estimated a minimum of 300 total samples per location to enable a realistic estimation for each location. To confirm specificity of the assays to detect SARS-CoV-2–specific antibodies, we included 25 prepandemic cat serum samples and 25 serum samples from cats that tested positive for feline coronavirus/feline infectious peritonitis (FCoV/FIP) by NovaTec VetLine (Novatec Immundiagnostica GmbH, https://www.novatec-id.com), a commercial antibody test, in the screening.

We tested all serum samples by VNT, as previously described (10). We considered serum samples positive when titers were >20, expressed as the reciprocal of the dilution that gave >80% reduction of stained cells in the plaque reduction neutralization test (PRNT80) (Appendix, https://wwwnc.cdc.gov/EID/article/27/12/21-1252-App1.pdf).

We also tested serum samples with an indirect ELISA we developed and validated inhouse. We used an ELISA previously used for detecting SARS-CoV-2 RBD antibodies in human serum (11) and replaced the anti-human IgG conjugate with an anti-cat IgG conjugate (Appendix).

We evaluated performance characteristics of the cat ELISA-RBD by using Pearson correlation of the results obtained by ELISA-RBD and Gaussian distribution analyses for the VNT. We also calculated

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1These authors contributed equally to this article.
diagnostic sensitivity and specificity of the ELISA-RBD compared with VNT. We conducted data analyses using R (R Foundation for Statistical Computing, https://www.r-project.org) and Prism version 9 (GraphPad Software Inc., https://www.graphpad.com). We calculated SARS-CoV-2 seroprevalence in cats separately for each country.

We found overall SARS-CoV-2 seroprevalence among cats was 4.2% in Germany, 3.3% in the United Kingdom, 4.2% in Italy, and 6.4% in Spain (Table 1; Figure). Among all 2,160 cat serum samples tested, 96 (4.4%, 95% CI 3.6%–5.4%) were positive by VNT and 92 (4.3%, 95% CI 3.4%–5.2%) by RBD-ELISA. The RBD-ELISA showed a diagnostic sensitivity of 90.6% (95% CI 90.0%–91.2%) and specificity of 99.8% (95% CI 99.8%–99.8%) compared with VNT (Table 2). Furthermore, correlation (r = 0.9, 95% CI 0.9–0.9) and Gaussian distribution analyses (r²>0.7) revealed high agreement between VNT and RBD-ELISA sensitivities. All 25 prepandemic serum samples and 25 FCoV/FIP-positive samples tested SARS-CoV-2-negative in both the VNT and RBD-ELISA (data not shown), confirming the specificity of the assay for measuring SARS-CoV-2-specific antibodies.

Our study of domestic cat serum from 4 selected countries showed that during the first COVID-19 wave in Europe, >4% of domestic cats had been infected with SARS-CoV-2, probably through their contacts with infected humans. Because serum samples were sent to the veterinary diagnostic laboratory for conditions unrelated to a suspected SARS-CoV-2 infection, our data might not fully represent the overall seropositivity of the domestic cat population in Europe.

We used a VNT and an RBD-ELISA based on the original SARS-CoV-2 wild-type isolate (Wuhan-Hu-1, GenBank accession no. MN908947.3). The RBD-ELISA proved to have a high sensitivity and specificity compared with the VNT (Table 2), but 5 low-titer (titer = 20) VNT-positive samples remained undetected by the RBD-ELISA. These samples might have remained undetected because of the high specificity of RBD-ELISA, which detects antibodies toward the single spike protein ectodomain. Unlike RBD-ELISA, VNT might identify a broader range of virus neutralizing antibodies, including those directed against other domains of the spike protein. Of note, the only correlation of virus protection we have to date is virus neutralization, which apparently correlates well with RBD-ELISA positivity. For serologic screening and for individual diagnostic testing of domestic cats, the RBD-ELISA could replace the VNT, thus avoiding

### Table 1. Overall VNT SARS-CoV-2 seroprevalence in cats by country during the first pandemic wave, Europe, April–August 2020*

<table>
<thead>
<tr>
<th>Location</th>
<th>No. tested</th>
<th>No. positive</th>
<th>% Positive (95% CI†)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germany</td>
<td>1,136</td>
<td>48</td>
<td>4.2 (3.1–5.6)</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>331</td>
<td>11</td>
<td>3.3 (1.7–5.9)</td>
</tr>
<tr>
<td>Italy</td>
<td>333</td>
<td>14</td>
<td>4.2 (2.3–7.0)</td>
</tr>
<tr>
<td>Spain</td>
<td>360</td>
<td>23</td>
<td>6.4 (4.1–9.4)</td>
</tr>
<tr>
<td>Total</td>
<td>2,160</td>
<td>96</td>
<td>4.4 (3.6–5.4)</td>
</tr>
</tbody>
</table>

*Seroprevalence determined by virus neutralization test (VNT). Similar results were found with RBD-ELISA, 4.3% (96/2,160; 95% CI 3.6%–5.4%) were seropositive (Table 2). RBD-ELISA, receptor-binding domain–specific ELISA; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; VNT, virus neutralization test. †Calculated by using 2-sided exact binomial test in R (R Foundation for Statistical Computing, https://www.r-project.org).
the use of live SARS-CoV-2 under Biosafety Level 3 laboratory conditions. We further confirmed specificities of the VNT and RBD-ELISA by showing that pre-pandemic and FCoV/FIP-positive cat serum samples were negative in both assays. This finding excluded the detection of cross-reactive antibodies against feline alphacoronaviruses (4) and alphacoronaviruses of other animal species that might infect cats (4,12). Our data contrast a heavily affected area in China at the onset of the pandemic from which seropositivity levels of domestic cats ranged ≤15% (1), although those results were from relatively fewer tested cats and used a different assay.

Conclusions
During the first COVID-19 pandemic wave, reported seroprevalence levels in domestic cats ranged from 0.4% in the Netherlands (4) to 23% among cats in COVID-19–positive households in France (13). Similar seroprevalence levels in cats and humans in the same areas found by us and others suggest that in the absence of another known source (4,13; C. Schulz, unpublished data) (Appendix Table), SARS-CoV-2 infections in cats are most likely due to human-to-cat contact transmission.

Most natural SARS-CoV-2 infections of cats appear to run a mild or subclinical course, with respiratory or gastrointestinal clinical signs reported in confirmed natural infections (6–8). Evidence from experimental studies suggests that cats are susceptible to SARS-CoV-2 infection and can maintain the virus within a cat population and spill the infection backward or forward to other species (2,3,14). However, no evidence of cat-to-human transmission, nor of cat-specific mutations or variants of SARS-CoV-2, has been detected thus far (8,12,15). This finding contrasts reports on minks kept in farms, where mink-to-human spillback infections and mink-specific mutations have been reported (5). Although no evidence currently suggests that domestic cats play a role in the epidemiology of human SARS-CoV-2 infection, clinicians and veterinary practitioners should recommend that SARS-CoV-2–infected persons avoid close contact with their domestic cats and practice the same nonpharmaceutical prevention measures toward cats as they do to prevent human-to-human infection.

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Table 2. Comparison of diagnostic sensitivity and specificity of the RBD-ELISA and VNT in a study of SARS-CoV-2 seroprevalence among domestic cats during the first pandemic wave, Europe, April–August 2020*

<table>
<thead>
<tr>
<th>Test results</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBD-ELISA sensitivity, % (95% CI)</td>
<td>90.6 (90.0–91.2)</td>
</tr>
<tr>
<td>RBD-ELISA specificity, % (95% CI)</td>
<td>99.8 (99.8–99.9)</td>
</tr>
<tr>
<td>No. positive, % (95% CI), n = 2,160</td>
<td></td>
</tr>
<tr>
<td>RBD-ELISA and VNT</td>
<td>87 (4.0; 3.2–4.9)</td>
</tr>
<tr>
<td>RBD-ELISA only</td>
<td>92 (4.3; 3.5–5.2)</td>
</tr>
<tr>
<td>VNT only</td>
<td>96 (4.4; 3.6–5.4)</td>
</tr>
</tbody>
</table>

*A total of 5 samples were positive with RBD-ELISA and negative with VNT; 9 samples were positive with VNT but negative with RBD-ELISA. RBD-ELISA, receptor-binding domain–specific ELISA; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; VNT, virus neutralization test.

About the Author
Dr. Schulz is a postdoctoral researcher at University of Veterinary Medicine Hannover, Hannover, Germany. Her research interests include the pathogenesis and epidemiology of emerging and vectorborne diseases. Dr. Martina is a senior researcher at Artemis One Health Research Foundation, Delft, the Netherlands. His research interests include the pathogenesis of and intervention strategies against emerging virus infections.

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etymologia revisited

Zika [zēkə] Virus

Zika virus is a mosquito-borne positive-sense, single-stranded RNA virus in the family Flaviviridae, genus Flavivirus that causes a mild, acute febrile illness similar to dengue. In 1947, scientists researching yellow fever placed a rhesus macaque in a cage in the Zika Forest (zika meaning “overgrown” in the Luganda language), near the East African Virus Research Institute in Entebbe, Uganda. A fever developed in the monkey, and researchers isolated from its serum a transmissible agent that was first described as Zika virus in 1952. It was subsequently isolated from a human in Nigeria in 1954. From its discovery until 2007, confirmed cases of Zika virus infection from Africa and Southeast Asia were rare. In 2007, however, a major epidemic occurred in Yap Island, Micronesia. More recently, epidemics have occurred in Polynesia, Easter Island, the Cook Islands, and New Caledonia.

Sources

https://wwwnc.cdc.gov/eid/article/20/6/et-2014_article
**Increased Incidence of Melioidosis in Far North Queensland, Queensland, Australia, 1998–2019**

Simon Smith, Peter Horne, Sally Rubenach, Richard Gair, James Stewart, Lee Fairhead, Josh Hanson

During January 1998–December 2019, the annual incidence of melioidosis in Far North Queensland, Queensland, Australia, more than doubled. Because climate and prevalence of predisposing medical conditions remained stable during that time, we hypothesize that the increased incidence was caused by urban expansion and increased construction, resulting in greater exposure to *Burkholderia pseudomallei*.

*Burkholderia pseudomallei*, an environmental gram-negative bacterium, causes the disease melioidosis. Although infection is frequently asymptomatic, melioidosis may be rapidly fatal for patients with underlying conditions that increase the risk for invasive disease. Modeling suggests that *B. pseudomallei* is ubiquitous in the tropics and that the global burden of disease is expected to rise (1). Indeed, increased melioidosis incidence has been documented in some countries (2). Although this increase may be associated with improved diagnostic capacity, it may also be explained by a growing burden of predisposing concurrent medical conditions or by greater *B. pseudomallei* exposure from environmental disruption (3,4). Changing weather patterns also have the potential to increase melioidosis incidence (5).

*Burkholderia pseudomallei* is endemic to Far North Queensland (FNQ), a region in the northernmost part of the state of Queensland, Australia (Figure 1). Incidence of melioidosis in the Torres Strait Islands in the region’s north is among the highest reported in published series of melioidosis cases in Australia (4,6). During the past 20 years, the FNQ population has grown rapidly, predominantly in the city of Cairns, the region’s major industrial hub, and in the nearby towns (Cairns area, in and around Cairns). This growth has necessitated substantial expansion of local infrastructure, including 2-phase development of a large motorway on the city’s southern outskirts during 2011–2017. Surveillance data suggest that this development coincided with a marked increase in the local incidence of melioidosis, primarily in the Cairns area. We aimed to determine if there was any temporospatial association between the motorway construction and the increasing incidence of melioidosis in the region or if there were other possible explanations for any observed change, with a particular focus in the Cairns area.

**The Study**

Cairns Hospital is the sole public microbiological service provider for FNQ, a region of >380,000 km² (7). We reviewed all culture-confirmed cases of *B. pseudomallei* infection identified in the hospital’s laboratory during January 1, 1998–December 31, 2019. Clinical details of each case were recorded as described by J.D. Stewart et al. (4); predisposing conditions were diabetes mellitus (glycated hemoglobin ≥6.5%), hazardous alcohol use, chronic lung disease, chronic renal disease, and immunosuppression. We used data from the Australian Bureau of Statistics to calculate disease incidence and from the Australian Bureau of Meteorology to record local climatic factors including rainfall, temperatures, cloud cover, dew points, and cyclones. For our analyses we used Stata version 14.2 statistical software (https://www.stata.com) and determined trends over time by using an extension of the Wilcoxon rank-sum test and using year of presentation as a continuous variable (8). We constructed maps by using MapInfo Pro 2019 Geographic Information System software.

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in the absence of a clear occupational or recreational exposure, we used participants’ residential addresses as the site of \textit{B. pseudomonas} exposure. The study was approved by the Far North Queensland Human Research Ethics Committee (HREC/15/QCH/46-977).

A total of 297 cases of melioidosis were diagnosed during the study period, of which 284 were acquired from FNQ and included in our analysis. The mean annual incidence in FNQ increased from 4.0 (95% CI 2.7–5.2) cases/100,000 population during 1998–2002 to 9.9 (95% CI 4.9–14.9) cases/100,000 population during 2012–2015.

### Table 1. Incidence, predisposing conditions, and outcomes of locally acquired melioidosis cases in Far North Queensland, Queensland, Australia, January 1998–December 2019

<table>
<thead>
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<tbody>
<tr>
<td>Far North Queensland population, mean (1998–2019)</td>
<td>220,814</td>
<td>232,598</td>
<td>256,852</td>
<td>272,055</td>
<td>283,178</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No. cases</td>
<td>44</td>
<td>41</td>
<td>31</td>
<td>56</td>
<td>112</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Annual incidence, cases/100,000 population, mean (95% CI)</td>
<td>4.0 (2.7–5.2)</td>
<td>3.5 (1.8–5.2)</td>
<td>3.0 (0–6.2)</td>
<td>5.1 (0.6–9.7)</td>
<td>9.9 (4.9–14.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age, y, median (interquartile range)</td>
<td>46 (32–58)</td>
<td>52 (40–63)</td>
<td>51 (38–62)</td>
<td>49 (42–64)</td>
<td>55 (47–65)</td>
<td>0.001</td>
</tr>
<tr>
<td>Predisposing condition, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any†</td>
<td>73</td>
<td>85</td>
<td>84</td>
<td>82</td>
<td>90</td>
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<tr>
<td>Diabetes mellitus</td>
<td>50</td>
<td>56</td>
<td>58</td>
<td>44</td>
<td>58</td>
<td>0.59</td>
</tr>
<tr>
<td>Hazardous alcohol use</td>
<td>34</td>
<td>39</td>
<td>45</td>
<td>46</td>
<td>31</td>
<td>0.60</td>
</tr>
<tr>
<td>Chronic lung disease</td>
<td>7</td>
<td>12</td>
<td>16</td>
<td>14</td>
<td>16</td>
<td>0.13</td>
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<tr>
<td>Chronic kidney disease</td>
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<td>15</td>
<td>15</td>
<td>23</td>
<td>17</td>
<td>0.48</td>
</tr>
<tr>
<td>Immunosuppression</td>
<td>7</td>
<td>12</td>
<td>16</td>
<td>20</td>
<td>13</td>
<td>0.21</td>
</tr>
<tr>
<td>Bacteremia, %</td>
<td>70</td>
<td>68</td>
<td>77</td>
<td>77</td>
<td>68</td>
<td>0.85</td>
</tr>
<tr>
<td>Case-fatality rate, %</td>
<td>27</td>
<td>15</td>
<td>3</td>
<td>11</td>
<td>9</td>
<td>0.004</td>
</tr>
</tbody>
</table>

*p value for trend calculated by using annual data with year as a continuous variable.
†Incomplete access to patient charts from early in the study period is likely to lead to overestimation of the proportion of cases with no predisposing factor.
during 2016–2019 (p<0.001) (Table 1). In the Cairns area, incidence rose from 0.6 (95% CI 0.1–1.1) cases/100,000 population during 1998–2002 to 6.6 (95% CI 3.0–10.2) cases/100,000 population during 2016–2019 (p<0.001) (Table 2; Figure 1).

During the study period, the proportion of patients in FNQ with different predisposing conditions for melioidosis did not change. The proportion of bacteremic patients also remained stable (Table 1). The case-fatality rate declined during the study period (Table 1).

In the Cairns area, where increased incidence was more marked, the small increases in mean temperature, cloud cover, and dew points in the final period of the study did not reach statistical significance. During the study period, 14 cyclones came within 200 km of Cairns, but only 1 occurred during 2016–2019 (p = 0.86) (Appendix, https://wwwnc.cdc.gov/EID/article/27/12/21-1302-App1.pdf).

Of the 284 cases included in the study, 111 (39%) were in the Cairns area; only 3 of these patients reported having an occupation in construction. Before commencement of the southern motorway expansion in the Cairns area in 2011, only 1/19 (5%) cases in the Cairns area were within 1,000 m of the existing road and 2/19 (11%) were within 2,000 m. However, after January 2012, a total of 92/168 (55%) cases occurred in the Cairns area, of which 15/92 (16%) were within 1,000 m of the highway construction and 27/92 (29%) within 2,000 m (Figure 2).

Conclusions
The incidence of melioidosis in FNQ is rising, increasing during the study period by ≥10-fold in the Cairns area. The proportion of bacteremic patients has not changed, suggesting improved diagnosis. Similarly, we found no statistically significant change in climate or frequency of cyclones. The proportion of patients who had the common predisposing conditions remained similar. However, urban expansion may be contributing because almost a third of cases in the past 8 years of the study period occurred within 2,000 m of development of a large motorway. Of note, the motorway is built predominantly through alluvial plain soils with moderate clay content and poor drainage, which favor *B. pseudomallei* growth (9).

Increased rainfall, dew points, cloud cover, and temperatures have been associated with increased melioidosis cases; however, these climatic factors were stable over our study period (5). Cyclones have been linked to increased melioidosis cases; however, we did not observe that association in FNQ (10). Indeed, since 2015 when melioidosis incidence in the Cairns area sharply increased, there has been only 1 cyclone within 200 km of the area.

Why the rates of bacteremic melioidosis remain higher in FNQ than in other parts of Australia remains unclear (11). The higher rates may be partly explained by fewer diagnoses of cutaneous disease in rural and remote communities; however, skin swab samples are frequently taken to identify other pathogens, and skin and soft tissue *B. pseudomallei* infections are uncommon in urban areas, where most new cases have been identified (12). Virulence factors in local *B. pseudomallei* strains may contribute (13). Despite the increasing incidence, the overall case-fatality rate from melioidosis in FNQ decreased significantly during the study period, which can probably be explained by early recognition and prompt access to multimodal intensive care unit support.

Among the limitations of our study, data collection was predominantly retrospective; in addition, in the absence of clear inoculation with *B. pseudomallei*, we assumed residential addresses to be the sites of

Table 2. Incidence, predisposing conditions, and outcomes of melioidosis cases near Cairns, Queensland, Australia, January 1998–December 2019

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cairns area population, mean</td>
<td>200,351</td>
<td>206,342</td>
<td>228,504</td>
<td>243,389</td>
<td>253,841</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No. cases</td>
<td>6</td>
<td>9</td>
<td>4</td>
<td>25</td>
<td>67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Annual incidence, cases/100,000 population,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean (95% CI)</td>
<td>0.6 (0.1–1.1)</td>
<td>0.9 (0.1–1.6)</td>
<td>0.4 (0–0.9)</td>
<td>2.5 (0–5.8)</td>
<td>6.6 (3.0–10.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age, y, median (interquartile range)</td>
<td>45 (30–62)</td>
<td>65 (55–69)</td>
<td>49 (39–58)</td>
<td>56 (43–66)</td>
<td>56 (49–66)</td>
<td>0.56</td>
</tr>
<tr>
<td>Predisposing condition, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any††</td>
<td>100</td>
<td>89</td>
<td>75</td>
<td>84</td>
<td>87</td>
<td>0.38</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>67</td>
<td>44</td>
<td>50</td>
<td>40</td>
<td>47</td>
<td>0.69</td>
</tr>
<tr>
<td>Hazardous alcohol use</td>
<td>67</td>
<td>22</td>
<td>75</td>
<td>40</td>
<td>28</td>
<td>0.046</td>
</tr>
<tr>
<td>Chronic lung disease</td>
<td>0</td>
<td>22</td>
<td>25</td>
<td>24</td>
<td>18</td>
<td>0.86</td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>17</td>
<td>22</td>
<td>0</td>
<td>32</td>
<td>18</td>
<td>0.99</td>
</tr>
<tr>
<td>Immunosuppression</td>
<td>33</td>
<td>44</td>
<td>0</td>
<td>24</td>
<td>19</td>
<td>0.25</td>
</tr>
<tr>
<td>Bacteremia, %</td>
<td>67</td>
<td>100</td>
<td>100</td>
<td>84</td>
<td>72</td>
<td>0.18</td>
</tr>
<tr>
<td>Case-fatality rate, %</td>
<td>0</td>
<td>33</td>
<td>0</td>
<td>8</td>
<td>10</td>
<td>0.60</td>
</tr>
</tbody>
</table>

*p value for trend value calculated using annual data with year as a continuous variable.
†Limited access to charts is likely to result in incomplete documentation of risk factors from early in the study period.

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exposure. Domestic gardens are a source of melioidosis in Australia, but it is possible that unrecorded patient movements may have resulted in exposure to \textit{B. pseudomallei} elsewhere (14). Additional confounding factors that increase the risk for melioidosis (e.g., socioeconomic disadvantage) may help explain regional variations in incidence, although the local geographic distribution of this socioeconomic disadvantage has not substantially changed in the past 20 years (15). In conclusion, although host factors and climate continue to influence the risk of acquiring melioidosis, we hypothesize that urban expansion and construction in soils harboring \textit{B. pseudomallei} may explain the recent rapid increase in Far North Queensland, Australia.

\textbf{Figure 2.} Cases of melioidosis occurring near to a motorway upgrade in southern Cairns, Far North Queensland, Queensland, Australia, 1998–2019.
About the Author
Dr. Smith is an infectious diseases and general internal medicine physician in Cairns, Queensland, Australia. His research interests include melioidosis, leptospirosis, and management of severe clinical manifestations of tropical diseases.

References


Address for correspondence: Simon Smith, Department of Medicine, Cairns Hospital, Cairns, QLD 4870, Australia; email: simon.smith2@health.qld.gov.au
We performed a large-scale severe acute respiratory syndrome coronavirus 2 screening campaign using 2 PCR-based approaches, coupled with variant genotyping, aiming to provide a safer environment for employees of Federal University in Curitiba, Brazil. We observed the rapid spread of the Gamma variant of concern, which replaced other variants in <3 months.

Ongoing screening for active severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections, coupled with contact tracing, can efficiently reduce viral transmission within the community (1). However, as new and more transmissible variants emerge, an increased number of cases can be observed across affected regions. This increase demands immediate action, such as consistent, uninterrupted genomic surveillance for regular evaluation of vaccination approaches (2), to curb virus spread.

Brazil has reaped the consequences of lack of genomic surveillance in a context of high seroprevalence. The variant of concern (VOC) Gamma (B.1.1.28/P.1) emerged in the city of Manaus, Brazil, at a time when three quarters of the population had tested positive for antibodies; it proceeded to spread across the city (3). The Gamma variant was able to replace its predecessor, the variant of interest (VOI) B.1.1.28/P.2, previously the dominant lineage in the region (4).

Routine SARS-CoV-2 mass screening programs of asymptomatic persons and follow-up genotyping of samples are necessary measures to control the number of cases and prevent further infection surges (5,6). Simplified approaches, such as multiplex quantitative PCR, provide a feasible, cost-effective way to discriminate samples and prioritize whole-genome sequencing efforts (5,7). To provide a safer environment for the university community, we performed a large-scale screening for SARS-CoV-2 infections and VOCs in the Federal University of Paraná (Curitiba, Brazil) community. The study was approved by the University Research Ethics Committee (approval no. CAAE: 31687620.2.0000.0096).

The Study
During October 10, 2020–May 24, 2021, asymptomatic and mildly symptomatic persons within the community of the Federal University of Paraná were called for voluntary participation through social media and email. Eligible participants were members of the academic community (students, technicians, professors, or outsourced employees) or their relatives (grandparents, parents, siblings, or children) or household members. Saliva samples were self-collected by using an individually wrapped plastic drinking straw, transferred to a prelabeled 2.0 mL microtube, and stored at 4°C. Samples were transported to the laboratory in <1 h; total turnaround time to results was <48 h.

Samples were homogenized and allowed to settle for 30 min or centrifuged for 2 min (2,000 × g). A quantity of 200 µL from each specimen was

Author affiliations: Brazilian Biosciences National Laboratory, Brazilian Center for Research in Energy and Materials, Campinas, Brazil (D. Adamoski); Universidade Federal do Paraná, Curitiba, Brazil (D. Adamoski, J. Carvalho de Oliveira, A.C. Bonatto, R. Wassem, M.B. Nogueira, S.M. Raboni, E.S. Trindade, E.M. de Souza, D.F. Gradia)

DOI: https://doi.org/10.3201/eid2712.211326

1These authors were co–principal investigators.
2Members of the group are listed at the end of this article.
then pooled (8) in groups of 5. We performed RNA extraction by using an automated magnetic EXTRACTA–RNA and DNA Viral kit (Loccus Biotecnologia, https://loccus.com.br). We performed amplification in 2 ways: on a QuantStudio5 instrument (Thermo Fisher Scientific, https://www.thermofisher.com) using AllPlex nCov-2019 reverse transcription PCR Master Mix Kit (SeeGene, https://www.seegene.com) (1) or Molecular SARS-CoV-2 EDx (Bio-Manguinhos/FioCruz, https://www.bio.fiocruz.br) (2). If the pool rendered a positive result, we reprocessed samples individually.

We further evaluated positive samples by using 2 probe-based genotyping systems to detect VOCs. The first one was the Vogels et al. (7) multiplex approach to detect Spike Δ69–70 and Orf1a Δ3675–3677 deletions as an outcome for distinguishing Alpha, Beta, or Gamma and wild-type or other lineages (7). For this approach, we also included the Centers for Disease Control and Preventon N1 target and defined a cycle threshold (Ct) of <28 on this particular target to evaluate the gene dropouts.

The second approach involved 3 allelic discrimination TaqMan assays (Thermo Fisher Scientific). The proposed readout was P.1 (K417T, N501Y, and E484K), P.2 (only E484K), B.1.1.7 (only N501Y), B.1.351 (N501Y and E484K, failure for K417T assay), and wild-type or others for the absence of mutated alleles (9). The discriminating power of this second assay made it possible to distinguish the B1.1.28/P.2 from the wild-type and the Beta/Gamma variants. We performed both assays by using GoTaq Probe 1-Step reverse transcription quantitative PCR System (Promega, https://www.promega.com) in the same instrument.

A total of 16 collection dates were recorded; 12,558 examinations were processed (Table) from the 7,249 persons who attended because some participants engaged in >1 day of collection. The number of attendees per collection date ranged from 162 to 1,737. The overall prevalence rate was 1.28% (161/12,558). Comparing these numbers to cases in the state of Paraná by the epidemiologic week of diagnosis (Figure 1, panel A), we found prevalence similar to the prevalence rate at the beginning of the state’s second wave of SARS-CoV-2 infections (Figure 1, panel A).

We also evaluated all SARS-CoV-2-positive cases by using multiplex and singleplex genotyping approaches (Figure 2). From all 161 positive cases evaluated, the Vogels et al. (7) multiplex assay was invalidated in 46 (28.6%) against 50 (31.1%) in a Thermo Fisher 3-assay allelic detection approach because of the high Ct values. Comparing the original Ct value of detection, performance depreciated in samples with Ct >30 (Figure 2, panel B), as stated in the Thermo Fisher manual. Nevertheless, all genotyped cases were concordant between the 2 assays, considering that the Vogels et al. (7) assay alone does not discriminate between wild-type and B1.1.28/P.2 VOI.

Detection of the Gamma variant occurred on January 21, 2021, <2 weeks after the collapse of the healthcare system in Manaus. Prevalence of the Gamma variant was 9.1% on this date and increased to 42.9% 2 weeks later (Figure 1, panel B). A possible explanation for this scenario is the increased transmission of this variant in >20% compared with the wild-type transmission rate (10), which is supported by the observation of a reproduction number of 1.5 in Paraná (http://shiny.leg.ufpr.br/elias/covid19time) in the weeks before the death peak, the highest reproduction number observed during the pandemic. This increase in cases could be correlated with the subsequent collapse of the Curitiba city healthcare system and a surge of coronavirus disease deaths in the Paraná state, reaching values >5% in the subsequent weeks. A similar scenario was also observed in Manaus, the origin of the Gamma variant; both SARS-CoV-2 cases and excess of burials in the city reached their highest levels during the pandemic to that point (4). Two factors could explain those observed surges: increased lethality of the Gamma variant—which is not yet defined (10)—and the actual collapse of healthcare systems, leading to poorer patient support. When testing activities resumed after the healthcare collapse, all cases became Gamma variant, completely displacing B1.1.28/P.2 VOI and wild-type cases in 3 months.

Table. Collection dates, engagement, and positivity rates for severe acute respiratory syndrome coronavirus 2 infection, Curitiba, Brazil

<table>
<thead>
<tr>
<th>Date</th>
<th>Total tested</th>
<th>No. (%) positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>2020 Oct 2</td>
<td>275</td>
<td>0</td>
</tr>
<tr>
<td>2020 Oct 19</td>
<td>279</td>
<td>0</td>
</tr>
<tr>
<td>2020 Nov 6</td>
<td>510</td>
<td>6 (1.18)</td>
</tr>
<tr>
<td>2020 Nov 24</td>
<td>1,265</td>
<td>34 (2.69)</td>
</tr>
<tr>
<td>2020 Dec 8</td>
<td>1,070</td>
<td>17 (1.59)</td>
</tr>
<tr>
<td>2021 Jan 12</td>
<td>1,692</td>
<td>23 (1.36)</td>
</tr>
<tr>
<td>2021 Jan 26</td>
<td>1,737</td>
<td>14 (0.81)</td>
</tr>
<tr>
<td>2021 Feb 9</td>
<td>1,615</td>
<td>16 (0.99)</td>
</tr>
<tr>
<td>2021 Mar 29</td>
<td>196</td>
<td>1 (0.51)</td>
</tr>
<tr>
<td>2021 Apr 12</td>
<td>157</td>
<td>4 (2.55)</td>
</tr>
<tr>
<td>2021 Apr 20</td>
<td>872</td>
<td>2 (0.23)</td>
</tr>
<tr>
<td>2021 Apr 26</td>
<td>162</td>
<td>4 (2.47)</td>
</tr>
<tr>
<td>2021 May 4</td>
<td>884</td>
<td>12 (1.36)</td>
</tr>
<tr>
<td>2021 May 10</td>
<td>177</td>
<td>1 (0.56)</td>
</tr>
<tr>
<td>2021 May 18</td>
<td>1,431</td>
<td>20 (1.4)</td>
</tr>
<tr>
<td>2021 May 24</td>
<td>236</td>
<td>7 (2.97)</td>
</tr>
<tr>
<td>Total</td>
<td>12,558</td>
<td>161 (1.28)</td>
</tr>
</tbody>
</table>
Conclusions

Analysis of saliva in pools as described in this study offers an inexpensive and easy-to-implement asymptomatic screening strategy. Thus, given the high rates of SARS-CoV-2 transmission, the risk for asymptomatic coronavirus disease spread and the importance of social distancing should continue to be stressed to the public until the vaccine is viable for large-scale application. Our mass testing program was intended to be accessible (every test was free-of-charge for the participant), reliable (all participants received their results and positive persons had a follow-up opportunity), and aimed to reach all social strata within the academic community (from professors to outsourced employees, which consisted mainly of socially, economically, and ethnically vulnerable groups), which are key characteristics of a strong mass testing system (11).
We found that both multiplex PCR and singleplex PCR approaches were valuable tools to evaluate the proportion of variants within genomic surveillance and were faster and less expensive than whole-genome sequencing approaches. Although those methods do not serve as substitutes for whole-genome sequencing, they could be an essential method to screen and select samples for further variant classification. Nevertheless, those approaches could demonstrate rapid spread of new variants and predict surges of SARS-CoV-2 infections, acting as a lighthouse for far-reaching public health decisions.

This article was preprinted at https://www.medrxiv.org/content/10.1101/2021.06.18.21258649v1.

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About the Author

Dr. Adamski is a substitute professor of genetics at the Curitiba Campus from Federal University of Paraná, now positioned as Researcher at National Laboratory of Biosciences. His primary research interests include mRNA binding proteins, splicing regulation, and genomics.

References


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Heartland virus (HRTV; Phenuviridae, Bandavirus) is an emerging human pathogen initially isolated from patients in Missouri, USA, during 2009 (1). Since then, >50 known human cases have been identified in Arkansas, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Missouri, North Carolina, Oklahoma, and Tennessee (2–5). Amblyomma americanum, the lone star tick, has been implicated in HRTV transmission and maintenance (6–8). Small-sized and medium-sized mammals and ground dwelling birds, such as wild turkeys (Meleagris gallopavo), serve as hosts for immature ticks. Adult ticks feed primarily on large mammals, such as coyotes (Canis latrans) and white-tailed deer (Odocoileus virginianus). Ticks at all 3 active developmental stages will bite humans (9). Serologic evidence in mammal hosts, including white-tailed deer, indicates that HRTV is distributed primarily in the Midwest and southeast United States, as well as the northeastern Atlantic coast (10–12).

During 2018, New York State Department of Health (NYSDOH) epidemiologists were notified that HRTV RNA was detected in an A. americanum nymph removed from a resident of Long Island, New York, USA. This infected tick was tested at the University of Massachusetts (https://www.tickreport.com).

In response, the NYSDOH and Suffolk County Department of Health Services conducted tick surveillance and performed HRTV serologic analysis on the person from whom the tick was removed. Analysis was also performed for a hunter-harvested white-tailed deer in Suffolk County.

The Study
Officials with the NYSDOH and Suffolk County Department of Health Services contacted a Long Island, New York, resident for a follow-up investigation after receiving notification that a tick removed from the resident and submitted for comprehensive pathogen testing was positive for HRTV RNA. The resident, a man in his 60s, removed the tick on August 8, 2018, and recalled having a low-grade fever (maximum temperature 100.5°F) and fatigue for 5 days beginning on August 15, 2018. He noted no other symptoms.

Serum was provided at multiple time points for serologic analysis. We tested serum samples by using a standard 90% plaque reduction neutralization test (PRNT90) for HRTV (strain M12-66) (8), provided by the Centers for Disease Control and Prevention. We tested samples at Wadsworth Center, NYSDOH, and results were confirmed by the Centers for Disease Control and Prevention. Neutralizing antibody titers were 1:20, 1:160, and 1:160 for samples collected at 8, 50, and 96 days after symptom onset (15, 57, and 103 days after removal of the tick), respectively, indicative of a recent infection with HRTV.


DOI: https://doi.org/10.3201/eid2712.211426

1These authors contributed equally to this article.
We initiated standardized drag and flag sampling of host-seeking *A. americanum* ticks on public lands for arbovirus surveillance during 2016, before HRTV detection. We found that 132 pools (containing 475 nymphs and 437 adults) from 4 Suffolk County locations were negative for HRTV by real-time reverse transcription PCR using established protocols (8). During 2018, tick surveillance at 5 locations yielded 102 pools (969 adults); all were negative for HRTV.

Increased efforts during the public health investigation conducted on August 23 and 24, 2018, yielded an additional 113 *A. americanum* ticks (92 larvae and 21 nymphs) from a location where tick exposure potentially occurred. All ticks collected during the investigation were negative for HRTV. No ticks were found during sampling of the property surrounding the residence of the case-patient.

During 2019 and 2020, tick surveillance in the towns of Brookhaven and Riverhead yielded 1,123 pools of *A. americanum* ticks (2,788 adults and 6,728 nymphs) (Figure 1). We found that 3 pools of unengorged nymphs collected from the Brookhaven site on June 14 (n = 1) and June 24 (n = 2), 2019, and 2 pools of unengorged nymphs collected from the same location on July 25 and August 5, 2020, were positive for HRTV RNA. We isolated virus from 2 tick pools after incubation on Vero cells. We found that testing of >1,100 *Ixodes scapularis* ticks (199 pools) collected during the surveillance campaign in Suffolk County, during 2018–2020, were negative for HRTV.

We extracted RNA from isolates by using established protocols (13). We developed primer pairs to amplify the small, medium, and large RNA segments by using a One-Step Superscript III Reverse Transcription PCR with Platinum Taq (Life Technologies, https://www.thermofisher.com) (Table 1). We performed 3 separate reactions using 5 µL of RNA, 1 µL of polymerase, and 0.2 µmol/L final concentration of primer pairs in a total reaction volume of 50 µL. We amplified products with the following thermocycler...
Table 1. Primer pairs for amplifying small, medium, and large RNA segments of Heartland virus genome

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence, 5′→3′</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRTV_S_F</td>
<td>TTACAAAAAGACCGCCCTTTGAAATATCA</td>
<td>Small</td>
</tr>
<tr>
<td>HRTV_S_R</td>
<td>CATCAAGGATGACTGCTGGCTGCAAT</td>
<td>Small</td>
</tr>
<tr>
<td>HRTV_M_F</td>
<td>AAGTAGGGTAAACCGGTAATCCACTGAGAT</td>
<td>Medium</td>
</tr>
<tr>
<td>HRTV_M_R</td>
<td>ACAAGGACCGCTATACAATTGGAAAAAC</td>
<td>Medium</td>
</tr>
<tr>
<td>HRTV_L1_F</td>
<td>GACGTCAGTGAATTTAGAAGCTTT</td>
<td>Large</td>
</tr>
<tr>
<td>HRTV_L1_R</td>
<td>CTATAGCTGCGCTGTGAGTTCTGC</td>
<td>Large</td>
</tr>
<tr>
<td>HRTV_L2_F</td>
<td>TGCAAGAGATGATGAGGACCTC</td>
<td>Large</td>
</tr>
<tr>
<td>HRTV_L2_R</td>
<td>AAGACCCTGCGATATCAACCTTAGG</td>
<td>Large</td>
</tr>
</tbody>
</table>

We conducted serologic testing of hunter-harvested white-tailed deer blood submitted for arbovirus serosurveys by using PRNT_{90} as described (14). We screened 686 serum samples at a dilution of 1:20 for neutralizing antibodies to HRTV (Figure 1) and serially diluted positive serum samples for endpoint titers. Overall, 9.8% of the deer were seropositive and had titers ranging from 1:20 to >1:640; 76% of the seropositive deer had titers >1:20. We tested 1,641 A. americanum ticks collected from 145 sampled deer for HRTV RNA but did not detect any virus.

Conclusions
Evidence of widespread HRTV transmission was demonstrated throughout Suffolk County, New York. Consistent with previous studies, A. americanum ticks were implicated in local transmission of HRTV. All positive pools were nymphal stage ticks, including the tick originally submitted for testing at the University of Massachusetts. Tick minimal infection rates ranged from 0% to 1.1%. It is unclear whether flat nymphs had acquired the virus as larvae feeding on viremic hosts, through cofeeding transmission, or transovarially because each of these modes has been demonstrated in the laboratory (7).

The lack of HRTV detection in adult ticks is notable if one considers that collections occurred at the same site across 3 seasons. Higher numbers of positive nymph pools were observed in Missouri, where 53/60 HRTV-positive tick pools collected at sites near the first described human cases were nymphs (6). Complete genome sequence analysis of the HRTV strains isolated during this study showed >98% amino acid identity to the strains isolated from patients in Missouri during 2009 (1) and a strain isolated in Tennessee during 2013 (2).

White-tailed deer are a sensitive sentinel model for many arboviruses, given their abundance, limited home range, and the frequency on which they are fed upon by hematophagous arthropods (10,11,14). Approximately 10% of the deer sampled during this study were seropositive against HRTV. Our serologic testing strategy differed from those of previous studies by using a more stringent PRNT_{90}. Suffolk County deer seropositive rates were similar to those reported in Vermont (10%), Maine (11%), and Florida (4%) deer (12). The rates are lower than those reported for deer tested in midwestern and southeastern states, areas with burgeoning populations of A. americanum ticks (10,11). To date, no competent vertebrate host, including deer, has been implicated in HRTV amplification (15).

Table 2. Pairwise genetic distance (% of variable positions) among fully sequenced isolates of Heartland virus, by segment (small/medium/large), for nucleotide (below diagonal) and amino acid (above diagonal) sequences

<table>
<thead>
<tr>
<th>Isolate (reference)</th>
<th>NY20-1782</th>
<th>NY20-1820</th>
<th>MO 2009-P1</th>
<th>MO 2009-P2</th>
<th>TN 2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>NY20-1782, MZ440344/MZ440346</td>
<td>0/0</td>
<td>0.54/0.46/0.43</td>
<td>0.72/1.67/0.34</td>
<td>1.26/0.46/0.19</td>
<td></td>
</tr>
<tr>
<td>/MZ440345*</td>
<td>0/0.03/0.61</td>
<td>0.54/0.46/0.43</td>
<td>0.72/0.43/0.34</td>
<td>1.26/0.46/0.19</td>
<td></td>
</tr>
<tr>
<td>NY20-1820, MZ440341/MZ440343/MZ440342*</td>
<td>1.41/1.43/1.84</td>
<td>1.41/1.46/2.45</td>
<td>0.54/1.77/0.77</td>
<td>1.26/0.37/0.62</td>
<td></td>
</tr>
<tr>
<td>MO 2009-P1 (1), JX005842.1/JX005844.1/</td>
<td>1.69/4.32/1.88</td>
<td>1.69/4.35/2.49</td>
<td>1.45/4.52/1.90</td>
<td>1.44/0.77/0.53</td>
<td></td>
</tr>
<tr>
<td>MO 2009-P2 (1), JX005843.1/JX005845.1/</td>
<td>2.34/2.45/1.98</td>
<td>2.34/2.48/2.50</td>
<td>2.06/2.22/2.00</td>
<td>2.57/4.91/1.95</td>
<td></td>
</tr>
<tr>
<td>TN 2013 (2), KJ740146.1/KJ740147.1/KJ740148.1*</td>
<td>2.34/2.45/1.98</td>
<td>2.34/2.48/2.50</td>
<td>2.06/2.22/2.00</td>
<td>2.57/4.91/1.95</td>
<td></td>
</tr>
</tbody>
</table>

*GenBank accession numbers.
Results from this study emphasize the need to include HRTV in surveillance programs wherever A. americanum ticks are distributed. Furthermore, clinicians should be aware of this pathogen and the potential for overlapping symptomologies (fever, fatigue, and loss of appetite) with other tickborne infections. Providers should request HRTV testing for patients who have clinical symptoms, including leukopenia and thrombocytopenia, and a history of tick exposure or travel to regions where A. americanum ticks are reported.

Acknowledgments
We thank Lauren Barlow and the Suffolk County Public Health staff for obtaining patient blood and submitting specimens and Dylan Bartlett, Lauren Rose, Anna Perry, and Jessica Stout for assisting with tick surveillance, processing, and testing. Cells for serologic assays and media production were provided by the Wadsworth Center Media and Tissue Culture Core. Genetic sequencing was conducted at the Wadsworth Center Applied Genomics Technology Core.

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References
Since the 2015 Zika virus outbreak in the Americas, transmission of this vectorborne disease has substantially decreased. But Zika virus doesn’t spread only through mosquito bites...it also spreads through sexual transmission, blood transfusions, breastfeeding, and even needlestick injuries in laboratories.

Stringent safety protocols minimize the risk of laboratory-associated exposures. But on rare occasions, researchers are accidently exposed to the disease they are trying to solve.

In this EID podcast, Dr. Susan Hills, a medical epidemiologist at CDC in Fort Collins, Colorado, describes the biosafety lessons exemplified by four cases of laboratory-associated Zika infection.

Visit our website to listen: https://go.usa.gov/xFZU2
As of August 2021, coronavirus disease (COVID-19) had caused >199 million cases and >4.2 million deaths worldwide (1). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes COVID-19, is being sequenced to document virus evolution and to inform vaccine efforts. In South Sudan, the COVID-19 index case was confirmed on April 4, 2020 (2); it was followed by 2 infection waves, in May–July 2020 and in February–March 2021 (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/27/12/21-1488-App1.pdf). As of August 3, 2021, a total of 11,063 cases and 119 deaths (1) had been reported in South Sudan. An earlier study from South Sudan reported that, after the second wave, 28% of the population showed serologic evidence of infection (3).

Chronic underdevelopment caused by prolonged conflicts has left South Sudan with a weak health system and population displacement. Large populations live in camps that may promote rapid spread and amplification of SARS-CoV-2, and poor socioeconomic conditions limit community-based COVID-19 prevention efforts. Monitoring the circulating viral genomic lineages in South Sudan is crucial, especially as vaccination is implemented and novel virus variants appear globally.

The Study

As part of South Sudan COVID-19 surveillance, samples were collected from community surveillance, point-of-entry screening, and sentinel site surveillance and tested for SARS-CoV-2 by real-time reverse transcription PCR (RT-PCR) at the National Public Health Laboratory (Juba, South Sudan) (4). During the second COVID-19 wave in February–March 2021 (Appendix Figure 1), we tested 56,014 samples for SARS-CoV-2; 6,645 samples tested positive (12% positivity). We selected a set of 70 (1%) of these positive samples for genomic sequencing with these inclusion criteria: diagnostic RT-PCR cycle threshold (Ct) values <31, from multiple locations (Figure 1), from new arrivals, from death cases, and from sites showing community transmission. We extracted nucleic acid from swab material and generated SARS-CoV-2 genome as previously described (5). A total of 45 complete genomes generated from samples collected in January–March 2021 showed a prevalence of 2 lineages: B.1.525 (Eta) and A.23.1 (Figure 1). The A.23.1 lineage, which was observed in October 2020 in Uganda (6) and has now spread globally to 26 countries, was one we observed in Juba and Nimule in early January 2021. The A.23.1 case-patients in Nimule, a South Sudan town on the border with Uganda (Figure 1), were travelers returning from Uganda. In Juba, the earliest-reported A23.1 case was in a traveler returning to South Sudan from Uganda. We detected A23.1 for only a short period; from the end of January to the end of March, we detected only B.1.525 genomes (Appendix Table). The B.1.525 lineage, reported earliest in

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DOI: https://doi.org/10.3201/eid2712.211488
the United Kingdom and Nigeria, has spread to 44 countries and is considered a variant of interest (7).

Phylogenetic analyses of the South Sudan genomes combined with the available global A.23.1 or B.1.525 genomes were performed to gain insight into the virus movement. The maximum-likelihood trees of both A.23.1 and B.1.525 genome sequences (Figure 2) suggested multiple importations of the strains into South Sudan; South Sudan strains belonged to several sublineages, rather than a single sublineage.

Both A.23.1 and B.1.525 lineages encoded changes in their spike protein (Appendix Figure 2) as well as other parts of the genome and substitutions or deletions in the nonstructural protein 6, open reading frame 3a and 8, and nucleocapsid genes (data not shown), which might be associated with higher transmission or immune evasion. Especially relevant, the A.23.1 genomes encoded spike P681R, which is adjacent to the small (S) 1/S2 furin cleavage site and is also present in the variant of concern B.1.617.2 (Delta) lineage, which is spreading in India and globally and may increase S1/S2 cleavage (10,11; B. Lubinski et al., unpub. data. http://biorxiv.org/lookup/doi/10.1101/2021.06.30.450632). A related P681H substitution is present in variants of concern B.1.1.7 (Alpha) and P.1 (Beta). The South Sudan B.1.525 genomes encoded a deletion in the N-terminal domain (NTD) at spike positions 69 and 79, which is also present in B.1.1.7 and many other global variants, and a deletion in the spike NTD in positions 141–146, which may help in evasion of host immune responses. The spike D614G substitution may alter the spike protein conformation; the Q677H substitution is near the furin cleavage site and may alter spike processing.

Conclusions
We describe the patterns of SARS-CoV-2 virus genomics in South Sudan in the second wave of infections during February–March 2021, showing circulation of B.1.525 (Eta) as well as the variant A.23.1. South Sudan faced high transmission of SARS-CoV-2 during this reporting period; our data suggest that the B.1.525 lineage spread widely and progressively increased in frequency in the country during the period. Data from Uganda and Rwanda retrieved from GISAID (https://www.gisaid.org) also showed the appearance of B.1.525 at this time.

A limitation of our study is that sample numbers are low and were limited by the challenges of procurement, shipment, and testing in a harsh and
Figure 2. Maximum-likelihood phylogenetic tree of severe acute respiratory syndrome coronavirus 2 viruses from South Sudan (red dots) and reference sequences. A) Lineage A.23.1. All sequences from South Sudan were combined with a subset of all available global A.23.1 genomes, algorithmically thinned. All available global A.23.1 genomes were retrieved from GISAID (https://www.gisaid.org) and aligned, and for the first genome, all genomes closer than 5 hamming distance were removed. This process was continued until the entire set was thinned. This global, thinned A.23.1 set was combined with all South Sudan A.23.1 genomes and used to infer the A.23.1 maximum-likelihood tree. The tree was rooted with the A.23 strain (UG109/PR_Amurug23(2020–08–14). B) Lineage B.1.525. The B.1.525 genome sequences were prepared in the same manner as those for A.23.1 except the hamming distance of 20. Maximum-likelihood phylogenetic trees were constructed in RaxML-NG (8) under the general time reversible plus gamma 4 plus invariable sites model as the best-fit model of substitution according to the Akaike information criterion determined by modelltestNG (9) and run for 100 pseudoreplicates and visualized using FigTree version 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree). For B.1.525, the tree was midpoint rooted for clarity. Scale bar indicates nucleotide substitutions per site.

resource-poor environment. Careful sample selection was performed to provide an unbiased description of the epidemic; however, not all positive samples yielded genome sequences. This lack of data could introduce bias in the reported genomes. Nonetheless, the study accurately describes SARS-CoV-2 lineages during the second wave of epidemic in South Sudan.

Substantial land-based traffic with neighboring countries makes it imperative to document the viruses circulating in this region. Careful monitoring of locally circulating viruses as vaccination becomes widespread is essential for interpreting vaccine function and for informing the healthcare systems whether the current vaccines are still a good match for the circulating viruses. We recommend continued genomic surveillance in South Sudan to help with public health responses, especially as new waves of infections come to the country and continent.

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References


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Want to stay updated on the latest news in *Emerging Infectious Diseases*? Let us connect you to the world of global health. Discover groundbreaking research studies, pictures, podcasts, and more by following us on Twitter at @CDC_EIDjournal.
Several new variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have emerged globally, most notably variants of concern Alpha (B.1.1.7) (1), Beta (B.1.351) (2), Gamma (P.1) (3), and most recently, Delta (B.1.617.2). Each variant is thought to pose an increased public health risk compared with the earlier wild-type strains that were circulating in 2020 because of >1 epidemiologic characteristics, such as higher transmissibility (4), greater immune escape properties toward antibodies from previous SARS-CoV-2 infection (5), lower response to current vaccines (6), or more severe outcomes or increased mortality rates (7). Detecting and monitoring these novel variants is essential in SARS-CoV-2 surveillance.

The Study

To assess the temporal epidemiologic dynamics among different variants of concern and identify spreading events and sources of SARS-CoV-2 cases detected in Finland, we began sequencing 400–1,000 virus samples per week collected during December 2020–May 2021 and analyzed the resulting genomes (n = 14,080), which are now available in the GISAID (https://www.gisaid.org) database. For quality control purposes, we removed all sequences with ≥2.0% gaps. We analyzed the resulting dataset (n = 9,160) with Pangolin (https://cov-lineages.org) (8) to identify lineages, from which we filtered Alpha and Beta variants for phylogenetic analyses. Each phylogenetic tree was computed from the filtered sequences and a global reference dataset consisting of 5 representative sequences, 1 sequence from the country of origin (England for Alpha, South Africa for Beta) and 4 randomly chosen from other countries containing the same lineage, for each date during December 2020–May 2021. The reference datasets included 841 genomes for Alpha variant and 775 genomes for Beta variant trees. We aligned sequences using MAFFT (https://mafft.cbrc.jp) (9) and removed gaps in the resulting alignments by trimming 50 characters from both the 5' and 3' ends.

We then used the aligned sequences to compute the trees with a SARS-CoV-2–specific version of IQ-TREE 2 (10) using ModelFinder to identify and use the optimal nucleotide substitution model,
performing 1,000 ultrafast bootstraps. We set the initial wild-type reference strain (GenBank accession no. NC_045512.2) as the outgroup. We assigned sequences to clusters using TreeCluster (11) based on an arbitrary branch length of 0.001 to identify major transmission chains. We collapsed clusters with ≤5 sequences for visualization purposes.

By May 2021, there had been 93,393 laboratory-confirmed SARS-CoV-2 infections reported in Finland (12); incidence peaks occurred in April and December 2020 and March 2021 (Appendix Figure 1, panel A, https://wwwnc.cdc.gov/EID/article/27/12/21-1631-App1.pdf). During this period, the weekly number of cases was as high as 4,900. National vaccinations began in late December 2020, and within 7 months, 3.5 million (62.8% of total population) persons had received first doses and 1.4 million (24.5% of total population) second doses (13). Seroprevalence remained low (<2%) until February 2021 (14) but increased because of growing vaccination coverage (Appendix Figure 1, panel B).

Throughout 2020, sequencing-based surveillance of the virus was conducted in the Hospital District of Helsinki and Uusimaa (HUS; Helsinki, Finland), which had the highest number of COVID-19 cases in the country (n = 21,742). Until December 18, 2020, only wild-type strains of SARS-CoV-2 had been detected, but the emergence of Alpha and Beta variants led to increased sequencing and sampling efforts at points of entry into Finland (i.e.,

![Figure 1. Phylogenetic tree of severe acute respiratory syndrome coronavirus 2 Alpha (B.1.1.7) variant clusters from Finland and sequence distribution. The tree (A) shows 86 clusters with ≥5 sequences (red circles), of which 84 contain 5,270 sequences sampled in Finland using TreeCluster, and 32 Finland singletons (white circles). The tree was constructed by using IQ-TREE 2 (10) with 1,000 ultrafast bootstraps. Each row in subsequent graphs is equivalent to a cluster and shows the number of sequences from Finland (B) and the proportion of sequences per region of Finland (C). Regions of Finland: 1, Åland Islands; 2, Central Finland Health Care District; 3, Central Ostrobothnia Hospital District; 4, East Savo Hospital District; 5, Hospital District of Helsinki and Uusimaa; 6, Hospital District of South Ostrobothnia; 7, Hospital District of Southwest Finland; 8, Kainuu Social and Health Care Joint Authority; 9, Kanta-Häme Hospital District; 10, Lääni-Pohja Healthcare District; 11, Lapland Hospital District; 12, North Karelia Hospital District; 13, North Ostrobothnia Hospital District; 14, North Savo Hospital District; 15, Päijät-Häme Hospital District; 16, Pirkanmaa Hospital District; 17, Satakunta Hospital District; 18, Social and Health Services in Kymenlaakso; 19, South Karelia Social and Health Care District; 20, South Savo Hospital District; 21, Vaasa Hospital District.](https://wwwnc.cdc.gov/EID/article/27/12/21-1631-App1.pdf)
SARS-CoV-2 Alpha and Beta Variants, Finland

Finland airports, harbors, land border crossing sites) starting in week 51 of 2020.

During December 2020–May 2021, a total of 14,080 SARS-CoV-2 genomes representing \( \approx 20.4\% \) of the PCR-confirmed SARS-CoV-2 infections (\( n = 65,921 \)) were sequenced. During this period, the Alpha variant (5,370 total detections) comprised 58.6% of all cases, and its proportion in weekly counts rapidly increased from 3 (6.0%) of 50 in week 51 of 2020 to 602 (69.1%) of 871 in week 11 of 2021 (Appendix Figure 2). The highest proportion of Alpha variant cases was 240 (82.2%) of 292 detections in week 17. Beta variant incidence rose later and at a slower rate (1,049 total detections, 19.5% of all cases); the proportion in weekly case counts rose from 2 (1.7%) in week 2 of 2021 to 181 (23.1%) by week 12. The proportions of Alpha and Beta variants started to diminish in week 13. Only 1 Gamma variant case was recorded, in week 10, and the first Delta variant samples in Finland were collected during week 17. In addition, several variants of interest (15) were detected beginning in early January 2021: B.1.429 (2 detections), B.1.525 (25 detections), B.1.526 (1 detection), B.1.617.1 (6 detections), and P.2 (1 detection). Of the variants being monitored (15), 18 cases of AT.1 and 29 of B.1.1.318 lineages were detected during this period.

The clustering analysis of Alpha variants (Figure 1) showed 86 distinct clusters, of which 84 contained 5,270 sequences from Finland (57.5% of all sequences). The 13 largest clusters from Finland (total \( n = \))

Figure 2. Phylogenetic trees of severe acute respiratory syndrome coronavirus 2 Beta (B1.351) variant clusters from Finland and sequence distribution. The tree (A) shows 76 clusters with \( \geq 5 \) sequences (red circles), of which 48 contain 898 sequences sampled in Finland using TreeCluster (11), and 23 Finland singletons (white circles) from 33. The tree was constructed by using IQ-TREE 2 (10) with 1,000 ultrafast bootstraps. Each row in subsequent panels is equivalent to a cluster and shows the number of sequences from Finland (B) and the proportion of sequences per region of from Finland (C). Regions of Finland: 1. Central Finland Health Care District; 2. East Savo Hospital District; 3. Hospital District of Helsinki and Uusimaa; 4. Hospital District of South Ostrobothnia; 5. Hospital District of Southwest Finland; 6. Kainuu Social and Health Care Joint Authority; 7. Kanta-Häme Hospital District; 8. Länsi-Pohja Healthcare District; 9. Lapland Hospital District; 10. North Karelia Hospital District; 11. North Ostrobothnia Hospital District; 12. North Savo Hospital District; 13. Päijät-Häme Hospital District; 14. Pirkanmaa Hospital District; 15. Satakunta Hospital District; 16. Social and Health Services in Kymenlaakso; 17. South Karelia Social and Health Care District; 18. South Savo Hospital District; 19. Vaasa Hospital District.
3,669, 69.6%) had 132–663 sequences each. We detected 32 singletons (0.6% of Alpha detections) from Finland, suggesting that the epidemic was largely seeded from a few introductions, which aligns with the super-spreading properties of SARS-CoV-2 epidemiology. Most Alpha sequences were from the HUS district (n = 3,476, 64.7% of cases). We included all available high-quality sequences from random populations from Finland and thus included data from both mild and severe cases. However, a proportion of the samples from the HUS region came from points of entry into Finland and other hospital districts. The proportions of these imported samples varied over the sampling period depending on travel restrictions and hospitalized case-patients, which may have led to nonrandomized sampling from the HUS region.

Beta variants formed 76 distinct clusters, of which 56 contained 910 sequences from Finland (9.9% of all sequences from Finland) (Figure 2). We also identified 33 singletons, of which 23 were from Finland (2.2% of Beta detections). In total, there might have been 79 introductions from other countries, which seeded 1 major cluster (≥100 Finland sequences) containing 167 sequences (15.9% of cases). Most Beta sequences were also from the HUS hospital district (n = 505, 48.1% of cases). Hospital district reports were based on data from the Finnish Institute for Health and Welfare (https://sampo.thl.fi), HUS, and Fimlab (https://fimlab.fi).

Conclusions
Altogether, our study shows both Alpha and Beta variants emerging early and rapidly beginning in December 2020. Most (98.2% Alpha, 86.8% Beta) formed clusters, and only a small proportion (0.6% Alpha, 2.2% Beta) were singletons. Because the singletons represent a small fraction of the sequences and many were transmitted directly from travelers, it is likely that a few introductions were able to seed the epidemic.

The Alpha and Beta variants dominated detected SARS-CoV-2 cases, although at lower numbers for Beta, during early 2021. Despite the rapid emergence of these variants, their incidence fell sharply (Appendix Figure 1, panel A). Incidence in Finland has been low compared with other countries in Europe, permitting use of more moderately restrictive prevention measures. Incidence, and therefore seroprevalence, remained relatively low until vaccines became available. Practices and policies enacted in Finland, including frequent testing, contact tracing, isolation, quarantine, and other nonpharmaceutical interventions, helped effectively interrupt chains of transmission, and ongoing national efforts have resulted in most of the population of Finland receiving at least the first vaccine dose. These findings suggest that with proper surveillance and preventative measures, along with moderate restriction compliance, the spread SARS-CoV-2 could be mitigated effectively.

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**etymologia revisited**

**Neospora caninum** [ne-os’ pa-rə ca-nin’ um]

From the *neo-* (Latin, “new”) + *spora* (Greek, “seed”) and *canis* (Latin, “dog”), *Neospora caninum* is a sporozoan parasite that was first described in 1984. It is a major pathogen of cattle and dogs but can also infect horses, goats, sheep, and deer. Antibodies to *N. caninum* have been found in humans, predominantly in those with HIV infection, although the role of this parasite in causing or exacerbating illness is unclear.

**Sources:**

https://wwwnc.cdc.gov/eid/article/25/6/et-2506_article
The genus Orthobunyavirus (family Peribunyaviridae) includes emerging arthropodborne viruses associated with human and animal disease worldwide (1). In 1966, orthobunyavirus Shuni virus (SHUV) was isolated from a cow, Culicoides midges, and a febrile child in Nigeria (2); SHUV recently emerged in Israel, where it has been associated with birth defects in ruminants (3). SHUV has been associated with neurologic disease in horses and wildlife (4,5) and was recently implicated in human cases of neurologic disease in South Africa (6). SHUV was detected in field-caught Culex theileri mosquitoes in the 1970s (5), and Culicoides midges have been suggested as vectors (7). We investigated mosquitoes collected in northeastern parts of South Africa to identify their potential as vectors of orthobunyaviruses in the Simbu serogroup of arboviruses, including SHUV.

The Study
We collected mosquitoes across 5 provinces of South Africa (Figure 1). Site selection was based on historical outbreaks of arboviruses, including SHUV, in animals (4,5) and humans (6). During January 2014–May 2017, we collected mosquitoes monthly; we performed additional collections in 2017 in and around the Kruger National Park (8). In 2018, we performed 1 collection per site during January–May.

We used multiple types of dry ice (carbon dioxide) baited traps: nets, CDC miniature light traps (https://www.johnwhock.com), and BG-Sentinel traps (https://www.bg-sentinel.com). We set traps during 3:30–6:00 PM and emptied them during 5:00–8:00 AM. We killed mosquitoes by freezing and then morphologically identified them to the species level. We pooled females (<50 individuals) by species, collection site, and month. We selected mosquitoes for screening from pools collected during January–June, which represents late summer and autumn, when arbovirus infections in animals and humans in South Africa increase. We obtained climate data from the South African Weather Service (http://www.weathersa.co.za).

For the virus assays, we produced homogenate pools by placing 5 sterile glass beads in microcentrifuge tubes containing 2 mL of reconstituted minimum essential medium, which we then vigorously shook and clarified. The resulted supernatant was stored at −80°C. To extract viral RNA from 200 μL homogenate, we used an RNeasy mini kit (QIAGEN, https://www.qiagen.com) according to the manufacturer’s instructions. We screened extracted RNA by using 2 PCRs, each targeting the nucleocapsid (NP) gene on the small (S) segment: a Simbu serogroup/orthobunyavirus–specific one-step TaqMan real-time reverse transcription PCR targeting a 152-bp fragment (4) and an SHUV nested real-time RT-PCR targeting a 460-bp fragment (9). In an attempt to obtain larger fragments, we performed an SHUV conventional PCR with published primers (10).

For mosquito barcoding (species identification), we extracted DNA from 50 μL of the homogenate by using a QIAGEN DNeasy Blood & Tissue Kit according
Mosquito Vectors for Shuni Virus, South Africa

All products of the expected size were sequenced by Sanger sequencing at the Forestry Agriculture Bioinformatics Institute, University of Pretoria (Pretoria, South Africa). We compared the resulting sequences by using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) with sequences available from GenBank, including SHUV strains from South Africa, Nigeria, and Israel and other representative members of Simbu serogroup. For the cytochrome oxidase gene, we selected representative mosquito sequences from GenBank and BOLD (https://v3.boldsystems.org). We compiled multiple sequence alignments by using MAFFT (https://mafft.cbrc.jp/alignment/software), produced maximum-likelihood trees by using MEGA 7.0 (https://www.megasoftware.net), and calculated maximum-likelihood estimates of mosquito infection rates by using PooledInfRate (https://www.cdc.gov/ncidod/dvbid/westnile/software.htm).

Of the 64,603 adult mosquitoes collected as described (8,12), we tested 39,035 females. A total of 11 pools were positive for SHUV (Table 1). No other orthobunyaviruses were detected. Positive pools for SHUV were detected in conservation areas (6/11, 54.5%) and rural areas (5/11, 45.5%) (Figure 1). Populations of the SHUV-positive mosquito species peaked with the heavy rains and with the highest mean air temperatures (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/27/12/20-3426-App1.pdf), which promote establishment of breeding sites and favorable habitats for developing stages and subsequent population growth.

The maximum-likelihood phylogeny based on the genus Orthobunyavirus PCR fragment of 152-bp of the S segment showed that all SHUV viruses from the mosquitoes clustered with the Simbu serogroup (Appendix Figures 1, 2) and were closest to SHUV on the basis of p-distance analyses (data not shown). For 5 samples, a larger region of the S segment could be amplified to confirm the clustering with SHUV strains previously identified in horses and wildlife from South Africa (Figure 2) and p-distances of 94%–100% with strains previously identified in South Africa, Israel, and Nigeria. Mosquito barcodes consisting of 517-bp were used to build a maximum-likelihood tree (Appendix Table 1, Figures 1–3). The barcoding confirmed all morphologic identifications except for a pool of damaged Aedes spp. mosquitoes and for Ae. subar- genteus mosquitoes (for which no other sequence was available in the databases).

Of the 11 pools of SHUV-positive mosquitoes, species belonged to the genera Mansonia (5 pools), Aedes (3 pools), Culex (2 pools), and Anopheles (1 pool) (Table 2). Previously, SHUV had been detected in
**Table 1. Mosquito species positive for Shuni virus, South Africa, January 2014–May 2018**

<table>
<thead>
<tr>
<th>Species</th>
<th>No. assayed</th>
<th>No. pools positive/no. pools tested</th>
<th>Infection rate, % (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anopheles pharoensis</td>
<td>27</td>
<td>1/4</td>
<td>39.0 (2.4–212.0)</td>
</tr>
<tr>
<td>Culex theileri</td>
<td>508</td>
<td>1/22</td>
<td>1.9 (0.1–9.0)</td>
</tr>
<tr>
<td>Cx. annulioris</td>
<td>120</td>
<td>1/7</td>
<td>6.7 (0.5–33.6)</td>
</tr>
<tr>
<td>Mansonia africana</td>
<td>340</td>
<td>3/13</td>
<td>8.7 (2.6–23.3)</td>
</tr>
<tr>
<td>Ma. uniformis</td>
<td>2,428</td>
<td>2/62</td>
<td>0.8 (0.1–2.7)</td>
</tr>
<tr>
<td>Aedes subgargenteus</td>
<td>1</td>
<td>1/1</td>
<td>Not applicable†</td>
</tr>
<tr>
<td>Ae. mcintoshi</td>
<td>3,653</td>
<td>1/87</td>
<td>0.3 (0.0–1.3)</td>
</tr>
<tr>
<td>Aedes spp.</td>
<td>273</td>
<td>1/25</td>
<td>3.6 (0.2–17.2)</td>
</tr>
<tr>
<td>Total</td>
<td>7,350</td>
<td>11/221</td>
<td></td>
</tr>
</tbody>
</table>

*Maximum-likelihood estimation: no. positive/no. mosquitoes assayed × 1,000.
†When all pools tested were positive for Shuni virus, the likelihood methods failed.

Cx. theileri mosquitoes collected in the 1970s near Johannesburg, South Africa (5). In that study, 2 pools of SHUV-positive Cx. theileri mosquitoes were also identified, although mosquitoes of this species were not abundant in the sites detected.

The highest rate of SHUV detection was in Mansonia uniformis mosquitoes, which were found in high numbers at the Shuni virus–positive pool collection sites. Three other arboviruses have been isolated from M. uniformis mosquitoes in South Africa: Wesselsbron, Ndumu, and Spondeweni (13). M. africana mosquitoes tested positive, but only small numbers of these mosquitoes were collected. Mansonia spp. mosquitoes can feed readily on humans and animals (13) and could have a potential epidemiologic role as bridge species for transmission between animals and humans.

**Figure 2.** Phylogenetic tree of SHUV-positive homogenate mosquito pools, South Africa, January 2014–May 2018 (black dots), based on 32 sequences and 328 bp of the nucleocapsid gene on the small segment. The tree was constructed with MEGA 7 software (https://www.megasoftware.net) by using the maximum-likelihood method and the Kimura 2-parameter model with 1,000 bootstrap replicates and includes members of the Simbu serogroup. The tree with the highest log likelihood (−299.13) is shown. GenBank accession numbers are indicated for the new and reference strains, which were selected from SHUV strains identified in South Africa among horses and wildlife (4,9) as well as strains from Nigeria and Israel available in GenBank. Numbers on internal branches indicate bootstrap values. RSA, South Africa; SHUV, Shuni virus.
Mosquitoes of other species that tested positive included *Aedes mcintoshii* and *Ae. subgarenteus*. Positive *Ae. mcintoshii* mosquitoes were collected from Mnisi, where they were the most abundant *Aedes* spp. at that site. They are considered nonspecific/opportunistic feeders and have a broad range of mammal hosts (14). *Ae. subgarenteus* mosquitoes are tree hole mammals and are either rare in South Africa (14) or are not attracted to the traps used in our study. Although little information about those mosquitoes is available, they might have a strong preference for biting humans (14).

Although SHUV has been detected in mosquitoes, recent studies have also implicated *Culicoides* spp. midges as potential competent vectors (15). An investigation of the vector competence of *Culicoides* midges and laboratory-reared *Cx. pipiens* and *Ae. aegypti* mosquitoes for SHUV (7) indicated that neither species of mosquito was susceptible but that *Culicoides* midges demonstrated the capacity to transmit SHUV. No *Ae. aegyptii* and *Cx. pipiens* field-caught mosquitoes tested positive for SHUV in this or other studies. Vector competence studies that used SHUV-positive species of mosquitoes identified in our study may define appropriate mosquito vectors and their role in the transmission of SHUV to animals and humans in Africa and the risk to areas where they are found outside the continent.

### Conclusions

Entomologic surveillance for orthobunyaviruses revealed a wide range of potential mosquito vectors for SHUV. We identified SHUV in different species of mosquitoes in South Africa, where cases with neurologic signs have been detected in animals (4,5) and humans (6). The identified mosquito species have also been associated with other arboviruses across Africa. SHUV recently emerged in Israel, where it is associated with neurologic disease and birth defects in animals (3). Mosquitoes of the identified species are potential vectors of SHUV and may be associated with SHUV outbreaks in Africa and further emergence in new regions.

### Acknowledgments

We are grateful to Lapalala Wilderness, Marataba Conservation, South African National Park, for logistical assistance and permission to collect mosquitoes. We acknowledge Leo Braack and Danny Govender for their valuable contribution to mosquito collection. We are grateful to the South Africa Weather Service for providing data for this study.

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### About the Author

Dr. Guarido completed her PhD degree and performs postdoctoral work at the Faculty of Veterinary Sciences, University of Pretoria. Her primarily research interest is the study of mosquitoborne viruses, with a focus on the entomology aspects of arbovirus transmission cycles.

### References


2. Causey OR, Kemp GE, Causey CE, Lee VH. Isolations of Simbu-group viruses in Ibadan, Nigeria 1964-69, including

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### Table 2. Mosquitoes positive for Shuni virus, South Africa, January 2014–May 2018*

<table>
<thead>
<tr>
<th>Species</th>
<th>Site</th>
<th>ID no.</th>
<th>Pool size</th>
<th>GenBank SHUV accession no.</th>
<th>GenBank COI accession no.†</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Anopheles pharoensis</em></td>
<td>Marakele</td>
<td>Mar16mp59</td>
<td>12</td>
<td>NA</td>
<td>MT428079</td>
</tr>
<tr>
<td><em>Culex theileri</em></td>
<td>Marakele</td>
<td>Mar16mp48</td>
<td>3</td>
<td>MN914125</td>
<td>MT428080</td>
</tr>
<tr>
<td><em>Cx. annulioris</em></td>
<td>Lapalala</td>
<td>Lap15mp15</td>
<td>4</td>
<td>MN914124</td>
<td>MT428086</td>
</tr>
<tr>
<td><em>Mansonia uniformis</em></td>
<td>KNP</td>
<td>Knp17mp758</td>
<td>9</td>
<td>NA</td>
<td>MT428087</td>
</tr>
<tr>
<td><em>Ma. africana</em></td>
<td>KNP</td>
<td>Knp17mp761</td>
<td>41</td>
<td>NA</td>
<td>MT428089</td>
</tr>
<tr>
<td><em>Ma. uniformis</em></td>
<td>KNP</td>
<td>Knp17mp755</td>
<td>2</td>
<td>NA</td>
<td>MT428088</td>
</tr>
<tr>
<td><em>Ma. africana</em></td>
<td>KNP</td>
<td>Knp17mp753</td>
<td>1</td>
<td>NA</td>
<td>MT434140</td>
</tr>
<tr>
<td><em>Aedes subgarenteus</em></td>
<td>Jozini</td>
<td>Kzn17mp108</td>
<td>2</td>
<td>NA</td>
<td>MT428082</td>
</tr>
<tr>
<td><em>Ae. mcintoshii</em></td>
<td>Mnisi</td>
<td>Kru16mp02</td>
<td>4</td>
<td>MT433095</td>
<td>MT428081</td>
</tr>
<tr>
<td><em>Aedes ssp.</em></td>
<td>Mnisi</td>
<td>Kru16mp05</td>
<td>8</td>
<td>MT433096</td>
<td>MT428085</td>
</tr>
<tr>
<td><em>Ma. uniformis</em></td>
<td>Mnisi</td>
<td>Kru17mp43</td>
<td>50</td>
<td>MT433097</td>
<td>MT428083</td>
</tr>
</tbody>
</table>

*COI, subunit I of the cytochrome oxidase gene; ID, identification; KNP, Kruger National Park; NA, not available because of sequence <200 bp; SHUV, Shuni virus.

†These sequences are available upon request.


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After infection with eastern equine encephalitis virus, the immune system races to clear the pathogen from the body. Because the immune response occurs so quickly, it is difficult to detect viral RNA in serum or cerebrospinal samples.

In immunocompromised patients, the immune response can be decreased or delayed, enabling the virus to continue replicating. This delay gave researchers the rare opportunity to study the genetic sequence of isolated viruses, with some surprising results.

In this EID podcast, Dr. Holly Hughes, a research microbiologist at CDC in Fort Collins, Colorado, describes a fatal case of mosquito-borne disease.

Visit our website to listen: https://go.usa.gov/xFUhU
Toscana virus (TOSV) is an emerging pathogen in the Mediterranean area and is neuroinvasive in its most severe form. Basic knowledge on TOSV biology is limited. We conducted a systematic review on travel-related infections to estimate the TOSV incubation period. We estimated the incubation period at 12.1 days.

The Study
We used PubMed and ISI Web of Knowledge search engines with no restriction on language and the phrase “Toscana AND virus AND (case report OR case-report OR travel* OR import*)”. We conducted a systematic search on ProMed and Google Scholar, as well as cross-reference checking. The inclusion criteria were laboratory-documented acute TOSV infection, indication of a travel-related infection in a TOSV-endemic area, and number of days between travel return and symptom onset. Two reviewers screened titles, abstracts, and full-text articles independently.

We extracted clinical and biologic elements from neuroinvasive TOSV case reports. For each patient, data related to the duration of travel and the time of symptom onset, gender, age, country in which case was reported, and country of infection were reported.

To estimate the IP, we used censored time-to-event models (10). Interval-censored observations related to travel duration represented the exposure time. Absence of a departure date was treated as left-censored data, whereas onset of illness during the travel period was considered right-censored. We performed data analysis by using R with the icenReg package.

1These authors contributed equally to this article.
package (R Foundation for Statistical Computing, https://www.r-project.org). We defined the data distribution with 4 parametric models (log-normal, log-logistic, Gamma, and Weibull). To determine the best model for our distribution, we calculated the Akaike information criterion. We used the nonparametric log-rank test from the interval package to assess the effect of age and gender as covariates. To check the result stability, we performed an additional Bayesian approach, fitting with the Weibull distribution.

Regarding imported case reports, 142 documents were identified on PubMed and 133 on Web of Knowledge. We removed 79 duplicates and excluded 118 records after screening titles and abstracts. A total of 42 articles were eligible after full-text reading. We then selected 22 documents for data extraction. A total of 24 cases were selected (Appendix Table 2, Figure). All travel-associated cases fulfilling the inclusion criteria were neuroinvasive; these cases were diagnosed in a non–TOSV-endemic area after a stay in a proven TOSV-endemic area (Figure 1).

We selected Weibull distribution because it presented the lower Akaike information criterion (Figure 2; Appendix Table 3). The median IP for neuroinvasive forms is estimated to be 12.1 (95% CI 10.2–14.4) days. In 5% of neuroinvasive cases, symptoms will develop by 6.8 (95% CI 3.8–9.9) days after an infectious bite; symptoms will develop in 95% of cases by 16.8 (95% CI 13.9–21.7) days after the infectious bite. We found no evidence of age or gender effect on the length of the IP (p value >0.05 by log-rank test). By using Bayesian analysis, we found an IP of 12.1 (95% CI 9.9–14.4) days (data not shown; results same as Figure 2).

**Conclusions**

In the literature, the IP values of TOSV are often heterogeneous, unsourced, or without evidence and therefore do not constitute a valid estimate for clinical or infection control decisions. Our literature review identified 24 neurologic cases of TOSV infection. Some travel durations were reported approximately in case reports and were not included in the analysis. All the data used were based on severe neurologic forms of the disease, which required hospitalization soon after the exposure period.

We estimated the median IP of TOSV at 12 (95% CI 10.2–14.4) days. Considering the delay from infection to symptom onset, this value is greater than that for most other arboviruses (11). Our estimate of the IP is evidence-based and relies on data from well-characterized cases. However, cases that cause milder symptoms, as opposed to neuroinvasive forms of the disease, might have a shorter IP (similar to...
other arboviruses). Other symptoms associated with paucisymptomatic forms of TOSV might not have been described yet and should be further investigated to improve case definition and diagnosis.

We also cannot exclude infections by other sandfly fever Naples phleboviruses because of cross-reaction risk in serologic analyses due to their close genetic relationships (12). However, the incidence in the population of other genetically similar phleboviruses is lower than TOSV, and TOSV remains the most common cause of neuroinvasive symptoms (3). Knowledge of TOSV genotypes and their aptitude to cause different clinical forms is limited (12). Analyzing this hypothesis was not possible because of the limited amount of available data. In addition to the genotype, other parameters may influence the IP, such as viral strain, patient’s immune status, or viremia (9). The amount of virus transmitted during bites (viral load) could also influence the IP and should be further investigated.

In addition, all other cases were diagnosed in countries or regions to which TOSV is not endemic (United States, United Kingdom, Sweden, Germany, Switzerland, Australia, and France). These imported cases represent a risk for emergence in these areas when vectors are established (13), as has been observed for other vectorborne diseases (14). Moreover, sand flies are known to spread in countries or regions to which TOSV is not endemic (15).

Currently, information on TOSV infections is lacking (12). Precise definitions of the IP should provide more information on the disease epidemiology and on its development in the human host. Moreover, because the IP is a key parameter for disease modeling (9), it would improve our understanding of the disease transmission dynamics. More reports of travel-related cases and standardization of data collection with reliable information (e.g., location and duration of the trips and precise dates of symptom onset) are clearly needed. The IP estimation will be improved with addition of new data.

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References


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Prions are proteinaceous infectious agents that can be transmitted through various components of the environment, including soil particles. We found that earthworms exposed to prion-contaminated soil can bind, retain, and excrete prions, which remain highly infectious. Our results suggest that earthworms potentially contribute to prion disease spread in the environment.

To investigate whether earthworms can act as carriers of infectious prions, we exposed groups of worms *Eisenia fetida* to soil previously mixed with brain homogenate (BH) from clinically diseased 263K Syrian golden hamsters (*Mesocricetus auratus*) (Harlan Envigo, https://www.envigo.com). For experiments, we homogenously mixed 375 g of Elliot soil (kindly provided by Joel Pedersen, Johns Hopkins University) with 25 mL of 10% wt/vol 263K brain homogenate. We assessed whether prions bind to worms or worm-associated soil by using protein misfolding cyclic amplification (PMCA) technology (5,6), which can detect prions down to the level of a single particle (7). Because PMCA efficiency can be severely affected by components in the inoculum (6), we first analyzed the effect of worm homogenate (WH) with or without soil on the efficiency of in vitro prion replication by PMCA (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/27/12/20-4236-App1.pdf). Our results indicated that whole WH does interfere with the reaction, but we could still obtain maximum amplification after 3 rounds of PMCA (Appendix Figure 1).

After verifying PMCA efficiency, we tested worms exposed to contaminated soil for different lengths of time. We collected worms from contaminated soil after 1, 3, 7, 14, and 28 days of exposure (Figure 1, panel A). PMCA results showed that worms exposed to prions take up PrPSc and efficiently sustain prion replication at all exposure times tested (Figure 1, panel B). We observed no PrPSc uptake in any worms exposed to control soil.

To study whether contaminated worms can transmit disease, we intraperitoneally injected hamsters with WH obtained from worms exposed to prion-soil mix for 28 days. To assess reproducibility, we used 3 different worms for this assay. Our results showed that worms exposed to prion-contaminated soil can transmit prion disease, albeit with variable efficiencies (Appendix Figure 2). Of the 3 worm extracts, 2 caused an attack rate of 4/5 and mean incubation periods of 237 (SE +39) and 255 (SE +25) days. A third WH transmitted disease to only 1/5 injected hamsters, which showed an incubation period of 272 days (Appendix Figure 2). For positive controls, we intraperitoneally injected groups of hamsters directly with 10% 263K BH. Terminal disease developed in all animals; the median incubation period was 151.4 (SE +30) days (Appendix Figure 2). We confirmed prion disease by biochemical detection of protease-resistant PrP (Figure 1, panel C). We did not detect a PrPSc signal in hamsters that did not show clinical signs, suggesting the absence of preclinical prion disease in
Results were many worm membrane proteins in each worm is equivalent to $1 \times 10^{-6}$ dilution of infected brain. This estimation also supported by the analysis of the data by using a semi-quantitative PMCA technique (8).

To investigate whether earthworms can retain infectious prions when exposed for different lengths of time to a prion-free environment, we exposed experimental subjects to prion-containing soil and subsequently transferred worms to naive soil (Figure 2, panel A). We collected worms from prion-containing soil after 7 days of exposure, thoroughly cleaned soil attached to the worms’ surface, and cultivated worms in naive soil for another 1, 3, 7, 14, and 28 days; we collected and analyzed 4 worms at each time point. PMCA results showed PrPSc-positive signal for all 4 worms immediately after exposure to prion-contaminated soil (Figure 2, panel B). We found that 25%–50% of worms exposed to prion-free naive soil retained PMCA-detectable PrPSc (Figure 2, panel B). We observed no clear trend with the time of incubation in naive soil, and even animals exposed to prion-free soil for 28 days retained prions in their bodies (Figure 2, panel B).

To evaluate whether prion-contaminated earthworms excrete PrPSc back into the environment, we analyzed worm castings by using PMCA. We collected 2 worms exposed to prion-contaminated soil for 7 days and thoroughly washed worms with water. For casting collection, we placed animals in petri dishes and collected 8 pieces of casting from the petri dish to analyze PrPSc content by PMCA (Figure 2, panel C). The results showed 6/8 casting samples were positive for PrPSc (Figure 2, panel D). Of note, 3 samples had large amounts of PrPSc detectable by just 2 rounds of PMCA, indicating that earthworms exposed to prions in soil can take up and release PrPSc competent for prion replication.

Finally, to study whether some PrPSc molecules taken up from the soil remain attached to the body of the animal, we contaminated 6 worms by exposure to contaminated soil for 7 days. After washing to remove outside soil, we dissected animals to completely remove all soil particles inside the animal. We thoroughly washed worm bodies, homogenized them, and then used the homogenate for PrPSc detection by PMCA. Of the 6 soil-void worms, 5 were positive for PrPSc after only 2 rounds of PMCA (Figure 2, panel E). The sixth worm became positive in the third PMCA round, as did control worms from which we...
did not remove internal soil (Figure 2, panel E). These results suggest that a substantial part of PrPSc taken up by worms from soil remained attached to the body of the animal and not merely in the soil particles that the worm acquired.

**Conclusions**

The mechanisms implicated in the natural spread of infectious prions are not completely known. Some prion diseases, such as chronic wasting disease and scrapie, are thought to be highly transmissible through

**Figure 2.** Detection of pathological prion protein (PrPSc) retention and dispersion by earthworms. A) Process for exposing earthworms to PrPSc–contaminated soil and analyzing for PrPSc retention. Worms were kept in PrPSc–contaminated soil for 7 days, then transferred to normal, prion-free soil and collected at various times. After collection, worms were thoroughly washed, homogenized, and used for PrPSc detection.

B) Western blot analysis of PMCA of worm samples after cultivation in 263K-contaminated soil for 7 days and exposure to normal soil for 0, 1, 3, 7, 14, and 28 days. Lane 0 is normal brain homogenate (NBH) used as positive control; lanes 1–4 indicate 4 different worms for each time point. C) Process for collecting castings excreted by prion-contaminated worms to analyze for PrPSc. D) PMCA results for castings collected from earthworms exposed to 263K-soil for 7 days. Samples 1–8 were harvested and subjected to 4 PMCA rounds. E) Detection of PrPSc attached to 6 earthworms after exposure to prion-contaminated soil for 7 days. After collection and thorough washing, worms were dissected, and soil was carefully removed from the inside of the animal (soil-devoid worms). Worm carcasses were homogenized and used for PMCA detection of PrPSc. As controls, we used 2 untreated worms, that is, worms for which no soil was removed. In panels B, D, and E, all samples were digested with proteinase K (Sigma Aldrich, https://www.sigmaaldrich.com) at 50 µg/mL for 1 h at 37°C, except the NBH used as a migration control of PrPSc. Numbers on the left indicate molecular weight markers. PMCA, protein misfolding cyclic amplification.
exposure to prion-contaminated environments (2,3). We previously demonstrated that infectious prions can attach to various components of the environment, including soil, plants, wood, and rock, and to several man-made surfaces, such as metals, plastic, and glass (9,10). However, little is known about how organisms living in the prion-exposed environment contribute to the spread of prions. In this study, we focused on earthworms (E. fetida) that live in close contact with known sources of prion infectivity in the environment, soil and diseased carcasses, and can move at a rate of 20–70 m/h (11,12). Our results demonstrate that earthworms can efficiently take up prions and act as vectors of prion disease transmission. In worms exposed to prion-contaminated soil, we noted PrPSc-competent for both in vitro prion replication and in vivo infectivity. Even a relatively short exposure of 1 day was enough to contaminate all exposed worms. Of note, within 1 day after moving contaminated worms into prion-free soil, many earthworms were free of infectious particles. However, 25%–50% of worms retained PMCA-detectable PrPSc even 28 days after living in noncontaminated soil. Dissection of the worm’s bodies to separate tissue from soil inside the animal showed that a substantial amount of PrPSc was in the worm bodies. Furthermore, analysis of the casting excreted by contaminated worms showed that 75% of the animal feces contained a relatively large quantity of PrPSc detectable by PMCA. These results suggest that earthworms exposed to prions remain potentially infectious for long periods and release prions back into the soil, therefore possibly contributing to the spread of infectious prions in nature.

Acknowledgments

We thank Joel Pedersen for providing Elliot soil used for these studies.

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C.S. is inventor on several patents related to the protein misfolding cyclic amplification (PMCA) technology and is currently founder, chief scientific officer, and member of the board of directors of Amprion, Inc. (https://amprionme.com), a biotech company focusing on the commercial utilization of PMCA for prion diagnosis. R.M. is listed as an inventor in a patent associated with the PMCA technology. S.P. also has a conflict of interest related to the PMCA technology and Amprion, Inc. The University of Texas System has licensed intellectual property to Amprion, Inc.

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Trichinella spiralis [tri·kuh·neh′·luh spr·a·luhs]

Monika Mahajan

Trichinella is derived from the Greek words trichos (hair) and ella (diminutive); spiralis means spiral. In 1835, Richard Owen (1804–1892) and James Paget (1814–1899) described a spiral worm (Trichina spiralis)–lined sandy diaphragm of a cadaver. In 1895, Alcide Raillet (1852–1930) renamed it as Trichinella spiralis because Trichina was attributed to an insect in 1830. In 1859, Rudolf Virchow (1821–1902) described the life cycle. The genus includes many distinct species, several genotypes, and encapsulated and non-encapsulated clades based on the presence/absence of a collagen capsule.

The smallest, viviparous nematode or pig parasite has sylvatic and domestic cycles and causes trichinellosis or trichinosis. Transmission occurs through the consumption of meat infected with pathogenic cysts, encasing larvae. Human-to-human transmission has not been reported.

Sources

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Experimental Oronasal Transmission of Chronic Wasting Disease Agent from White-Tailed Deer to Suffolk Sheep

Eric D. Cassmann, S. Jo Moore, Justin J. Greenlee

Chronic wasting disease (CWD) is a fatal prion disease of cervids. We examined host range of CWD by oronasally inoculating Suffolk sheep with brain homogenate from a CWD-positive white-tailed deer. Sixty months after inoculation, 1/7 sheep had immunoreactivity against the misfolded form of prion protein in lymphoid tissue. Results were confirmed by mouse bioassay.

Transmissible spongiform encephalopathies (TSEs), also known as prion diseases, are a group of fatal neurologic diseases caused by a misfolded form of the prion protein (PrPSc). Several TSEs affect livestock, including scrapie in sheep and chronic wasting disease (CWD) in cervids.

Susceptibility of sheep to the agent of scrapie is determined by the host prion protein genotype. Three polymorphisms at codons 136, 154, and 171 of the prion protein gene occur in sheep. The haplotype A136R154R171 is associated with resistance to scrapie, whereas VRQ is linked with susceptibility. Likewise, the deer prion protein genotype GG96 is overrepresented in cases of CWD.

CWD was identified in captive mule deer in Colorado, USA, in 1967 (1). Since then, CWD has been reported in ≥24 states in the United States, 2 provinces in Canada, and South Korea (2,3). During 2016, CWD was reported in Europe, and it has since been detected in 3 Nordic countries (Norway, Sweden, and Finland), although CWD strains in Europe were recently shown to be distinct from strains in North America (4). Because of human consumption of cervid meat products and intermingling of various livestock species with wild cervid populations, there is major interest in characterizing the possible host range of CWD.

Scrapie has been implicated as the possible source of CWD in cervids (5). This finding is supported by in vitro conversion of sheep prion protein by infectious CWD prions (6) and glycoprofile similarities between scrapie and CWD prions (7). Another similarity between scrapie and CWD is prominent lymphoid accumulation of PrPSc in both species affected (5). Experimental transmission of mule deer CWD to Suffolk sheep by intracranial inoculation, a highly artificial route of transmission, has been performed (8). Widespread peripheral lymphoid accumulation of PrPSc is retained in intracranially CWD inoculated sheep.

The objective of this study was to test the oronasal susceptibility of sheep to the agent of CWD. We report the preliminary findings of an ongoing multi-year study.

The Study

Initially, we oronasally inoculated (9) seven Suffolk lambs (3–4 months of age) with the V136R154Q171/ARQ (n = 2), ARQ/ARQ (n = 4), or ARQ/ARR (n = 1) prion protein genotype and 0.1 g of 10% (wt/vol) brain homogenate from a GG96 white-tailed deer that had CWD. The sheep were housed indoors in a Biosafety Level 2 agriculture facility separate from scrapie-affected sheep. At 60 months postinoculation, the initial experimental endpoint, sheep were asymptomatic, and all 7 sheep were culled.

We performed a postmortem examination on each sheep and collected a full spectrum of tissues, which we froze and stored in 10% neutral-buffered formalin. To evaluate lymphoinvasion and neuroinvasion, we tested tissues from the brainstem at the obex and pons, third eyelid, palatine tonsil, lymph nodes (mesenteric and retropharyngeal), spleen, and ileum. We processed the formalin-fixed tissues, embedded in paraffin, and sectioned at optimal thickness (brain, 4 µm; lymphoid, 3 µm; and other tissues, 5 µm) for subsequent staining with hematoxylin and eosin and immunohistochemical (IHC) analysis. We used a
cocktail of PrPSc monoclonal antibodies (F89/160.1.5 and F99/97.6.1; 5 µg/mL) for IHC.

Examination of IHC-stained tissues showed PrPSc in the retropharyngeal lymph node (Figure 1, panel A) and palatine tonsil (Figure 1, panel B) of 1 sheep inoculated with the ARQ/ARQ genotype. The retropharyngeal lymph node was also positive by enzyme immunoassay (EIA) (HerdChek; IDEXX Laboratories, https://www.idexx.com) at initial (optical density 0.99; negative cutoff value 0.186) and repeat (optical density 0.559; negative cutoff value 0.178) tests. The palatine tonsil was negative by EIA.

To confirm prion disease infectivity in the retropharyngeal lymph node, we performed bioassays in Tg12 cervidized (10) and Tg338 ovinized (11) transgenic mice. Mice expressed the transgene for the elk prion protein polymorphism MM132 (Tg12) and the ovine prion protein polymorphisms V136R154Q171 (Tg338). We homogenized fresh frozen lymph nodes to 10% (wt/vol) and enriched them by repeated rounds of differential centrifugation; we intracranially inoculated mice with 20 µL of 10% (wt/vol) equivalent enriched homogenate. The Tg12 bioassay had a partial attack rate of 20% (1/5) of mice. Most (4/5) dead Tg12 mice were strongly positive by EIA (optical density 4.0) and had an average incubation period of 511 days.

Western blots of these 4 Tg12 mice confirmed the presence of proteinase K-resistant PrPSc in the brains (Figure 2). The positive EIA results were obtained from brain homogenates in Tg12 mice; the spleens were negative for PrPSc. For the Tg338 bioassay (n = 15), brains and spleens were negative by EIA. Four Tg338 mice that died or were euthanized because of intercurrent disease at 254, 462, 629, and 657 days postinoculation were negative by EIA. The rest of the Tg338 mice were negative at the study endpoint, 700 days postinoculation.

**Conclusions**

The oronasal susceptibility of sheep to the agent of CWD is a major finding in light of its possible effect on risk assessment and understanding possible transmission of CWD to noncervid species in field conditions. Interspecies transmission of TSEs is less likely when the experimental species barrier between hosts is strong (12). One study demonstrated that the CWD agent does not readily transmit to transgenic ovinized mice (13). However, another study reported lifelong replication of PrPSc in the spleen after intracranial inoculation of the CWD agent in Tg338 ovinized mice (14). The finding of extraneuronal PrPSc in 1 sheep 5 years after oral inoculation suggests that sheep are unlikely to develop neurologic disease after natural exposure to the agent of CWD, but they might serve as asymptomatic carriers under the right conditions.

In this study, we used a relatively low dose (0.1 g) of brain homogenate. These results are intriguing, but they do not assess potential modes of transmission that could occur in the field, such as nose-to-nose contact or...

![Figure 1. Immunoreactivity against misfolded form of the prion protein (red) in lymphoid tissue from a sheep oronasally inoculated with the agent of chronic wasting disease from white-tailed deer. A) Retropharyngeal lymph node (original magnification ×100.) B) Palatine tonsil (original magnification ×40). We used a cocktail of monoclonal antibodies (F89/160.1.5 and F99/97.6.1).](image)

![Figure 2. Western blot analysis showing proteinase K–resistant misfolded form of the prion protein (PrPSc) in brains of 4 Tg12 mice. Mice were intracranially inoculated with a homogenate made from retropharyngeal lymph node of a sheep oronasally inoculated with the agent of chronic wasting disease. Tg12 brain was prepared as a 10% (wt/vol) homogenate with phosphate-buffered saline. A total of 1 mg of tissue equivalent was treated with proteinase K (90 µg/mL) before electrophoresis. Immunodetection of PrPSc was performed overnight at 4°C with monoclonal antibody Sha31 (dilution 1:10,000). Left lane, molecular mass ladder. kDa, kilodaltons.)](image)
environmental contamination. In our ongoing multi-year study, 1 sheep had PrPSC-positive lymphoid tissue but no evidence of neuroinvasion 5 years postinoculation. This time interval is an extremely protracted incubation period. Had we continued this experiment, it is unknown how long the sheep would have remained asymptomatic or whether they would have eventually developed clinical disease. Because PrPSc was detected in lymphoid tissues of the head, the possibility that this sheep might have been shedding infectivity into the environment cannot be ruled out.

Positive bioassay events in Tg12 mice confirm CWD infectivity in the lymph node. Negative results in Tg338 mice could be explained by a donor/host mismatch between the ARQ donor sheep and VRQ CWD infectivity in the lymph node. Negative results in lymphoid tissues of the head, the possibility that this sheep might have been shedding infectivity into the environment cannot be ruled out.

Interspecies transmission events might increase the pathogenicity of an infectious prion on subsequent transmission to other species (15). Thus, exploration of potential new host ranges of this CWD isolate and performing human health risk assessments will provide useful information for this prion.

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Geographic expansion of Rift Valley fever virus (RVFV) associated with health and socioeconomic losses is of great concern for veterinary and public health professionals worldwide (1). In South Africa, major human Rift Valley fever (RVF) epidemics occurred in 1950–1951, 1974–1975, and 2010–2011 (2–4), but single outbreaks are reported only sporadically (5). RVF outbreaks in South Africa primarily have occurred on the temperate central plateau of the country (6), but historic data suggest circulation of RVFV in both humans and animals in the northern, tropical part of KwaZulu-Natal Province (7–9). Results of recent studies in this region show high RVFV seroprevalence in domestic goats (31.7%) and cattle (34%) (10) and in wild ruminants (35%) (II), without reported epizootics. To investigate the possibility of undetected RVFV infections in humans, we tested patients visiting healthcare facilities in northern KwaZulu-Natal for RVFV antibodies.

The Study

Because of recent active circulation of RVFV in livestock and wildlife (10,11), we selected the uMkhanyakude Health District for active RVFV surveillance during April 2018–August 2019. Many households keep livestock composed of indigenous Nguni chickens, cattle, goats, or ducks. Participating locations were 4 hospitals, Manguzi, Bethesda, and Mseleni, and Ndumo clinic attached to Mosvold hospital, and associated clinics, Mahlungulu, Makathini, and Mbazwana (Figure). The study was performed in accordance with protocols approved by the Human Research Ethics Committee of the University of Witwatersrand (Johannesburg, South Africa; approval nos. HREC M170606, M160667, and M161005) and provincial department of health (reference no. KZ_201709–037).

Enrolled participants comprised persons ≥5 years of age of either sex who had measured axillary temperature of ≥37.5°C at examination or history of symptoms ≤7 days before examination, or at the time of examination, such as rash, headache, myalgia, arthralgia, and conjunctivitis. Study controls were persons from the same selected health facilities who were seeking healthcare for noninfectious conditions or for chronic care, and who had no history of fever ≤7 days. Case-controls were matched to age groups of enrolled participants as much as possible. Nurses conducted interviews and collected and recorded survey data on a case investigation form at the time the blood was drawn. Data were transferred into data gathering tool built on a tablet computer by using REDCap software (https://projectredcap.org), which is powered by Vanderbilt University (Nashville, Tennessee, USA). For analysis, we downloaded data from respective servers into Excel software (Microsoft, https://www.microsoft.com).
Nurses drew 5 mL of whole blood from participants 5–12 years of age and 10 mL from participants >12 years of age. Blood specimens were transported daily from clinics to their associated hospital laboratory for processing and temporary storage until transported for testing to the National Institute for Communicable Diseases of the National Health Laboratory Service (Johannesburg). We enrolled and collected samples from a total of 1,395 volunteers during April 2018–August 2019.

We first tested serum samples by inhibition RVFV ELISA (12), then tested all positive samples by IgG sandwich ELISA and IgM capture ELISA, as previously described (13). We tested IgM-positive serum samples by using real-time reverse transcription PCR (rRT-PCR) (14). Of note, RVF and malaria can have similar clinical manifestations in patients, such as fever, arthralgia, and headache. Thus, we also tested specimens collected during April 2018–January 2019 for malaria antigen by using an ICT Malaria Combo Cassette Test (ICT International, https://www.ictdiagnostics.com), according to manufacturer instructions. We performed statistical analyses by using Stata version 13 (StataCorp LLC, https://www.stata.com) and Excel. We determined univariable statistics by using Fisher exact test for variables associated with RVFV seropositivity, such as sex, age, time outdoors, and agriculture activities. We used ArcGIS ArcMap 10.2 (Esri, https://www.esri.com) to create distribution and choropleth maps of RVF occurrence.

Among participants, 72.6% (997) were female and 27.4% (377) were male; no sex was recorded for
21 participants. The average age among participants was 35.3 (SD 17.0, range 5–96) years, and median was 33 (interquartile range 22–46) years.

Of 1,395 volunteers tested, 39 tested RVFV positive by inhibition ELISA, of which 11 were positive for RVFV IgM and 9 for RVFV IgG (Table 1). The overall seroprevalence adjusted for facility clustering was 2.8% (95% CI 1.45%–5.34%), and seropositivity differed significantly between facilities (p = 0.03) (Table 1). RVFV seropositivity was higher among groups >10 years of age compared with those 5–9 years old (p = 0.001) but was not significantly associated with sex (p = 0.481), spending time outdoors (p = 0.263), or working in agriculture (p = 0.161). None of the 11 IgM seropositive persons tested positive by RVFV rRT-PCR; 6 had fever at clinical examination at the healthcare facility. The most frequently observed symptoms were headache, myalgia, and arthralgia, and 3 participants had conjunctivitis (Table 2). Among IgM-positive participants, 3 were tested for malaria infection, and 2 tested positive.

The east coast, the border with Mozambique, the Ndumo area in the north, Umhlabonela towards the south of the district where Bethesda is located, and the southeast near the uSimangaliso had higher RVFV seroprevalence, suggesting that more favorable conditions for RVFV circulation and human exposure exist in these areas. Of 11 IgM seropositive participants, 7 were seen in the Ndumo clinic, located in the northern section of the Jozini municipality and at the southern edge of Ndumo Game reserve and adjacent Tembe Elephant Park, part of the Lubombo Transfrontier Conservation and Resource area with Mozambique.

### Conclusions

Our serosurvey confirms recent exposure and indicates endemic circulation of RVFV in humans residing in the tropical coastal plain of northern KwaZulu-Natal Province in South Africa. The RVFV seropositivity we noted in our study is lower than that reported in the temperate inland of South Africa (15). The central plateau of South Africa is prone to RVF outbreaks, and more frequent and intense RVF outbreaks have occurred in the central plateau than the eastern coastal area (6). The inland of South Africa has the largest and most concentrated sheep farming regions. Sheep farms are not common in northern KwaZulu-Natal, and households keep livestock comprised mostly of indigenous cattle and goats. Among livestock, sheep, particularly newborn lambs, are most susceptible to RVFV infection (1,6). Most confirmed cases during the 2008–2011 RVF outbreak in South Africa were caused by physical contact with infected animals, either through disposal of dead animals or aborted fetuses, or slaughtering (4,15). No RVF outbreaks have been reported in northern KwaZulu-Natal, either in humans or animals, but recent findings suggest year-round virus transmission in cattle, goats (10), and wild antelopes (11) are associated with high RVFV seroconversion rates in domestic ruminants (10).

### Table 1. Rift Valley fever virus IgG and IgM seropositivity in survey participants by healthcare facility and uMkhanyakude district, northern KwaZulu-Natal, South Africa, 2018–2019*

<table>
<thead>
<tr>
<th>Healthcare facility</th>
<th>No. tested</th>
<th>No. (%) seropositive</th>
<th>No. (%) IgG positive†</th>
<th>No. (%) IgM positive‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mbazwana</td>
<td>185</td>
<td>(4.3)</td>
<td>(3.8)</td>
<td>(0.5)</td>
</tr>
<tr>
<td>Ndumo-Mosvold</td>
<td>377</td>
<td>(4.2)</td>
<td>(3.7)</td>
<td>(1.9)</td>
</tr>
<tr>
<td>Bethesda</td>
<td>178</td>
<td>(2.8)</td>
<td>(2.8)</td>
<td>(0.6)</td>
</tr>
<tr>
<td>Manguzi-Mahlungulu</td>
<td>207</td>
<td>(2.4)</td>
<td>(2.4)</td>
<td>(0.5)</td>
</tr>
<tr>
<td>Mseleni</td>
<td>178</td>
<td>(2.3)</td>
<td>(2.3)</td>
<td>(0.6)</td>
</tr>
<tr>
<td>Makhathini</td>
<td>270</td>
<td>(0.4)</td>
<td>(0.4)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>1,395</td>
<td>(2.8)</td>
<td>(2.6)</td>
<td>(0.8)</td>
</tr>
</tbody>
</table>

*Serum tested by an inhibition ELISA with 99.47% diagnostic sensitivity, 99.66% diagnostic specificity. This assay measures total Rift Valley fever virus antibody but does not discriminate between IgG and IgM (12).

†Serum tested by an IgG-sandwich ELISA with 100% diagnostic sensitivity, 99.95% diagnostic specificity (13).

‡Serum tested by an IgM-capture ELISA with 96.47% diagnostic sensitivity, 99.44% diagnostic specificity (13).

### Table 2. Symptoms and signs in Rift Valley fever virus in IgM-positive participants by health care facility, uMkhanyakude district, northern KwaZulu-Natal, South Africa, 2018–2019*

<table>
<thead>
<tr>
<th>Healthcare facility</th>
<th>Age, y/sex</th>
<th>Fever</th>
<th>Rash</th>
<th>Headache</th>
<th>Myalgia</th>
<th>Arthralgia</th>
<th>Conjunctivitis</th>
<th>Vomiting</th>
<th>Malaria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mbazwana</td>
<td>30/M</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Ndumo-Mosvold</td>
<td>55/F</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>39/F</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td>50/F</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>71/F</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>27/M</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>72/F</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>25/F</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>NT</td>
</tr>
<tr>
<td>Bethesda</td>
<td>67/M</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>NT</td>
</tr>
<tr>
<td>Manguzi-Mahlungulu</td>
<td>15/F</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>NT</td>
</tr>
<tr>
<td>Mseleni</td>
<td>47/F</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>NT</td>
</tr>
</tbody>
</table>

*NT, not tested.
Study participants had detectable IgG and IgM to RVFV, and most IgM-positive samples were collected from participants with no recent history of travel beyond the study area. Our study indicates that RVFV infections in northern KwaZulu-Natal could be misdiagnosed or underreported, highlighting the urgent need for improved diagnostic testing and awareness of RVF and other arbovirus diseases in this part of South Africa. Moreover, our results suggest the possible role of the northern KwaZulu-Natal wildlife-livestock-vector host reservoir system in maintaining RVFV endemicity, including the potential to drive large-scale emergence and spread of the virus to other parts of the country. Because clinical manifestations of RVF in humans mimic those of malaria, RVFV surveillance can reduce potential misuse of antimalaria treatments. Our findings underscore the need for improved and active arbovirus biosurveillance in humans, wildlife, livestock, and mosquito vectors to mitigate associated transmission risk and potential RVF epidemics.

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We thank the healthcare workers of the facilities and the uMkhanyakude community in KwaZulu-Natal Province for supporting the study. We also thank the staff of the Center for Emerging Zoonotic and Parasitic Diseases, NICD/NHLS for the technical and laboratory assistance for testing of the samples.

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In an effort to understand the health impacts of endemic typhoid, the Severe Typhoid Fever Surveillance in Africa Program (SETA) detects and records cases of surgically confirmed intestinal perforations, a relatively rare but severe complication of *Salmonella enterica* serovar Typhi infection (1). Since the beginning of the coronavirus disease (COVID-19) global pandemic in early 2020, SETA surveillance has found an alarming increase in surgically confirmed intestinal perforations cases in Madagascar. This increase, which does not correlate with an increase in blood culture–confirmed typhoid cases found through SETA surveillance, may insinuate the serious effects on healthcare-seeking behavior and healthcare quality that the COVID-19 pandemic has had in the country.

Typhoid intestinal perforation is a severe complication of untreated or mismanaged infection that disproportionately affects low-income countries (2). Delay in diagnosis and proper antibiotic treatment of typhoid is frequently cited as a major factor contributing to typhoid intestinal perforation incidence and associated deaths (3–6). As such, increases in intestinal perforation cases may suggest deterioration in the quality of healthcare or changes in the healthcare-seeking behavior of the community.

SETA sentinel sites represent both primary and tertiary healthcare facilities, where all incoming patients are screened for febrile illness, clinically suspected typhoid, and gastrointestinal perforations. Once a patient is enrolled in the study, cultures of their blood, stool, and (in the case of surgery) tissue are used to detect *Salmonella Typhi* and other bacteremia. Over a 4-year period of SETA observation, we detected a marked increase in the rate of surgically confirmed typhoid intestinal perforations after the onset of the COVID-19 pandemic in early 2020 (Figure, panel A).

During the coronavirus disease pandemic, we observed a 6.4-fold increase in typhoid intestinal perforation incidence in Antananarivo, Madagascar. Thirteen perforations and occurred within 6 months (February 2020–July 2020) compared with 13 perforations during the previous 41 months (August 2016–January 2020). The increase may be attributable to delayed healthcare seeking during the pandemic.

SETA Madagascar enrolled case-patients with suspected typhoid intestinal perforations in the town of Imerintsiatosika, 43 km from the capital city of Antananarivo, as well as case-patients from tertiary care facilities in Antananarivo (1). Clinical and demographic data were systematically collected from enrolled participants at entry into the study. We observed participants daily until their hospital discharge. We detected a total of 26 intestinal perforation cases of any etiology during August 2016–September 2020. The mean age of the patients was 28.5 years (SD ± 19.1 years); men and boys accounted for 69% of the total patients. Of the 26 patients with perforation, 9 died and 17 were discharged. The overall case-fatality rate was 50% among women and girls and 28% among men and boys. Of note, all 4 deaths among women and girls occurred during the prepandemic period, and all 5 deaths among men and boys occurred during the pandemic.

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pandemic period. During August 1, 2016–January 31, 2020, the pre–COVID-19 pandemic period, 13 perforations (2.1/100,000 person-years of observation [PYO]) and 4 deaths (0.6/100,000 PYO) occurred. These incidence rates contrast with the remainder of 2020, the COVID-19 pandemic period, during which 13 perforations (13.2/100,000 PYO) and 5 deaths (5.1/100,000 PYO) occurred. This change represents a 6.4-fold (95% CI 3.0–13.7-fold) increase in the incidence of intestinal perforations during the COVID-19 pandemic period (p<0.05). Although we noted no statistically significant difference in mean age of onset for intestinal perforation patients detected before versus after the onset of the pandemic (30 vs. 27 years; p = 0.75 by t-test), intestinal perforation patients identified during the pandemic seem more likely to be middle-aged (20–50 years of age) (Figure, panel A).

We suspect that the immediate increase in incident intestinal perforations observed since February 2020 may be an externality of delayed treatment for mild typhoid fever because of changes in healthcare-seeking behavior, healthcare quality, or both during the initial COVID-19 pandemic outbreak. However, COVID-19 investigations to date indicate that COVID-19 can affect various organs, including the gastrointestinal tract; hence, the possibility of SARS-CoV-2 having a direct effect on the risk for perforations cannot be ruled out at this stage and warrants further research.

As of Oct 19, 2021, a total of 42,898 COVID-19 cases and 958 COVID-19–related deaths had been reported in Madagascar (7). Well before the first 3 cases of COVID-19 were reported in-country on March 20, 2020, media coverage of the global pandemic was substantial (8,9). Like the rest of the international community, Madagascar watched with collective anxiety and apprehension as the novel coronavirus outbreak unfolded. Although the surge of reported perforation cases predates regional lockdowns, which were first imposed in July 2020 (10), we cannot rule out ad hoc closures of healthcare centers affecting the community’s ability to seek regular care in addition to the unknowns of potential social stigma that raises barriers to the already low levels of healthcare-seeking observed during nonpandemic conditions in the community (11,12).

Before future in-depth qualitative research can provide a comprehensive picture of healthcare in Madagascar during the COVID-19 pandemic, SETA screening records may provide the first hint at a disruption of individual healthcare-seeking behavior as shown by a reduction in the number of overall patient hospital visitations beginning in January 2020 (Figure, panel B). SETA records all patients who visit any of the sentinel health centers for any concern and screens those patients for study eligibility; consequently, SETA screening numbers can be understood as a proxy for hospital visitation numbers.
The COVID-19 pandemic is likely having broad impacts on other preventable diseases in an already struggling healthcare system; widespread availability of COVID-19 vaccines in Madagascar is expected only in 2023 (13). The observed increase in illness and deaths from treatable diseases and disruption of routine primary care should not be neglected (14,15). Although the SETA program has only investigated intestinal perforations in the capital city, delayed healthcare-seeking might be an even larger problem in more remote areas of the country. The public health community must remain vigilant about maintaining routine healthcare services and ensuring that healthcare facilities are safe and usable. In particular, public trust in the healthcare system amidst the pandemic is essential for encouraging persons with potentially life-threatening conditions to seek healthcare.

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Evidence of Human Exposure to Tamdy Virus, Northwest China

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The species Tamdy orthornairovirus (genus Orthonairovirus, family Nairoviridae) includes 5 viruses: Tamdy virus (TAMV), Burana virus (BURV), Tácheng tick virus 1 (TcTV-1), Huángpí tick virus 1 (HpTV-1), and Wēnzhōu tick virus (WzTV) (1). TAMV and BURV were initially isolated from ticks in countries in central Asia (2–4), but little is known about their medical and veterinary importance. TcTV-1, HpTV-1, and WzTV were putative viruses identified by virome sequencing from ticks in China (5); however, their virologic properties and pathogenesis potential remain unclear. One study (6) reported TcTV-1 isolated from a febrile patient in northwest China, providing evidence of the potential public health threat from these viruses. We report TAMV isolated from ticks in northwest China and demonstrate serologic evidence of infection in humans.

The Study

During April and May of 2016 and 2017, we collected Hyalomma asiaticum ticks (n = 4,123) from Xinjiang in northwest China and divided the ticks into 55 groups according to the sampling location (n = 50–100 ticks/group) (Figure 1; Appendix Table 1, https://wwwnc.cdc.gov/EID/article/27/12/20-3532-App1.pdf). We isolated the virus from homogenates of each tick group in suckling mice. After the first inoculation, we observed symptoms in mice including loss of balance, limb paralysis, tremors, and articulo mor-tis from 4 (36.37%) of 11 pooled samples from Yuli County, 1 (14.29%) of 7 from the city of Karamay, 3 (60%) of 5 from Luntai County, and 17 (53.13%) of 32 from the city of Wujiqiu (Appendix Table 1). We performed a second inoculation using brain samples from diseased mice from Luntai and Wujiqiu Counties, in which >50% of the mice experienced illness onset after first inoculation. Similar symptoms were reproducibly observed in 1 group from Luntai and 4 groups from Wujiqiu (Appendix Table 1).

Subsequently, we prepared 3 RNA pools of diseased mouse brains and obtained a total of 196,946,814 reads by RNA sequencing. We found TAMV contigs in all 3 pools (Appendix Table 2), confirming findings using real-time reverse transcription PCR (rRT-PCR) (data not shown). We used homogenates of 2 TAMV RNA-positive brain samples from the A-M6 pool (Appendix Table 2) to isolate viruses in Vero E6 cells. As indicated by immunofluorescence assays (IFA) (Appendix), we observed increasing TAMV infection from first to fourth passages in Vero E6 cells, suggesting successful isolation (Appendix Figure 1). Negative-stain electron microscopy revealed an enveloped spherical viral morphology with a diameter of ≈90–110 nm (Figure 2, panel A). We observed viral particles in cytoplasm and vesicles of infected cells (Figure 2, panel B). Although this screening was not exhaustive, IFAs showing varied susceptibility of different cells lines indicate that TAMV seems to have a broad host range, including humans, monkeys, sheep, dogs, and mice (Appendix Figure 2).

We report the isolation of Tamdy virus from Hyalomma asiaticum ticks in northwest China and serologic evidence of human Tamdy virus infection in the same region. These findings highlight the need to further investigate a potential causal relationship between Tamdy virus and febrile illnesses of unknown etiology in that region.
The 2 TAMV isolates shared very high sequence similarities (99.96% for large, 100% for medium, and 99.95% for small segments). We named the 2 TAMV strains YL16082 and YL16083, including an abbreviation (YL) for the geographic location (Yuli County) where the original tick samples were collected. TAMV genome sequences (YL16082) shared 37%–59% identity with other members of the *T. orthonairovirus* species and 34%–49% identity with Crimean-Congo hemorrhagic fever virus (CCHFV), another *Orthonairovirus* species. Protein sequences shared 44%–62% identity with other members of the *T. orthonairovirus* species and 33%–40% with CCHFV (Table 1). Phylogenetic trees based on nucleotide sequences of RNA-dependent RNA polymerase, glycoprotein, and nucleoprotein (NP) genes all confirmed the close taxonomic relationships with currently known TAMV strains and other members of the species *T. orthonairovirus* (Appendix Figure 3).

To investigate potential human infection by TAMV in northwest China, we conducted a seroprevalence study using archived serum samples from 725 healthy persons (collected in 2005 from Fukang City, 2014 from Aksu City, and 2017 from Usu City) and 87 febrile patients (collected in 2007 from Bachu County, which has a history of CCHFV prevalence) (Appendix). Of the 87 febrile patients, 21 (24.14%) were TAMV IgG positive and 17 (19.54%)...
IgM positive (Table 2; Appendix Table 4), whereas only 1 (0.13%) of the 725 healthy participants we tested IgG positive (data not shown). Neutralization (titers 16–64) was demonstrated in serum samples from 6 febrile patients (6.9%) (Table 2). Moreover, of the 24 tick groups from the same locations as the febrile patients, 10 groups (41.76%, 6 identified in sheep and 4 in fields) tested positive for TAMV RNA by rRT-PCR (data not shown). Partial sequences of large segments from these positive groups clustered together with TAMV strains (Appendix Figure 4). These results showed serologic evidence of human exposure to TAMV and evidence of TAMV presence in Hy. asiaticum ticks in northwest China as early as 2007, which warranted more in-depth investigation to establish the potential causal relationship between TAMV and febrile illnesses of unknown etiology in regions where TAMV is present.

Finally, because another T. orthonairovirus, TcTV-1, had been identified in a febrile patient in northwest China (6), we thought it important to determine the potential serologic cross-reactivity between these 2 viruses. However, TAMV and TcTV-1 shared limited protein sequence identity (49%–60%) (Table 1), suggesting limited cross-reactivity, if any. This result was confirmed by conducting serologic testing using recombinant NP proteins from the 2 viruses. As shown by both IFA and Western blot analyses, TAMV NP antibodies had no cross-reaction with TcTV-1 NP (Appendix Figure 5, panels A, B). In addition, human serum samples that were positive for TAMV IgM or IgG, or both, showed reactivity with TAMV NP, but not with TcTV-1 NP (Appendix Figure 5, panel C).

### Conclusions

TAMV was initially found in ticks in countries in central Asia, including Kazakhstan, Kyrgyzstan, Uzbekistan, and Turkmenistan (3,4). Its infection status in humans and livestock animals was not well characterized. Our data, together with reports of TAMV isolated from ticks in Xinjiang, China (7), and identified in Turkey (8), shows that the geographic distribution of TAMV is much wider than originally recognized. In addition, we provide strong serologic evidence of human exposure in TAMV-affected regions.

Findings of a potential role of TcTV-1 in causing human febrile disease (6) suggest that >1 virus in this species group may have the potential to cause

### Table 1. Sequence identity of TAMV isolate YL16082 from China compared with other members of the species Tamdy orthonairovirus and Crimean-Congo hemorrhagic fever virus*

<table>
<thead>
<tr>
<th>Virus</th>
<th>Nucleotide identity, %</th>
<th>Amino acid identity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L segment</td>
<td>M segment</td>
</tr>
<tr>
<td>Wēnzhōu tick virus</td>
<td>59</td>
<td>50</td>
</tr>
<tr>
<td>Táchēng tick virus 1</td>
<td>57</td>
<td>46</td>
</tr>
<tr>
<td>Huángpí tick virus 1</td>
<td>55</td>
<td>45</td>
</tr>
<tr>
<td>Burana virus†</td>
<td>59</td>
<td>47</td>
</tr>
<tr>
<td>Crimean-Congo hemorrhagic fever virus</td>
<td>49</td>
<td>34</td>
</tr>
</tbody>
</table>

*G, glycoprotein; L, large; M, medium; NP, nucleoprotein; RdRp, RNA-dependent RNA polymerase; S, small; TAMV, Tamdy virus.
†Partial sequences were available for analyses.
diseases in humans. However, our data did suggest the possibility of such a relationship because the TAMV-positive ratio was much higher among febrile patients than healthy persons in the study from the same region. In addition, at least 2 febrile patients had both TAMV IgM and IgG at the time of sampling, during or not long after acute illness; 1 of them had neutralization to TAMV (Appendix Table 3).

Among study limitations, the nature of using archived samples limited our ability to provide direct evidence of a causal relationship between TAMV and human febrile illnesses. Also, it is possible that the high TAMV antibody-positive ratio might have resulted not from the recent cases but from a small outbreak of human TAMV infection in northwest China in 2007.

In summary, our study strongly suggests the potential of TAMV as a human pathogen and supports an urgent need to conduct in-depth epidemiologic and pathogenesis investigations into this group of viruses in China, central Asia, and beyond. While the world’s attention is currently on coronavirus disease and batborne viruses, our study highlights the need to pay attention at the same time to emerging zoonoses of tick origin to prevent future outbreaks.

Acknowledgments

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Table 2 Seroprevalence of TAMV among 87 febrile patients with samples collected in 2007 from Bachu County, Xinjiang, China*  

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Serum samples, no.</th>
<th>TAMV IgG positive, %</th>
<th>TAMV IgM positive, %</th>
<th>Neutralization activity, %</th>
<th>Neutralization titers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>20</td>
<td>3 (15.0)</td>
<td>7 (35.0)</td>
<td>1 (5.0)</td>
<td>64</td>
</tr>
<tr>
<td>F</td>
<td>29</td>
<td>6 (20.7)</td>
<td>5 (17.2)</td>
<td>3 (10.3)</td>
<td>16, 32, 32</td>
</tr>
<tr>
<td>NA</td>
<td>38</td>
<td>12 (31.6)</td>
<td>5 (13.6)</td>
<td>2 (5.3)</td>
<td>16, 16</td>
</tr>
<tr>
<td><strong>Age, y†</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18</td>
<td>6</td>
<td>2 (33.3)</td>
<td>1 (16.7)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>18–30</td>
<td>20</td>
<td>1 (5.0)</td>
<td>8 (40.0)</td>
<td>1 (5.0)</td>
<td>16</td>
</tr>
<tr>
<td>31–45</td>
<td>7</td>
<td>2 (28.6)</td>
<td>1 (14.3)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>46–60</td>
<td>11</td>
<td>2 (18.2)</td>
<td>0</td>
<td>1 (9.1)</td>
<td>32</td>
</tr>
<tr>
<td>&gt;60</td>
<td>5</td>
<td>2 (40.0)</td>
<td>2 (40.0)</td>
<td>2 (40.0)</td>
<td>32, 64</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>87</td>
<td>21 (24.1)</td>
<td>17 (19.5)</td>
<td>6 (6.9)</td>
<td>16–64</td>
</tr>
</tbody>
</table>

*NA, not available; TAMV, Tamdy virus.
†Age stratification based on 49 patients with recorded age information.

References


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SARS-CoV-2 B.1.1.7 Variant Infection in Malayan Tigers, Virginia, USA

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We report infection of 3 Malayan tigers with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) B.1.1.7 (Alpha) variant at a zoologic park in Virginia, USA. All tigers exhibited respiratory signs consistent with SARS-CoV-2 infection. These findings show that tigers are susceptible to infection with the SARS-CoV-2 B.1.1.7 variant.

On April 4, 2021, a 5-year-old male Malayan tiger (Panthera tigris jacksoni) at the Virginia Zoo (Norfolk, VA, USA) began exhibiting lethargy, labored breathing, coughing, intermittent upper respiratory sounds, hypoxemia, and mucoid nasal discharge. On April 7, another 5-year-old male Malayan tiger began experiencing labored breathing, cough, clear nasal discharge, and hypoxemia. On April 10, a third Malayan tiger, a 10-year-old male, had cough and later clear nasal discharge. The tigers’ clinical signs resolved by April 15, eleven days after the outbreak began.

Zoo staff collected nasal swab and fecal samples from the 5-year-old tigers on April 9 and the 10-year-old tiger on April 13 and submitted these to Cornell University’s Animal Health Diagnostic Center (AHDC; Ithaca, NY, USA). AHDC tested samples for Bordetella sp., Chlamydia felis, Mycoplasma cynos, M. felis, Streptococcus equi subspecies zooepidemicus, influenza virus, pneumovirus, feline calicivirus, and feline herpesvirus; all results were negative. All samples tested positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by EZ-SARS-CoV-2 Real-Time RT-PCR Test (Tetracore, Inc., https://tetra-core.com). We isolated SARS-CoV-2 from respiratory and fecal specimens from the first tiger. Testing at the US Department of Agriculture National Veterinary Services Laboratories (Ames, IA, USA) confirmed SARS-CoV-2 infection. We screened the tiger samples using TaqPath COVID-19 RT-PCR Kit (Thermo Fisher Scientific, https://www.thermofisher.com), which revealed a spike gene dropout in samples from all 3 tigers; only the nucleoprotein and open reading frame 1ab gene targets were detected, suggesting B.1.1.7 variant infection.

We performed whole-genome sequencing on all samples by using MinION (Oxford Nanopore Technologies, https://nanoporetech.com), as previously described (1). We assembled reads using the ARTIC nCov-2019 protocol (ARTIC Network, https://artic.network) and Medaka (Oxford Nanopore Technologies) for variant calling. We obtained near-complete (29,702–29,710-bp) assemblies from all nasal swab specimens (GenBank accession nos. MZ305031–3) but no assemblies from fecal samples. We identified respiratory specimen genomes as lineage B.1.1.7 (Alpha variant) by using Pangolin version 2.4.2 (https://github.com/cov-lineages/pangolin). We used Nextstrain (https://nextstrain.org) for phylogenetic analysis of tiger-derived sequences and other B.1.1.7 sequences downloaded from GISAID (https://www.gisaid.org) on April 15, 2021 (2,3). Tiger-derived sequences all were identical, except 1 manually corrected homopolymer repeat error, and fell into a clade defined by a C4900T mutation containing other samples collected primarily in the United States. Tiger-derived sequences differed from others in the clade by 1 single-nucleotide polymorphism in the spike gene (K558N) (Figure, panel A). Using the vdb tool (4), we found 46 additional B.1.1.7 sequences that had the K558N mutation in GISAID on July 22, 2021; all were collected from Virginia during March 27–July 7, 2021. However, phylogenetic analysis of these sequences and the tiger-derived sequences showed divergence of 11 single-nucleotide polymorphism, minus the divergence producing the K558N mutation (Figure, panel B), indicating the sequences are not related epidemiologically.

The source of the tigers’ infection is unknown. The zoo has been open to the public, but transmission from a visitor is unlikely because tiger exhibit areas are separated from visitors by either a glass enclosure or >9 m distance. The most plausible explanation is that ≥1 tiger acquired the virus from a keeper because they had close contact. However, no employees tested positive for SARS-CoV-2 nor had symptoms during the 4 weeks before the tigers’ symptom onset. Nine keepers were responsible for the animals’ daily care; 2 other persons prepared animal diets daily. Employees were required to wear facemasks always, indoors and outdoors; everyone wore standard 2-ply surgical masks or homemade cloth facemasks. Staff also were required to wear gloves when handling and preparing food and when servicing animal areas. Furthermore, staff were
required to step into an accelerated hydrogen peroxide disinfectant footbath when entering the tiger building and diet kitchen. The 3 tigers might have been infected by an employee, or 1 tiger was infected, then transmission occurred to the others. Two tigers lived in the same enclosure and had no direct contact with the third, but all 3 rotated through common enclosure spaces.

After identification of the tiger infections, 4 additional zoo animals were tested: 1 lion (Panthera leo) with lethargy and hyporexia ≈1 week after SARS-CoV-2 diagnosis in the tigers; another asymptomatic lion because of age and proximity to the first lion; and 2 degus (Octodon degus) that died in late March and had interstitial pneumonia on necropsy. AHDC tested nasal swab samples from the lions and frozen spleen and cecum samples from the degus by reverse transcription PCR; all results were negative for SARS-CoV-2.

Our findings underscore felid susceptibility to SARS-CoV-2, which also has been detected in captive snow leopards (Panthera uncia) and pumas (Puma concolor) (5). Other nonhuman species, including gorillas (Gorilla gorilla), minks (Neovison vison), and ferrets (Mustela putorius furo), have acquired SARS-CoV-2; additional species have been shown to be susceptible experimentally (5–7). Domestic cats and dogs in the United Kingdom and United States reportedly had B.1.1.7 infections, suggesting that mutations characterizing this lineage are not constrained to a host range (8; L. Ferasin et al., unpub. data, https://doi.org/10.1101/2021.03.18.435945).

Monitoring animals for SARS-CoV-2 infection is critical to determining potential host range, particularly as new virus variants emerge and spread.

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References
1. Caserta LC, Mitchell PK, Plocharczyk E, Diel DG.
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Postmortem Stability of SARS-CoV-2 in Mouse Lung Tissue

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The infectivity of severe acute respiratory syndrome coronavirus 2 in deceased persons and organisms remains unclear. We studied transgenic K18-hACE2 mice to determine the kinetics of virus infectivity after host death. Five days after death, virus infectivity in the lung declined by >96% and RNA copies declined by 48.2%.

The safe handling and disposal of bodies of persons who have died of coronavirus disease (COVID-19) is vital for infection control. Although cremation or burial practices are mainly dictated by religious and societal customs, deaths associated with contagious illness warrant appropriate precautions. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, is rapidly inactivated (>2 log10) within hours on nonporous surfaces (1). In addition, several studies have detected viral RNA by reverse transcription PCR (RT-PCR) of nasopharyngeal and pharyngeal mucosal swab specimens, skin swab specimens, and tissue samples collected during autopsies at different times after death (2–5). Furthermore, infectious virus was isolated in 2 of 4 cases at 4–17 days post-mortem; however, this study did not quantify virus titers to determine the loss of virus infectivity (6). A separate study found that infectious virus was undetectable after exhumation at 3–4 months postmortem (7). Overall, RNA detection by RT-PCR might not directly correlate with virus infectivity or duration of symptomatic disease.

Transgenic K18-hACE2 mice provide a surrogate model to study the kinetics of SARS-CoV-2 viral replication during infection (8) and after host death. In humans and K18-hACE2 mice, little evidence exists for extrapolatory dissemination of SARS-CoV-2, except for neurotropism in younger mice, a finding that has not been demonstrated reliably in humans. We investigated the temporal decay of infectious SARS-CoV-2 in postmortem tissues of infected K18-hACE2 mice. All experimental procedures were conducted in accordance with the standards and approved by the Committee on the Use of Live Animals in Teaching and Research (approval no. 5511-20) at The University of Hong Kong (Hong Kong, China).

We infected twelve 14–20-week-old mice with 1 × 10^4 50% tissue culture infectious dose (TCID50)/25μL SARS-CoV-2 by the intranasal route. Five days later, after the mice had lost 18.8% (SD 7.77%) of their body weight, we euthanized them by ketamine/xylazil anesthesia. We wrapped each carcass in a sealable plastic bag, similar to the storage of human corpses, and stored them intact at 4°C, which is standard mortuary temperature. On days 0, 1, 5, and 14 after death, we dissected 3 carcasses and tested the lung tissue for coronavirus nucleoprotein (N) by histological and immunohistochemistry assays (9) (Appendix Figure, panels A–H, https://wwwnc.cdc.gov/EID/article/27/12/21-1612-App1.pdf). We quantified infectious virus by culture (Figure, panel A) and viral RNA by RT-PCR (Figure, panel B) (Appendix).
Viral decay, measured using TCID\textsubscript{50} for infectious virus and RNA copies of the N gene detected by RT-PCR, occurred over a 14-day period (Table). At day 1 we observed a 50% reduction of infectious virus and 48.8% loss of viral RNA (Figure, panels A, B). By day 5, levels of infectious virus had fallen by 96.5%, whereas viral RNA remained at 48.2% compared with day 0 (Figure, panels C, D). At day 14 only 0.7% of the initial infectious virus and 17% of viral RNA remained. Plenzig et al. (7) detected viral RNA in 2 exhumed corpses at 3 months postmortem, despite an absence of infectious virus. We used hematoxylin and eosin staining to detect viral nucleoprotein in lung tissue. We observed persistent antigen staining until day 5; by day 14, only 1 of 3 samples had detectable staining (Appendix Figure).

We euthanized the mice 5 days after infection, when the lungs had a high viral load. However, COVID-19 deaths usually occur during later stages of disease, by which time infectious viral load has decreased from the peak usually seen early during the symptomatic phase of the illness (10). We detected virus antigen in the lungs of all mice at 5 days postmortem; infectious virus had declined by 96.48%, but viral RNA declined by only 48.21%. Our results shows that infectious virus declines earlier than viral RNA or antigen in postmortem tissues.

These findings have implications for the safe handling of deceased COVID-19 patients. Infectious virus can persist on inanimate surfaces for up to 14 days at lower temperatures (<4°C), but rapidly decays in postmortem tissue samples. We observed a 96.5% decrease in infectious virus by day 5 and a 99.3% decrease by day 14. Most published postmortem studies in humans have reported viral load at the time of death using cycle threshold values rather than N gene copies as we have done; results range from 17–36 for cycle threshold values and 0–5.49 log\textsubscript{10} for N gene copies (11). Therefore, the maximum potential risk of transmission from an infected

### Table. Postmortem viral loads in K18-hACE2 mice lung tissue after 5 days of infection with severe acute respiratory syndrome coronavirus 2*

<table>
<thead>
<tr>
<th>Day</th>
<th>N gene copies, log\textsubscript{10}</th>
<th>% Reduction of N gene copies†</th>
<th>TCID\textsubscript{50}/mL, log\textsubscript{10}</th>
<th>% Reduction of TCID\textsubscript{50}/mL†</th>
<th>Lung inflammation score‡</th>
<th>SARS-CoV-2 N protein antigen score§</th>
<th>Positive for SARS N protein¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.28 ± 0.53</td>
<td>NA</td>
<td>5.48 ± 0.44</td>
<td>NA</td>
<td>9.33 ± 1.53</td>
<td>3.66 ± 1.15</td>
<td>3 (100.0)</td>
</tr>
<tr>
<td>1</td>
<td>6.39 ± 1.59</td>
<td>−48.85 ± 48.14</td>
<td>4.54 ± 1.48</td>
<td>−50.86 ± 58.82</td>
<td>7 ± 2</td>
<td>2.66 ± 2.31</td>
<td>2 (66.6)</td>
</tr>
<tr>
<td>5#</td>
<td>7.05 ± 0.36</td>
<td>−48.21 ± 45.43</td>
<td>4.00 ± 0.41</td>
<td>−96.48 ± 3.54</td>
<td>5.33 ± 4.61</td>
<td>4 ± 1.4</td>
<td>2 (100.0)</td>
</tr>
<tr>
<td>14</td>
<td>5.53 ± 1.43</td>
<td>−82.95 ± 29.13</td>
<td>2.78 ± 1.21</td>
<td>−99.35 ± 0.86</td>
<td>10.33 ± 1.53</td>
<td>1.66 ± 2.88</td>
<td>1 (33.3)</td>
</tr>
</tbody>
</table>

*Values are mean ±SD, except as indicated. NA, not applicable; TCID\textsubscript{50}, 50% tissue culture infectious dose.
†Compared with day 0.
‡Scale of 0–16, in which 16 represents most severe inflammation.
§Scale of 0–5, in which 5 represents highest amount of antigen.
¶Values are no. (%).
#Days 0.1, and 14 values reflect 3 mice. Day 5 values reflect 2 mice for histology and 3 mice for TCID\textsubscript{50} and reverse transcription PCR.
corpse is during the first 24 hours after death. By day 5, the amount of infectious virus has decreased by 96.48%. If proper biosafety precautions and personal protective equipment are used to handle the corpse during autopsy or preparation for burial or cremation, we believe that the burial or cremation process is unlikely to spread disease.

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Guillain-Barré Syndrome Associated with COVID-19 Vaccination

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We conducted a multi-institutional study in Taiwan and a systematic review of the literature for reports of Guillain-Barré syndrome after coronavirus disease vaccination. This condition, mostly the classic form and the acute inflammatory demyelinating polyneuropathy subtype, has been reported in 39 cases and has occurred within 2 weeks of vaccine administration.

Guillain-Barré syndrome (GBS), an immune-mediated polyradiculoneuropathy with a 5% mortality rate, has an incidence worldwide of 0.81–1.91 cases/100,000 person-years (1). GBS has been

1These authors contributed equally to this article.
reported to be associated with coronavirus disease (COVID-19) vaccination, but a comprehensive summary regarding this rare adverse event is still lacking. To determine clinical features of GBS associated with COVID-19 vaccination, we conducted hospital-based investigations in Taiwan along with a systematic review of published case reports.

We analyzed electronic medical records data from Taiwan’s largest multi-institutional healthcare system, including 9 branches of Chang Gung Memorial Hospital (2), where healthcare workers received first-priority COVID-19 ChAdOx1-S vaccine (Oxford/AstraZeneca, https://www.astrazeneca.com) starting March 22, 2021. We included healthcare workers vaccinated during March 22–May 31 and followed them for 30 days after vaccination. We identified GBS cases on the basis of code G610 from the International Classification of Disease, 10th Revision, Clinical Modification, or spontaneous adverse drug reaction reporting systems within the hospitals. Two authors (C.H.W. and S.C.L.) confirmed diagnosis and classification of GBS cases through chart reviews (3,4). This study was approved by the Institutional Review Board of Chang Gung Medical Foundation (approval no. 202101087B0).

To summarize clinical features of published cases from literature, we searched PubMed and Embase for reports posted through August 17, 2021, using relevant key terms such as “COVID-19,” “Guillain-Barré syndrome,” and “vaccine” with suitable MeSH terms. Two independent reviewers (S.C.S., C.H.W.) performed the study selection and data extraction; a third-reviewer (S.C.L.) settled any differences between them. We excluded cases with coexisting COVID-19 or preexisting GBS. We included only publications with reports of clinical features related

![Figure. Systematic review of published literature in study of Guillain-Barré syndrome associated with coronavirus vaccination, 2021. GBS, Guillain-Barré syndrome.](image-url)
to GBS. We described basic characteristics, laboratory data, pathologic reports, treatment patterns, and prognosis of GBS cases associated with COVID-19 vaccination. The study protocol of this systematic review is published on PROSPERO (https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=265479).

We included 18,269 healthcare workers (mean age 40.6 years, range 18–87 years; 67.5% were women) who received ChAdOx1-S vaccine during the study period. After these 18,257 first-dose and 544 second-dose vaccinations, we identified 1 GBS case after a first dose of ChAdOx1-S vaccine in 1 of the hospitals participating in the study.

After a systematic review of published literature (Figure), we included 17 publications reporting an additional 38 cases of GBS related to COVID-19 vaccination (India, 10 cases; United Kingdom, 11 cases; Mexico, 7 cases; United States, 3 cases; France, 1 case; Italy, 3 cases; Malta, 1 case; Turkey, 1 case; and Qatar, 1 case) (Appendix Table, https://wwwnc.cdc.gov/EID/article/27/12-211634-app1.pdf). Including the case in Taiwan, these 39 cases occurred in persons with a mean age of 57.8 (range 20–86) years; 56.4% were male. Most of the reported case-patients received ChAdOx1-S (25/39), followed by BNT162b2 (12/39) (Pfizer-BioNTech, https://www.pfizer.com), Ad26.COV2.S (1/39) (Johnson & Johnson, https://www.jnj.com), and CoronaVac (1/39) (Sinovac Biotech, http://www.sinovac.com). The GBS rate after COVID-19 vaccination ranged from 1.8 to 53.2 cases/1 million doses. The initial symptoms of GBS included myalgia (12/39), paraparesis (5/39), quadriplegia (22/39), parasthesia (28/39), and facial palsy (23/39), and symptoms of dysautonomia also were observed during hospitalizations (3/39). The average time from vaccination to symptom onset was 11.3 days. A total of 34 case-patients received lumbar puncture; 30 had manifestations of albuminocytologic dissociation in the cerebrospinal fluid.

On the basis of the clinical diagnostic classification of GBS, we found that most case-patients had the classic form (22/39), followed by bilateral facial palsy with paresthesia (12/39), the paraparetic form (4/39), and GBS–Miller Fisher syndrome overlap variant (1/39). We defined all classic and paraparetic forms of GBS (26/26) as level 1 or 2 on the basis of the Brighton criteria (5). We identified the GBS subtype in 33/39 cases by electrophysiological examination; most reported case-patients had a diagnosis of acute inflammatory demyelinating polyneuropathy (23/33), followed by acute motor and sensory axonal neuropathy (4/33) and acute motor axonal neuropathy (3/33). For GBS management, 33 case-patients received intravenous immunoglobulin and 2 received plasmapheresis. One case-patient died; 9 case-patients required mechanical ventilation during hospitalization. The scores on the GBS disability scale (5) were only available for 30 cases; 12 scored ≥4 (i.e., indicating bedridden or chair-bound status) during follow-up or after discharge.

Similar to previous reviews on GBS associated with COVID-19, we found that both COVID-19 and COVID-19 vaccination mostly cause the classic form of GBS (under the clinical diagnosis classification) and the acute inflammatory demyelinating polyneuropathy subtype (based on electrodiagnostic features) within 2 weeks of infection or vaccination (6–8). However, the bilateral facial palsy with paresthesia variant and initial onset symptoms of facial diplegia were more frequently found in GBS case-patients after COVID-19 vaccination.

Case series and reports can indicate safety issues and outline clinical features of diseases, but they cannot establish robust causal relationships between COVID-19 vaccination and GBS. Despite the benefits (e.g., increase in the number of persons not susceptible to infection and decrease in severe outcomes after infection) of COVID-19 vaccination far outweighing the potentially severe adverse events after infection (9), our findings highlight the need for vigilance in patients with neurologic symptoms after COVID-19 vaccination and for postvaccination surveillance programs to assess causality of GBS.

Acknowledgments
We thank Cheng-Yang Hsieh and Wen-Mei Cheng for their insightful opinions on this study.

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References
3. Uncini A, Vallat JM, Jacobs BC. Guillain-Barré syndrome in
Limited Protection of Inactivated SARS-CoV-2 Vaccine against Wild-Type Strain and Variants of Concern


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In vitro determination of severe acute respiratory syndrome coronavirus 2 neutralizing antibodies induced in serum samples from recipients of the CoronaVac vaccine showed a short protection period against the original virus strain and limited protection against variants of concern. These data provide support for vaccine boosters, especially variants of concern circulate.

Circulation of novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants capable of evading vaccine-derived protection is challenging the efficacy of coronavirus disease (COVID-19) vaccines (1). The inactivated SARS-CoV-2 vaccine CoronaVac (Sinovac Biotech, http://www.sinovac.com), 1 of 2 COVID-19 vaccines licensed in Thailand, has been widely administered to health care workers. Clinical studies show CoronaVac efficacy against symptomatic COVID-19 ranging from 51% (Brazil) to 65.9% (Chile) and 100% against severe illness and illness requiring hospitalization (2,3). However, data on CoronaVac efficacy against variants of concern are very limited. Our study was approved by the Research Ethics Review Committee, Faculty of Medicine, Chulalongkorn University (Bangkok, Thailand) and recorded in the Thai Clinical Trial Registry (TCTR20210325003). Investigators adhered to U.S. Department of Defense AR 70–25 policies for protection of human subjects.

For this study, we enrolled 207 health care workers in Thailand who were fully vaccinated with 2 doses of CoronaVac (0.5 mL/dose, 2–4 wk between doses); all had received their first dose during February 22–March 12, 2021. Median age was 39 (interquartile range 30–51) years of age; 67 (49.6%) were men. Among study participants, 58 (28%) provided blood samples only at baseline (when the first dose was administered), 93 (44.0%) both at baseline and 2–3 weeks after the second dose, and 56 (27.0%) at baseline and at 2–3 weeks and 10–12 weeks after the second dose. Using an in vitro system (Appendix, https://wwwnc.cdc.gov/EID/article/27/12/21-1772-App1.pdf), we evaluated the ability of the serum of CoronaVac
recipients to neutralize SARS-CoV-2. We measured circulating serum neutralizing antibodies to the original SARS-CoV-2 wild-type strain by using a cPass receptor binding domain antigen-based surrogate virus neutralization test (sVNT) ELISA (GeneScript, https://www.genscript.com) and using a microneutralization assay (MNA) for SARS-CoV-2 wild-type strain and Alpha, Beta, and Delta neutralizing antibodies. Seroconversion rates for CoronaVac-vaccinated participants, determined by sVNT ELISA using 30% inhibition as cutoff, were 85.2% (78.2% mean inhibition level) at 2–3 weeks and 35% (25.4% mean inhibition level) at 10–12 weeks. The MNA seropositivity cutoff was set at ≥50%.

At 2–3 weeks after the second dose, 61.1% (91/149) of participants were seropositive against the wild-type strain, 35.6% (53/149) against Alpha variant, 3.4% (5/149) against Beta, and 8.7% (13/149) against Delta (Figure). Mean neutralizing rate at 2–3 weeks was 49.3% (95% CI 44.9%–53.6%) against the wild-type strain, 40.9% (95% CI 37.8%–43.9%) against Alpha variant, 9.0% (95% CI 6.1%–11.8%) against Beta, and 10.8% (95% CI 7.1%–14.5%) against Delta. At 10–12 weeks after the second dose, the proportion of seropositive participants fell to 50% (28/56) against Wild-type strain and was significantly reduced (p<0.001) to 17.9% (10/56) against Alpha variant, 1.8% (1/56) against Beta, and 1.8% (1/56) against Delta. Mean neutralizing rates at 10–12 weeks were 48.0% (95% CI 39.9%–56.1%) against the wild-type strain, 21.8% (95% CI 37.8%–43.9%) against Alpha variant, 1.2% (95% CI 3.5%–8.8%) against Beta, and 1.0% (95% CI 2.9%–7.5%) against Delta.

Comparing sVNT ELISA results between the 2 time points, Wild-type strain antibodies appear to have a half-life of 83.4 days (95% CI 76.6–90.3 days). However, when the MNA was used, neutralizing antibodies waned in a time- and variant-dependent manner. The half-life of neutralizing antibodies was as low as 47.2 days (95% CI 37.4–56.9 days) for the wild-type strain, 38.6 days (95% CI 37.5–39.5 days) for Alpha variant, 6.8 days (95% CI 5.3–8.8 days) for Beta, and 12.3 days (95% CI 8.3–16.3 days) for Delta.

Table. Results of in vitro testing by surrogate virus neutralization test ELISA and microneutralization assay of CoronaVac-induced neutralizing wild-type strain and Alpha, Beta, and Delta variants of severe acute respiratory syndrome coronavirus 2

<table>
<thead>
<tr>
<th>Neutralization test detection method</th>
<th>Slope coefficient (95% CI)</th>
<th>Half-time coefficient, d (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surrogate virus neutralization test ELISA</td>
<td>−0.645 (−0.751 to −0.538)</td>
<td>83.42 (76.55–90.29)</td>
</tr>
<tr>
<td>Microneutralization assay</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>0.008 (−0.141 to 0.159)</td>
<td>47.17 (37.48–56.86)</td>
</tr>
<tr>
<td>Alpha</td>
<td>−0.187 (−0.302 to −0.072)</td>
<td>38.57 (31.16–45.99)</td>
</tr>
<tr>
<td>Beta</td>
<td>−0.063 (−0.121 to −0.006)</td>
<td>6.88 (3.20–10.57)</td>
</tr>
<tr>
<td>Delta</td>
<td>−0.125 (−0.211 to −0.040)</td>
<td>12.27 (6.78–17.77)</td>
</tr>
</tbody>
</table>

*CoronaVac vaccine by Sinovac Biotech (http://www.sinovac.com).
CI 31.2–45.9 days) for Alpha variant, 6.9 days (95% CI 3.2–10.6 days) for Beta, and 12.3 days (95% CI 6.8–17.8 days) for Delta (Table). These data indicate the possibility that SARS-CoV-2 variants are able to escape humoral induced by wild-type prototype inactivated vaccines, which is consistent with results of other recent studies (4,5). Our findings support administering vaccine boosters, especially where these variants circulate.

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Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation or publication. The opinions or assertions contained herein are the private views of the authors, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense. The investigators have adhered to the policies for protection of human subjects as prescribed in AR 70-25.

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References

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Breakthrough Infections of E484K-Harboring SARS-CoV-2 Delta Variant, Lombardy, Italy

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The Delta variant of concern of severe acute respiratory syndrome coronavirus 2 is dominant worldwide. We report a case cluster caused by Delta sublineage B.1.617.2 harboring the mutation E484K in Italy during July 11–July 29, 2021. This mutation appears to affect immune response and vaccine efficacy; monitoring its appearance is urgent.
Since the beginning of 2021, a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant originally described in India has become the predominant circulating variant of the coronavirus disease pandemic. This variant of concern (VOC) was renamed Delta by the World Health Organization and consists of date of 5 different sublineages (B.1.617.2, AY.1, AY.2, AY.3, and AY.3.1, according to PANGOLIN phylogeny) that share T478K and L452R as the main mutations of concern (MOCs) within the spike protein. B.1.617.2 (also known as VUI-21APR-02) is by far the most represented Delta sublineage. None of the 5 sublineages are to date characterized by the occurrence of the other MOC E484K, which causes resistance to monoclonal antibodies and reduced vaccine efficacy. However, given the widespread convergent evolution of the spike protein observed across clades, the occurrence of MOC E484K and its widespread circulation is largely expected. A clade simultaneously harboring all such MOCs is likely to be of extreme concern because of theoretical increased immune escape. We report a cluster of B.1.617.2 and E484K occurring in Lombardy, Italy. All cases were first tested by real-time reverse transcription PCR and, if positive, sequenced as previously reported (1).

On July 11, 2021, a 41-year-old man from a small village in northern Lombardy (vaccinated with BNT162b2 [Pfizer-BioNTech, https://www.pfizer.com] on June 12 and July 12) began experiencing cough, fever, and malaise; a nasopharyngeal swab specimen tested positive on July 14 by the SARS-CoV-2 Variants Elite MGB Kit (EliTech Group, https://www.elitechgroup.com); cycle threshold (Ct) was 21 for open reading frame (ORF) 1ab gene and 21 for the nucleocapsid (N) gene. He fully recovered without need for hospital admission; whole-genome sequencing confirmed B.1.617.2 that harbored E484K. His 80-year-old mother (vaccinated with mRNA-1273 [Moderna, https://www.modernatx.com] on April 9 and May 7) experienced fatigue, headache, myalgia, and dyspnea beginning July 17 and tested positive on July 24 (Ct 22 for ORF1ab gene and Ct 21 for N gene). She likely further infected (while playing cards) a 77-year-old man (vaccinated with BNT162b2 on April 26 and May 17) who began experiencing fever July 21 and tested positive on July 23 (Ct 20 for ORF1ab gene and Ct 19 for N gene) and an 83-year-old woman (vaccinated with BNT162b2 on April 3 and April 24) who experienced fever, fatigue, ageusia, and anosmia beginning July 21 and tested positive July 24 (Ct 18 for both genes). None required hospital admission. An unrelated patient from the same village, an 81-year-old woman (vaccinated with mRNA-1273 on May 7 and June 9), experienced dyspnea, fever, myalgia, and fatigue beginning July 24. On July 29, she tested positive for SARS-CoV-2 RNA (Ct 23 for ORF1ab gene and Ct 21 for N gene), and she was admitted to the hospital. All sequences obtained in this study have been deposited into GISAID (https://www.gisaid.org; accession nos. EPI_ISL_3462078, EPI_ISL_3462074, EPI_ISL_3462072, EPI_ISL_346208).

E484K is the hallmark MOC of VOCs Beta and Gamma, in addition to having been reported in a minor sublineage of VOC Alpha, in variants of interest Eta and Iota, and at frequencies >50% in 38 more strains. E484K causes resistance to many class 2 RBD-directed antibodies (2), including bamlanivimab (3). The most potent mRNA vaccine-elicited monoclonal antibodies were ≥10-fold less effective against pseudotyped viruses carrying the E484K mutation (Z. Wang et al., unpub. data, https://www.biorxiv.org/content/10.1101/2021.01.15.426911v2). As of August 12, 2021, GISAID reported E484K in 52 of 408,781 B.1.617.2 sequences, 2 of 549 AY.1 sequences, and 32 of 19,996 AY.3 (Delta) sequences; none of these reports were in Italy. E484K has been additionally reported in 1 of 6,011 B.1.617.1 (Kappa variant) sequences (4).

Nasopharyngeal swab specimens positive for the Delta variant have ≈4-fold higher viral loads than non-VOC or Alpha variants (C. von Wintersdorff et al., unpub. data, https://www.researchsquare.com/article/rs-762916/v1) and a shorter incubation time of 4 days (B. Li et al., unpub. data, https://www.medrxiv.org/content/10.1101/2021.07.07.21260122v1). It is resistant to REGN10933 (T. Tada et al., unpub. data, https://www.biorxiv.org/content/10.1101/2021.07.19.452771v3) and bamlanivimab (M. Hoffman et al., unpub. data, https://www.biorxiv.org/content/10.1101/2021.05.04.442663v1; P. Arora et al., unpub. data, https://www.biorxiv.org/content/10.1101/2021.06.23.449568v1), whereas neutralization by antibodies were >10-fold less effective against pseudotyped viruses carrying the E484K mutation (Z. Wang et al., unpub. data, https://www.biorxiv.org/content/10.1101/2021.01.15.426911v2). All sequences obtained in this study have been deposited into GISAID (https://www.gisaid.org; accession nos. EPI_ISL_3462078, EPI_ISL_3462074, EPI_ISL_3462072, EPI_ISL_346208).

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E484K mutation represents a critical evolutionary event that leads to immune escape, although its consequences on viral fitness are unclear. Surveillance by genome sequencing should be maintained (T. Farinholz et al., unpub. data, https://www.medrxiv.org/content/10.1101/2021.06.28.21258780v4).

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Dr. Baj is a medical research scientist at University of Insubria, Varese, Italy. Her research interests include persistent and emerging viral infections.
Phylogenetic analysis of a clinical isolate associated with subclinical *Burkholderia pseudomallei* infection revealed probable exposure in the British Virgin Islands, where reported infections are limited. Clinicians should consider this geographic distribution when evaluating possible infection among persons with compatible travel history.
other bacteria and are not confirmatory for this bacterium. Even 16S rRNA gene sequencing can be inadequate depending on the segment queried (1). The Ohio Department of Health Laboratory confirmed B. pseudomallei by using CDC’s Laboratory Response Network algorithm (https://emergency.cdc.gov/lrn/index.asp).

Because the patient could not tolerate optimal eradication therapy (5), she received intensive therapy with intravenous meropenem for 14 days, then completed a 3-month course of oral doxycycline. Computed tomography images shortly after completing the treatment course showed no evidence of active infection.

Figure. Dendrogram of Burkholderia pseudomallei isolated in a patient who traveled to the British Virgin Islands, 2018. Bold text indicates patient isolate; reference genomes predominantly are from the Western Hemisphere. The tree was generated by using MEGA 7.0 software (http://www.megasoftware.net). Single-nucleotide polymorphism analysis was performed by using Parsnp in the Harvest 1.3 package (https://github.com/marbl/harvest). Scale bar indicates nucleotide substitutions per site.
During interviews with public health officials, the patient reported traveling to the British Virgin Islands (BVI) twice a year for ≈3 weeks at a time and had visited 2–3 months before the identification of lymphadenitis. She also reported trips of <1 month duration to China and Singapore, where \( B. \text{pseudomallei} \) is endemic, within the previous 10 years (1,3). No known exposures to \( B. \text{pseudomallei} \) were reported. However, she recalled landscaping activities in BVI that resulted in noticeable dust in her residence, but she did not know on which BVI visit this exposure to aerosolized soil occurred. CDC performed whole-genome sequencing of the patient’s \( B. \text{pseudomallei} \) isolate, OH2018, for comparison to reference genomes that have well-established geographic origins. The isolate’s genome sequence is available at the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov) under Bioproject accession no. PRJNA575632. Multilocus sequence typing classified the isolate as sequence type 92, which previously has been observed in several isolates originating from the Western Hemisphere (6,7). Phylogenetic single-nucleotide polymorphism analysis demonstrated OH2018 groups with reference genomes from the Caribbean, especially the US Virgin Islands and BVI (Figure).

Whole-genome sequencing of the isolate was essential to determining potential exposure risk because the patient traveled to multiple regions where \( B. \text{pseudomallei} \) is endemic. The patient likely was exposed to \( B. \text{pseudomallei} \) in BVI 2–3 months before infection was identified, as ascertained through molecular epidemiology and supported by her report of travel and exposure to aerosolized soil in this location. The case provides additional evidence that \( B. \text{pseudomallei} \) is endemic to the Caribbean and, more specifically, BVI, where reported infections are limited. Only 1 other infection associated with BVI has been reported in the literature (8), and no environmental isolates have been reported. To support prompt identification and treatment for melioidosis, clinicians and public health officials should be aware of this geographic distribution when considering possible infection among persons with compatible travel history.

Acknowledgments
We thank Hillary Van Heule for providing information about patient isolates and the local health department and public health officials who assisted in the epidemiologic investigation.

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Dr. Dewart is a registered nurse and infectious disease epidemiologist. She served as a CDC Epidemiology Intelligence Service officer during 2019–2021 and is currently a field assignee to the Ohio Department of Health, Columbus, Ohio, USA, through CDC’s Center for Preparedness and Response. Her primary research interests include healthcare-associated infections and antimicrobial resistance.

References

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SARS-CoV-2 Sequence Analysis during COVID-19 Case Surge, Liberia, 2021

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Before May 2021, Liberia reported <10 coronavirus disease (COVID-19) cases per day among its population of ≈5 million (1). Thereafter, case numbers, hospitalizations, and deaths rapidly increased and peaked to >200 cases and 10–15 deaths per day in mid-July 2021 (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/27/12/21-1818-App1.pdf). To determine whether the rapid case surge was associated with the introduction of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants of concern or newly emerging variants, we collected nasopharyngeal swab samples from 267 hospitalized patients countrywide during March–July 2021 for high-throughput sequencing.

We collected samples in viral transport media from Bomi, Bong, Grand Cape Mount, Lofa, Margibi, Maryland, Montserrat, and Nimba Counties (Appendix Figure 2). We noted sample collection date and site and sex and median age of patients from whom samples were obtained (Table; Appendix Table). We used Buffer AVL (QIAGEN, https://www.qiagen.com) lysis buffer to extract total nucleic acid and performed PCR by using the Triplex-CI-SARS-CoV-2 rRT PCR assay (2). We conducted further high-throughput sequencing on 89/267 (33.3%) samples that had cycle threshold values <33 (Appendix Table).

To prepare libraries, we used the Kapa Hyperplus Kit (Roche, https://www.roche.com) on first strand cDNA synthesized from 89 RNA samples (3), then we enriched for SARS-CoV-2 by using myBaits Custom RNA-Seq Kit (Daicel Arbor Biosciences, https://arborbiosci.com). We sequenced captured libraries on Nextseq 2000 or Nextseq 550 (Illumina, https://www.illumina.com), which yielded 5–8 million 220-bp reads per sample. We mapped reads to a SARS-CoV-2 reference sequence (GenBank accession no. NC_045512) to determine variants (Table; Appendix Table).

Of the 89 RNA samples, 77 (86.5%) yielded complete coding sequences with a minimum depth of ≈15× (GISAID accession nos. EPI_ISL_3547663–705, EPI_ISL_3560291, and EPI_ISL_4232122–52). Using high-throughput sequencing data, we generated consensus fasta sequences of 77 SARS-CoV-2 genomic sequences and further analyzed sequences by using Geneious R10 (https://www.geneious.com), Next-Strain (4), and GISAID (5).

Among 77 genomes recovered, 4 (5.2%) were Alpha variant (B.1.1.7); 6 (7.8%) were Beta variant (B.1.526); 6 (7.8%) were Eta variant (B.1.525); and 56 (72.7%) were Delta variant (B.1.617.2) viruses (Table). We identified Delta variant viruses in samples collected in early March and in April and May 2021, from Bong County. Delta variant viruses were co-circulating with Alpha, Beta, Eta, Iota, and other 20B variant viruses in Liberia. All 44 sequences recovered during June–July 2021 were from Delta variant viruses (Table). We used complete polyprotein coding sequences from Liberia, other representative SARS-CoV-2 sequences, and variant reference sequences to create a maximum-likelihood, nucleotide-based phylogenetic tree in MEGA X (6) (Figure).

Using reference sequence NC_045512 as a baseline, we found 3 Alpha variant–specific amino acid deletions (H69del, V70del, Y144del) in the surface glycoprotein of all Alpha variant genomes and 3 Beta variant–specific amino acid deletions (L241del, L242del, A243del) in the surface glycoprotein of all Beta variant genomes. All 56 Delta variant genomes had the 2 variant-specific amino acid deletions, F157del and R158del, and 8 of 9 other Delta variant-

1These first authors contributed equally to this article.
The population was vaccinated in Liberia. The infections were caused by multiple separate introductions. Strains were circulating after March 2021 and suggest that Alpha variant viruses (B. Shobayo, unpub. data) were Alpha variant viruses (B. Shobayo, unpub. data) in <3 months. Mutation and phylogenetic analyses further indicate that several Delta variant strains were circulating after March 2021 and suggest multiple separate introductions.

Before June 2021, only a small percentage of the population was vaccinated in Liberia. The infections we report occurred in unvaccinated persons. The Ministry of Health, Liberia, initiated a vaccination drive in August 2021. By September, >130,000 persons, >2% of the population, had received a single dose of the Johnson & Johnson/Janssen vaccine (https://www.jnj.com). The COVID-19 vaccination campaign is ramping up as <30 cases/day are reported in Liberia, but the currently circulating Delta variants are a concern because they contain mutations and deletions in the surface glycoprotein that might influence vaccine efficacy (9). Liberia should continue surveillance for SARS-CoV-2 variants of concern to determine whether additional vaccination or public health measures are needed to curb severe disease and future case surges in the country.

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Table. Characteristics of 77 clinical samples collected before and during COVID-19 case surge that yielded complete SARS-CoV-2 coding genomic sequences, Liberia, 2021

<table>
<thead>
<tr>
<th>Month collected</th>
<th>Total no. of samples</th>
<th>Patient sex, no.</th>
<th>Average age, y (SD)</th>
<th>County</th>
<th>No. of samples, county</th>
<th>Delta</th>
<th>Alpha</th>
<th>Beta</th>
<th>Eta</th>
<th>Iota</th>
<th>20B</th>
</tr>
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<tbody>
<tr>
<td>Mar</td>
<td>4</td>
<td>2M, 2F</td>
<td>39.25 (6.05)</td>
<td>Montserrado</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Apr</td>
<td>11</td>
<td>10M, 1F</td>
<td>42.54 (11.52)</td>
<td>Montserrado</td>
<td>10</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Grand Cape Mount</td>
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<td>Mount</td>
<td>14</td>
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<td></td>
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<td></td>
<td></td>
<td>Nimba</td>
<td>2</td>
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<tr>
<td>May</td>
<td>18</td>
<td>9M, 9F</td>
<td>40.11 (16.82)</td>
<td>Bong</td>
<td>1</td>
<td>1</td>
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<td>Margibi</td>
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<td>Montserrado</td>
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<td>Lofa</td>
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<td>Maryland</td>
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<td></td>
<td>Montserrado</td>
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<td>Nimba</td>
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</table>


Specific amino acid substitutions in the surface glycoprotein (T19R, G142D, E156G, L452R, T478K, D614G, P681R, and D950N). The A222V surface glycoprotein mutation was absent in only 2/56 Delta variant genomes, LIB-0226 and LIB-0217, collected from Monteserrado County in May 2021. We observed another mutation in the surface glycoprotein, V367L, in 14 sequences: 1 from Bong, 2 from Margibi, 1 from Maryland, 9 from Montserrado, and 1 from Nimba. No sequences recovered from Lofa County had the V367L mutation. We noted the R724K mutation in the open reading frame 8 region of 2 sequences from Monteserrado County and had 2 amino acid deletions in the open reading frame 8 region (position 120–121).

Recent surges in COVID-19 in many countries have been associated with the emergence of highly transmissible Delta variant viruses (7,8). In March 2021, the National Public Health Institute of Liberia sequenced 10 random samples from hospitalized COVID-19 patients in Monteserrado; all sequences were Alpha variant viruses (B. Shobayo, unpub. data).

A limitation of our study is the small sample sets used for analysis; nonetheless, our findings suggest that Alpha and other circulating variant viruses were replaced by Delta variant viruses countrywide in Liberia in <3 months. Mutation and phylogenetic analyses further indicate that several Delta variant strains were circulating after March 2021 and suggest multiple separate introductions.
About the Author

Dr. Shobayo is public health and medical research scientist and deputy director at National Public Health Institute of Liberia, Monrovia, Liberia, and member of Partnership for Research on Infectious Diseases in Liberia. His research interests include surveillance and investigation of emerging and re-emerging viruses and their impacts on public health.

Figure. Phylogenetic analysis of 77 nasopharyngeal swab samples collected during coronavirus disease case surge, Liberia, March–July 2021, and reference sequences. We created a maximum-likelihood nucleotide phylogenetic tree of the complete polyprotein coding region by using MEGA X (https://www.megasoftware.net), with a bootstrap value of 100 and used Tamura-Nei 93 (TN93) as a substitution model with a discrete gamma distribution (+G) for evolutionary rate; the rate variation model allowed some sites to be evolutionarily invariable (+I). Numbers along the branches are bootstrap values of 100 bootstrap resamplings. Teal indicates samples collected in March 2021; purple indicates samples collected in April 2021; pink indicates samples collected in May 2021; blue indicates samples collected in June 2021; orange indicates samples collected in July 2021; brown indicates variants of concern or variants of interest; black indicates other circulating variants; green indicates severe acute respiratory syndrome coronavirus 2 reference sequence and other early parental sequences from 2020.
References:
4. GISAID. Tracking of variants [cited 2021 Sep 15]. https://www.gisaid.org/hcov19-variants

Real-Time Projections of SARS-CoV-2 B.1.1.7 Variant in a University Setting, Texas, USA

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We used the incidence of spike gene target failures identified during PCR testing to provide an early projection of the prevalence of severe acute respiratory syndrome coronavirus 2 variant B.1.1.7 in a university setting in Texas, USA, before sequencing results were available. Findings from a more recent evaluation validated those early projections.

Identification of the highly transmissible novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant B.1.1.7 (Alpha variant) in the United Kingdom raised concerns for renewed pandemic surges worldwide (1,2). B.1.1.7 likely arrived in the United States by October 2020 (1); it was first detected in December 2020 and declared the dominant strain in April 2021, as projected in January 2021 (3). However, the regional prevalence of B.1.1.7 was largely unknown in early 2021 because of limited molecular surveillance for SARS-CoV-2 (4). To provide local situational awareness at that pivotal moment in the coronavirus disease (COVID-19) pandemic, we estimated the prevalence of B.1.1.7 on the basis of 17,003 student SARS-CoV-2 PCR test results reported through the Proactive Community Testing Program at the University of Texas (UT; Austin, Texas, USA), a large public university located in a metropolitan area with a population >2 million, during January 16–February 12, 2021 (K.E. Johnson et al., unpub. data, https://doi.org/10.1101/2021.03.05.21252541. Those early estimates were subsequently validated by using PCR data through April 9, 2021.

Mutations in the B.1.1.7 spike protein result in a failure to detect the spike gene probe in standard SARS-CoV-2 quantitative reverse transcription PCR (qRT-PCR). In estimating the prevalence of B.1.1.7 from local quantitative PCR data, we initially assumed US estimates for the proportion of spike gene target failures (SGTF) attributable to B.1.1.7 (4) and, in our retrospective analysis, update that proportion on the basis of local sequencing data. We used a Bayesian model to estimate the local growth rate of B.1.1.7 among all SARS-CoV-2 infections and applied a compartmental susceptible-exposed-infected-recovered model of SARS-CoV-2 transmission to project the effect of B.1.1.7 on future COVID-19 prevalence.

These authors contributed equally to this article.
We previously estimated that the relative frequency of B.1.1.7 among positive SARS-CoV-2 samples was growing logistically at a daily rate of 0.077 (95% CI 0.017–0.140), corresponding to an early doubling time of 9.0 days (95% CI 5.0–41.0 days) (K.E. Johnson et al., unpub. data, https://doi.org/10.1101/2021.03.05.21252541). At the time, we projected that B.1.1.7 would comprise most cases at UT by March 5 (95% predictive interval [PI] February 20–March 28) (Figure, panel A).

Subsequent estimates of B.1.1.7 prevalence based on quantitative PCR data from February 20 through April 9 fell within 95% PIs of the early projections (Figure, panel A) but suggested a lower daily growth rate of 0.037 (95% CI 0.026–0.048) and a corresponding doubling time of 18.7 days (95% CI 14.3–26.7 days). As of April 9, we estimated that B.1.1.7 comprised 61.2% (95% CI 48.5%–72.6%) of SARS-CoV-2 infections, consistent with our initial projections that B.1.1.7 would become the dominant variant by March 28 (95% CI March 20–April 10) and that B.1.1.7 is 24% (95% CI 17%–32%) more transmissible than the wild-type virus.

Based on those local estimates, scenario-based projections suggested that B.1.1.7 might cause 6.2%
Emergence in cities throughout the United States. Forts have provided sentinel warning of B.1.1.7. During January 16–March 5, UT confirmed rapid detection of B.1.1.7 emergence from widely available quantitative PCR data when sequence confirmation was not available or delayed, while quantifying uncertainty in the variant growth rate and fraction of SGTF samples that were positive for B.1.1.7. During January 16–March 5, UT confirmed 22 of 23 sequenced SGTF SARS-CoV-2 specimens as the B.1.1.7 variant, corroborating our reliance on SGTF data (Appendix). Our findings reinforce the urgent need for expanded molecular surveillance capacity. In the absence of widespread and rapid sequencing efforts, quantitative PCR data from large-scale testing efforts have provided sentinel warning of B.1.1.7 emergence in cities throughout the United States.

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Correlation between Buruli Ulcer Incidence and Vectorborne Diseases, Southeastern Australia, 2000–2020

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Researchers have hypothesized that mosquitoes are vectors involved in Mycobacterium ulcerans transmission. Previous findings of a correlation between incidence of M. ulcerans, which causes Buruli ulcer, and locally acquired vectorborne diseases in southeastern Australia further strengthened this argument. However, our updated data indicate that this correlation has not continued beyond 2008.

Mycobacterium ulcerans infection causes skin and soft tissue destruction and is classified by the World Health Organization as a neglected tropical disease. Internationally, disease caused by M. ulcerans infection is known as Buruli ulcer (BU) and has been reported in 33 countries (1), mostly in central and western Africa. However, in southeastern Australia, the epidemic is worsening; disease incidence and severity of infection have increased rapidly since 2015 (2). Although the mode(s) of transmission of M. ulcerans remain(s) unclear, a study published in 2009 showed a correlation between BU incidence and locally acquired vectorborne diseases in southeastern Australia (3). This finding strengthened the hypothesis that mosquitoes may be involved in M. ulcerans transmission. We examined data to determine if this correlation continued beyond 2008.

In Africa, M. ulcerans is thought to be transmitted by water bugs, and a report of a case in southeastern Australia suggested that a BU lesion first appeared at the site of a mosquito bite (4). Subsequently, the probability of mosquitoes being M. ulcerans positive by PCR has been associated with the degree of BU endemicity in southeastern Australia (4,5). Furthermore, being bitten by a mosquito substantially increases the odds of BU developing, and using insect repellent and protective clothing reduces the odds (6). However, this association does not necessarily imply that mosquitoes are involved in M. ulcerans transmission because covering limbs with clothing would also help to protect against other potential environmental sources of M. ulcerans, such as possum excreta or soil contamination of wounds. Furthermore, mosquitoes rarely act as vectors for bacteria, and no other species of Mycobacteria are known to have arthropod vectors.

The apparent role of mosquitoes in the transmission of M. ulcerans may be explained by mechanical vectoring. Within M. ulcerans–endemic areas of southeastern Australia, the geographic locations of BU cases are highly focal. Cases are often clustered together, and adjoining communities only a few kilometers away may be spared (7). However, Aedes camptorhynchus mosquitoes, one of the main species thought to be involved in M. ulcerans transmission, are widespread within M. ulcerans–endemic and –nonendemic areas, and many species of mosquito may have the capacity to fly distances that would take them outside of BU-affected regions. Therefore, the transmission model for M. ulcerans may not be explained by mosquitoes alone.

In coastal regions of southeastern Australia, Ross River virus (RRV) and Barmah Forest virus (BFV) cause locally acquired vectorborne diseases. RRV and BFV are transmitted by Ae. camptorhynchus mosquitoes, which were the main species captured during an M. ulcerans outbreak in Point Lonsdale, southeastern Australia (4). The incidence of RRV and BFV peaks sporadically, especially during years of above average rainfall or La Niña events, as occurred during the 2020–21 summer in Australia (8). These environmental changes often favor increased mosquito population sizes, thus giving rise to RRV and BFV outbreaks (9). It is also thought that BU incidence is associated with environmental factors; increased incidence has lagged 12 months behind periods of greater rainfall (10). However, no association has been found between rainfall and BU cases on the Mornington Peninsula, the main M. ulcerans–endemic site driving the increased incidence of BU in southeastern Australia.

During 2002–2008, BU incidence correlated with combined RRV/BFV incidence in Victoria, southeastern Australia ($r^2 = 0.52$) (3). It was argued that this correlation strengthened the link between mosquitoes and M. ulcerans transmission in southeastern Australia. However, this observation was made over a short time. Using the square of the Pearson product-moment correlation coefficient (coefficient of determination) analysis over a 21-year period (2000–2020), we found little to no correlation between BU and combined RRV/BFV incidence in southeastern Australia ($r^2 = 0.05; p = 0.69$) (Figure) (Appendix, https://wwwnc.cdc.gov/EID/
For comparison, during this same period there was no correlation between BU and infection with *M. tuberculosis* (the other main mycobacterial disease in southeastern Australia) or *Legionella* (water-associated bacteria).

A lack of correlation between BU incidence and locally acquired vectorborne diseases does not disprove that mosquitoes are involved in *M. ulcerans* transmission. Nevertheless, this lack of correlation may suggest that the worsening BU epidemic in southeastern Australia is not caused by increased mosquito populations or other environmental changes that favor RRV and BFV outbreaks. We believe that other independent factors may be driving the increased BU incidence, although the effects of recent La Niña events on BU incidence in 2021 are not yet known.

Planning and implementing successful public health interventions to control *M. ulcerans* are substantially hindered by lack of knowledge of the mechanism of disease transmission.

**About the Author**

Mr. Linke is a final-year doctor of medicine student at the Geelong Clinical School, Deakin University. His research interests include mosquito-borne flaviviruses and cellulitis.

**References**


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**Figure.** Incidence (cases/100,000 population) of Buruli ulcer compared with that of other notifiable diseases in Victoria, Australia, during 2000–2020. Victoria is located in southeastern Australia. “Other diseases” on left y-axis indicates TB, legionellosis, and RRV and BFV incidence combined. The shaded area (2002–2008) denotes a period when Buruli ulcer incidence correlated with RRV/BFV incidence (3). In Australia, these infections are notifiable and incidence rates are publicly available (8). BFV, Barmah Forest; RRV, Ross River virus; TB, tuberculosis.
**Borrelia miyamotoi in Human-Biting Ticks, United States, 2013–2019**

Guang Xu, Chu-Yuan Luo, Fumiko Ribbe, Patrick Pearson, Michel Ledizet, Stephen M. Rich

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During 2013–2019, *Borrelia miyamotoi* infection was detected in 19 US states. Infection rate was 0.5%–3.2%; of *B. miyamotoi*-positive ticks, 59.09% had concurrent infections. *B. miyamotoi* is homogeneous with 1 genotype from *Ixodes scapularis* ticks in northeastern and midwestern states and 1 from *I. pacificus* in western states.

**Table. Human-biting tick species positive for Borrelia miyamotoi and *B. burgdorferi* sensu lato, United States, 2013–2019**

<table>
<thead>
<tr>
<th>Tick species</th>
<th>Total no. tested</th>
<th>No. <em>B. miyamotoi</em> positive</th>
<th>No. <em>B. burgdorferi</em> s.l. positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amblyomma americanum</td>
<td>1,167</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A. cajennense</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A. maculatum</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dermacentor andersoni</td>
<td>60</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D. occidentalis</td>
<td>91</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D. variabilis</td>
<td>1,060</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Haemaphysalis leporispalustris</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>H. longicornis</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Ixodes affinis</em></td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>I. angustus</em></td>
<td>55</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>I. cookei</em></td>
<td>123</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>I. dentatus</em></td>
<td>48</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td><em>I. marxi</em></td>
<td>26</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>I. muris</em></td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>I. pacificus</em></td>
<td>1,497</td>
<td>14</td>
<td>25</td>
</tr>
<tr>
<td><em>I. scapularis</em></td>
<td>34,621</td>
<td>594</td>
<td>11,287</td>
</tr>
<tr>
<td><em>I. spinipalpis</em></td>
<td>63</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Rhipicephalus sanguineus</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>38,855</td>
<td>608</td>
<td>11,324</td>
</tr>
</tbody>
</table>

*Borrelia miyamotoi*, a relapsing fever spirochete (1), was first isolated from *Ixodes persulcatus* ticks in Japan in 1995 (2) and later detected in *Ixodes* ticks in the United States and Europe (3–5). Although *B. miyamotoi* bacteria have been mainly detected in *I. ricinus* species complex ticks that transmit *B. burgdorferi* worldwide, the vector specificity needs further study because investigators have found *B. miyamotoi* in multiple tick species (6). *B. miyamotoi* has 3 geographically distinct genotypes: Asian, European, and American. In the United States, *B. miyamotoi* bacteria have been found in field-collected *I. scapularis* ticks in the northeastern and northern midwestern regions, where the average infection rate is 1.9% (7). However, an expanded geographic study of the prevalence of *B. miyamotoi* in human-biting ticks, its genotypes, and concurrent infections with other tickborne pathogens is warranted.

Human-biting ticks were submitted to the public tick testing program at the University of Massachusetts (Amherst, Massachusetts, USA) during May 2013–December 2019. We extracted DNA from individual ticks using the Epicenter Master Complete DNA and RNA Purification Kits (Lucigen, https://www.lucigen.com). We performed a species-specific quantitative PCR (qPCR) for differentiation of *I. scapularis* and *I. pacificus* ticks (8). To detect *Borrelia* bacteria, we first applied a genus-specific detection assay, followed by specific qPCR assays for *B. burgdorferi* sensu lato and *B. miyamotoi*. We detected the tickborne pathogens *Anaplasma phagocytophilum*, *Babesia microti*, *B. mayonii*, and *Ehrlichia muris*-like agent (EMLA) by a multiplex qPCR assay targeting different genes. We used a qPCR assay targeting tick 16S mtDNA gene as an internal control (8). We sequenced 3 partial gene fragments, 16S rDNA (16S) (9), flagellin (fla) (6), and glycerophosphodiester phosphodiesterase (glpQ) (6), for *B. miyamotoi* samples that were positive by qPCR.

We received and tested 39,198 ticks found on humans for *B. miyamotoi* during May 2013–December 2019. Of those, 38,855 (99.12%) ticks originated from the continental United States, comprising 18 tick species (Table). Although *Ixodes* ticks are the main vectors for *B. miyamotoi*, we did not detect *B. miyamotoi* DNA in *I. affinis*, *I. angustus*, *I. cookei*, *I. dentatus*, *I. marxi*, *I. muris*, or *I. spinipalpis* ticks. We detected *B. miyamotoi* in *I. pacificus* (14/1,497, 0.94%) and *I. scapularis* (594/34,621, 1.72%) ticks.

*B. miyamotoi* was found in 19 states; infection rates were 0.5%–3.2% (Figure). In the western...
United States, *B. miyamotoi* was found in *I. pacificus* ticks in Oregon and California (14/1,497, 0.94%). Although *I. scapularis* ticks are distributed across the eastern United States, no *B. miyamotoi*–positive ticks were detected south of Virginia. *B. miyamotoi*–positive ticks were concentrated in the Northeast and upper Midwest (594 of 34,621, 1.72%) (Figure). Lyme disease remains the principal public health concern; the causative agent, *B. burgdorferi* (11,287/34,621; 32.60%, 95% CI 32.1%–33.1%), was 19 times more prevalent than *B. miyamotoi* (594/34,621, 1.72%) in *I. scapularis* ticks.

On average, prevalence of *B. miyamotoi* infection in *I. scapularis* ticks (1.72%, 95% CI 1.58%–1.86%) was higher than in *I. pacificus* ticks (0.94%, 95% CI 0.51%–1.56%). The prevalence of *B. miyamotoi* in *I. pacificus* ticks was 1.00% (95% CI 0.53%–1.7%) in adults (13/1,300), 0.53% (95% CI 0.01%–2.9%) in nymphs (1/190), and 0.00% (95% CI 0%–40.1%) in larvae (0/7). The prevalence of *B. miyamotoi* in *I. scapularis* ticks was 1.80% (95% CI 1.64%–1.97%) in adults (456/25,376), 1.54% (95% CI 1.29–1.83%) in nymphs (133/8,615), and 0.79% (95% CI 0.26%–1.84%) in larvae (5/630).

Of 594 *B. miyamotoi*–positive *I. scapularis* ticks, 351 (59.09%) had concurrent infections. We found 293 (49.33%) *I. scapularis* ticks had a dual infection with *B. miyamotoi*: 220 (37.04%) were also infected with *B. burgdorferi* s.l., 43 (7.24%) with *A. phagocytophilum*, and 30 (5.05%) with *B. microti*. We further found 52 (8.75%) had a triple infection with *B. miyamotoi*: 23 (3.87%) were also infected with *B. burgdorferi* s.l. and *A. phagocytophilum*, and 7 (1.18%) with *A. phagocytophilum* and *B. microti*. Six (1.01%) of the *B. miyamotoi*–positive ticks had a quadruple infection with *B. miyamotoi*, *B. burgdorferi* s.l., *A. phagocytophilum*, and *B. microti*. No ticks with *B. mayonii* or EMLA were additionally infected with *B. miyamotoi*.

Multilocus sequence typing of the 16S, *fla*, and *glpQ* genes revealed 2 distinct *B. miyamotoi* genotypes separated by their tick vectors, *I. scapularis* ticks in the Northeast and upper Midwest and *I. pacificus* ticks in the West (Appendix, https://wwwnc.cdc.gov/EID/article/27/12/20-4646-App1.pdf). Whereas the 16S gene sequences were identical among all isolates, variable sites were found among *fla* and *glpQ* nucleotide sequences. Among 14 *I. pacificus* tick–borne
B. miyamotoi isolates, all fla and glpQ sequences were identical. A previously reported A/G substitution in B. miyamotoi fla sequences from I. pacificus ticks (5,9) was outside of our sequenced fla fragment (Appendix). The genetic identity between the 2 tick species-specific genotypes was 0.996 for fla and 0.986 for glpQ. Unlike heterogeneous B. burgdorferi populations, B. miyamotoi appears to be very homogeneous within its respective tick vectors.

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References

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Wohlfahrtiimonas chitiniclastica
Monomicrobial Bacteremia in a Homeless Man

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We report a case of septic shock attributable to monomicrobial bloodstream infection secondary to Wohlfahrtiimonas chitiniclastica infection. This case suggests that W. chitiniclastica likely possesses the virulence to cause severe disease. Culture-independent techniques were essential in the identification of this organism, which enabled selection of appropriate therapy.

In August 2020, a 63-year-old homeless man with a history of deep vein thrombosis and chronic venous insufficiency was found in his truck, unconscious and covered in feces and maggots. He reportedly had been parked in a single parking spot in rural Maryland, USA, for 3 days. His blood pressure in the field was too low to be quantified, and he was admitted to a community hospital in septic shock. Blood cultures were drawn before establishing intravenous access for administration of vancomycin, piperacillin/tazobactam, and crystalloid. After being stabilized, he was transferred to our hospital, a tertiary care center in Baltimore, Maryland, USA, where surgeons performed superficial surgical debridement of his lower extremities and removed maggots by using a scrub brush with the patient under anesthesia in the operating room. We discarded the maggots, and they were not submitted for identification.

The patient’s leukocyte count on arrival was 38.6 K/µL (reference range 4.5–11.0 K/µL), his creatinine 6.86 mg/dL (reference range 0.7–1.5 mg/dL), and his lactic acid 3.5 mmol/L (reference range 0.5–2.2 mmol/L). He had elevated transaminases, bilirubin 38.6 µmol/L (reference range 0.5–2.2 µmol/L), and his alkali phosphatase 577.4 KU/L (reference range 0–49 KU/L). He was transferred to our hospital, a tertiary care center in Baltimore, Maryland, USA, for 3 days. His blood pressure in the field was too low to be quantified, and he was admitted to a community hospital in septic shock. Blood cultures were drawn before establishing intravenous access for administration of vancomycin, piperacillin/tazobactam, and crystalloid. After being stabilized, he was transferred to our hospital, a tertiary care center in Baltimore, Maryland, USA, where surgeons performed superficial surgical debridement of his lower extremities and removed maggots by using a scrub brush with the patient under anesthesia in the operating room. We discarded the maggots, and they were not submitted for identification.

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On day 2 of hospitalization, transient hemodynamic instability necessitated initiation of

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vasopressor support and continuous renal replacement therapy; however, these treatments were rapidly tapered off. We identified gram-negative rods in the anaerobic blood culture from the community hospital, and we narrowed the patient’s antibiotics to piperacillin/tazobactam monotherapy. On hospital day 5, we identified the gram-negative rods as W. chitiniclastica by using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. We changed the patient’s intravenous antibiotics to 2 g of ceftriaxone daily and then, on hospital day 9, changed the regimen to 750 mg of oral levofloxacin daily to complete a 21-day course of treatment. We were unable to follow up with the patient after his discharge, but we proceeded with reporting about his case after it was deemed to be exempt by the Institutional Review Board at the University of Maryland Baltimore.

In 2008, W. chitiniclastica was first isolated from larvae of the parasitic fly Wohlfahrtia magnifica (1). Since 2008, a total of 11 cases of W. chitiniclastica bloodstream infections have been described (2–10; Appendix references 11,12, https://wwwnc.cdc.gov/EID/article/21/12/21-0327-App1.pdf).

### Table. Published cases of Wohlfahrtimonas chitiniclastica bloodstream infection

<table>
<thead>
<tr>
<th>Country of origin (reference)</th>
<th>Age, y/sex; housing status; presentation</th>
<th>Bacteria identified on blood cultures</th>
<th>Microbiology tools used</th>
<th>Antimicrobial agents and duration of treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>France (2)</td>
<td>60/F; homeless; fatigue and ulcers to the scalp</td>
<td>W. chitiniclastica</td>
<td>16S rRNA sequencing</td>
<td>Ceftriaxone; duration not defined</td>
<td>Survival</td>
</tr>
<tr>
<td>Argentina (3)</td>
<td>70/M; homeless; altered mental status, septic shock, and plaques in the inguinal region</td>
<td>W. chitiniclastica</td>
<td>16S rRNA sequencing</td>
<td>Ciprofloxacin and ampicillin/sublactam; duration not defined</td>
<td>Death</td>
</tr>
<tr>
<td>Washington, USA (4)</td>
<td>57/M; stable home; wet gangrene of the ankle, septic shock, and multi-organ failure</td>
<td>Propionibacterium acnes, Staphylococcus hominis, and Wohlfahrtimonas species</td>
<td>MALDI-TOF mass spectrometry and 16S rRNA sequencing</td>
<td>No mention of antimicrobials used</td>
<td>Death</td>
</tr>
<tr>
<td>Ohio, USA (5)</td>
<td>41/F; stable home; abdominal pain and sacral osteomyelitis</td>
<td>Proteus mirabilis and W. chitiniclastica</td>
<td>MALDI-TOF mass spectrometry</td>
<td>Vancomycin, cefepime, and metronidazole; duration of 6 wks</td>
<td>Death from Clostridioides difficile infection</td>
</tr>
<tr>
<td>Indiana, USA (6)</td>
<td>37/M; not specified; necrotizing infection of lower extremities</td>
<td>W. chitiniclastica, Ignatzschnieri Indica, and Providencia stuartii</td>
<td>Not specified</td>
<td>Piperacillin/tazobactam, clindamycin and vancomycin, then cefepime; duration of 10 d</td>
<td>Survival</td>
</tr>
<tr>
<td>United Kingdom (7)</td>
<td>82/F; stable home; unconscious</td>
<td>W. chitiniclastica, P. mirabilis, Providencia rettgeri, and Staphylococcus aureus</td>
<td>MALDI-TOF mass spectrometry and 16S rRNA sequencing</td>
<td>Cefuroxime, metronidazole, and clindamycin, then fluoxacillin; duration of 7 d</td>
<td>Survival</td>
</tr>
<tr>
<td>Australia (8)</td>
<td>54/M; stable home; unconscious, septic shock and myasis of the foot and toes</td>
<td>W. chitiniclastica and Morganella morganii</td>
<td>MALDI-TOF mass spectrometry</td>
<td>Piperacillin/tazobactam, then meropenem, then ciprofloxacin; duration of 3 wks</td>
<td>Survival</td>
</tr>
<tr>
<td>Hawaii, USA (9)</td>
<td>72/M; stable home; unconscious, septic shock, and myasis of the umbilical cord</td>
<td>Escherichia coli and W. chitiniclastica</td>
<td>16S rRNA sequencing</td>
<td>Piperacillin/tazobactam, clindamycin, and vancomycin; duration not specified</td>
<td>Death</td>
</tr>
<tr>
<td>Japan (10)</td>
<td>75/M; homeless, unconscious</td>
<td>Peptoniphilus harei on initial blood cultures. On day 20, P. mirabilis, M. morganii, Streptococcus anginosus, Streptococcus agalactiae, Bacteroides fragilis, and W. chitiniclastica</td>
<td>MALDI-TOF mass spectrometry and 16S rRNA sequencing</td>
<td>Cefazolin, then vancomycin, cefepime, and metronidazole; duration not specified</td>
<td>Survival</td>
</tr>
<tr>
<td>North Dakota, USA (Appendix reference 11)</td>
<td>70/M; stable home; fall</td>
<td>W. chitiniclastica</td>
<td>Not specified</td>
<td>Levofloxacin; duration not specified</td>
<td>Survival</td>
</tr>
<tr>
<td>Pennsylvania, USA (Appendix reference 12)</td>
<td>82/M; stable home; fall and confusion, myasis of the lower extremities and toes</td>
<td>Staphylococcus aureus, W. chitiniclastica, and l. indica</td>
<td>MALDI-TOF mass spectrometry</td>
<td>Daptomycin for 6 wks</td>
<td>Survival</td>
</tr>
</tbody>
</table>

(Table). Our patient shares risk factors observed in other cases, including homelessness and chronic venous insufficiency (Appendix reference 13). The pathogenicity of W. chitiniclastica has remained uncertain in previous case reports secondary to its identification in polymicrobial infections. This severe case of monomicrobial W. chitiniclastica BSI is similar to a previous report of a 70-year-old man in Argentina who had septic shock with multiorgan failure secondary to the same bacteria (3). Taken together, these 2 cases challenge the hypothesis that other bacteria present in polymicrobial infections are primarily responsible for the disease associated with BSI attributable to W. chitiniclastica infection (9) and instead suggest that this pathogen may cause severe disease.

For our patient, W. chitiniclastica was first identified on MALDI-TOF mass spectrometry from a positive anaerobic blood culture. In all 9 cases for which detailed microbiologic methods are reported, W. chitiniclastica was identified from blood or tissue cultures by using MALDI-TOF mass spectrometry (5,8; Appendix reference 12), 16S rRNA sequencing (2,3,9), or both (4,7,10) (Table). This pattern demonstrates that W. chitiniclastica is extremely difficult to identify from clinical specimens without culture-independent techniques and highlights the utility of these techniques in clinical care.

Published case-reports demonstrate a heterogeneous approach to the clinical management of patients with W. chitiniclastica BSI. Often, selection of antibiotics was dictated by the other pathogens present in a polymicrobial infection. Generally, most studies report the use of β-lactams (2,3,5–10; Appendix reference 12) as initial therapy, with fluoroquinolones available as second-line or step-down therapy (3,7,8). The duration of treatment ranges from 7 days to 6 weeks (5–8; Appendix reference 12). Given that our patient rapidly improved and the presumed source of his infection had been controlled with debridement of his lower extremities, we opted for a 3-week course of treatment.

Acknowledgments
We thank the healthcare team that participated in this patient’s care at the University of Maryland Medical Center and the University of Maryland Midtown Campus.

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Septic Polyarthritis Caused by Streptobacillus moniliformis

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DOI: https://doi.org/10.3201/eid2712.210649

Streptobacillus moniliformis is a pleomorphic, fastidious gram-negative bacillus commonly found in the nasopharynges of rats and other rodents (1). It is transmitted to humans through rat bites, scratches, or ingestion of food or water contaminated with rat feces (2), as exemplified by a 1926 outbreak in Haverhill, Massachusetts, USA (3). Symptoms usually comprise fever, headache, pharyngitis, myalgia, migratory arthralgia, and vomiting, followed by a maculopapular rash on extensor surfaces. Arthralgia related to reactive polyarthritis develops in ≈50% of patients (4). Wang et al. (5) reported a well-documented case of septic arthritis and reviewed 11 cases in the literature; 5 of 12 cases had local signs of arthritis but not fever or general sepsis (5).

In January 2021, a 59-year-old cleaning woman sought treatment for 3 consecutive days at the emergency department before she was admitted for 2 months of progressively worsening left knee pain. She did not have a rash or fever. Her medical history included ovarian cancer, which was treated surgically 20 years before, and cervical stenosis after C2-T1 fusion. Radiographs of her knee showed mild arthritis; arthrocentesis conducted at the first emergency department visit produced synovial fluid with no organisms visible by Gram staining. The patient was prescribed steroids for inflammatory arthritis, but joint pain and swelling did not improve. At her third visit to the emergency department, she was afebrile and had tender, warm, and swollen knees, wrists, right shoulder, and left ankle; these joints also showed a decreased range of motion (Figure). She did not have a rash or lymphadenopathy. Seven days after admission, a second arthrocentesis produced synovial fluid with 40,000 leukocytes/mL, no organisms visible by Gram staining, and no crystals. Other rheumatic results were within reference ranges. On

Figure. Tender, warm, and swollen knees (A), wrists (B), and left ankle (C) with decreased range of motion in a patient with septic polyarthritis caused by Streptobacillus moniliformis infection, United States.
day 11, tiny colonies grew poorly on sheep blood agar (Appendix Figure, https://wwwnc.cdc.gov/EID/article/27/12/21-0649-App1.pdf). We did not observe growth on chocolate, MacConkey, or Columbia colistin-nalidixic acid agars. Microscopic examination of a Gram-stained smear revealed gram-negative rods with bulbar swellings (Appendix Figure). We used matrix-assisted laser desorption/ionization time-of-flight mass spectrometry to confirm the colonies as S. moniliformis (score 2.35); we did not conduct susceptibility testing.

We diagnosed subacute polyarticular septic arthritis, which has a recommended treatment of penicillin G (200,000 units 2×/d for 5–7 days); the alternative option is a 4-week course of ceftriaxone. We stopped steroid treatment and prescribed ceftriaxone because the patient had a severe penicillin allergy. She responded very well to intravenous treatment, and her joint pain and swelling improved remarkably. Two months before symptom onset, she had cleaned a research laboratory housing rats and homes that had mousetraps. She was not aware of any bites or scratches. We obtained informed consent for her participation in this research.

S. moniliformis is the etiologic agent of rat-bite fever, which usually causes fever, rash, and arthralgia. However, this patient and others had polyarticular involvement without fever or rash (5,6). Previous case reports have described S. moniliformis as favoring synovial and serosal surfaces (7,8).

S. moniliformis is difficult to identify because of its fastidious nature and slow growth on culture; as a result, it is sometimes misdiagnosed as inflammatory arthritis. An informed diagnosis requires raised clinical awareness and attention to patient social history. Arthrocentesis should be conducted in any case of suspected septic arthritis. As shown in this case, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry is a useful tool for diagnosing S. moniliformis infection.

Acknowledgment
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References

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Coxiella burnetii in 3 Species of Turtles in the Upper Midwest, United States

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Two studies have identified *Coxiella burnetii* in poikilotherms (vertebrates that cannot regulate body temperature physiologically); both studies originated in India. Two tortoises had antibodies to *C. burnetii* by capillary agglutination testing of their serum samples in Uttar Pradesh (1). Additional reptiles, including snakes and skinks, had serum samples positive for *C. burnetii* in a separate study in Karnataka (2). Although both studies are useful in clarifying how this bacterium might interface with reptiles, there is no other evidence to support the role played by this large class of vertebrates (with reptiles, there is no other evidence to support). In clarifying how this bacterium might interface in Karnataka (2), samples positive for *C. burnetii* in a separate study in Karnataka (2). Although both studies are useful in clarifying how this bacterium might interface with reptiles, there is no other evidence to support the role played by this large class of vertebrates (3). Furthermore, serologic assays applied to species that they were not designed for are difficult to interpret (Appendix, https://wwwnc.cdc.gov/EID/article/27/12/21-1278-App1.pdf).

Serologic testing, typically using indirect immunofluorescence assay, is the primary method used to diagnose *C. burnetii* infection, which causes Q fever in humans and coxiellosis in domestic ruminants (4). Additional serologic testing includes complement fixation and ELISA (5). Serologic assay benefits include commercial availability and insights into acute, treated, and chronic patients, depending on titers (6). Several PCR-based assays have been developed for detection of *C. burnetii* in samples from nontraditional mammals, birds, and arthropods (7). PCR provides a simple and reliable method for detection of the bacterium even retrospectively from tissues (6). Therefore, we tested turtles from multiple locations in Illinois and Wisconsin, USA, for *C. burnetii*.

As part of these annual surveys, turtle species collected have various morphometric data, blood samples, or oral and cloacal swab specimens obtained before being released. Several diagnostic tests are performed with these samples, such as PCR screening for several pathogens, including *C. burnetii*. Other pathogenic organisms include *Amblyomma tigrinum* virus, Bohe iridovirus, Terrapene herpervirus 1, Terrapene herpesvirus 2, epizootic hematopoietic necrosis virus, *Erythromyces testavornans*, frog virus 3, Emydid herpesvirus 1, Emydoidea herpervirus 1 (in Blanding’s turtles), *Mycoplasma agassizii*, *M. testudineum*, *Salmonella* spp., and Testudinid herpesvirus 2 (9).

We extracted DNA from frozen, combined oral/cloacal swab specimens from each turtle by using the DNA Blood Mini Kit (QIAGEN, https://www.qiagen.com). We assessed spectrophotometrically DNA concentration and purity by using NanoDrop 1000 (Thermo Fisher Scientific Inc., https://www.thermo-fisher.com). We performed quantitative PCR by using a QuantStudio3 Real Time PCR System (Applied Biosystems, https://www.thermofisher.com) and a TaqMan primer–probe assay targeting the *C. burnetti icd* gene as described (10).

We assayed all samples, standards, and non-template controls in triplicate and quantified positive samples by using a 7-point standard curve (10^1–10^7 target copies). Samples were considered positive if all 3 replicates had a lower cycle threshold value than the lowest detected standard dilution. We used a highly sensitive and specific quantitative PCR for *C. burnetii*.

During 2019, samples from 5/605 turtles encountered across 8 counties showed positive results for quantitative PCRs, indicating presence of *C. burnetii* (Figure). We collected positive samples from 3 Blanding’s turtles (*Emydoidea blandingii*), 1 painted turtle (*Chrysemys picta*), and 1 ornate box turtle (*Terrapene ornata*). These positive turtles were found in Kane and Lee Counties in Illinois and Sauk County in Wisconsin. We did not perform serologic analysis for these animals. One Blanding’s turtle had a microchip and transmitter, was sampled again during 2020, and showed a negative PCR result. All of these turtles were found within a 1-hour drive to the Illinois–Wisconsin state border within protected preserves. However, the 3 locations in which the 5 turtles varied in proximity to farms, livestock, industry, residential areas, and major highways; we found no geographic associations. All other screening tests showed negative results for pathogenic organisms for these 5 animals.

*C. burnetii* is a ubiquitous bacterium that has been found in many different species, often...
without pathogenicity (4). A variety of species of turtles are sampled annually in Illinois and surrounding areas through the Wildlife Epidemiology Laboratory. Over time, the testing for various organisms has expanded, especially as additional tests are validated. Screening for the bacterium that causes Q fever has been conducted for many species but infrequently in poikilotherms. These results show that the bacteria can be detected in these species and should be further researched to understand additional sources of this reportable disease, including potential management or regulatory decisions.

Continued investigation and screening in poikilotherms for zoonotic pathogens should be prioritized to understand the potential risk from additional hosts. The pet trade is a potential avenue of risk for exposure between humans and turtles. As these pathogens of concern are better characterized, the implications of different and varied hosts will drive the need for continued One Health research and dialogue between environmental, animal, and human health professionals.

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References
Reassortant Influenza A(H1N1)pdm09 Virus in Elderly Woman, Denmark, January 2021

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A case of human infection with influenza A(H1N1)pdm09 virus containing a nonstructural gene highly similar to Eurasian avian-like H1Nx swine influenza virus was detected in Denmark in January 2021. We describe the clinical case and report testing results of the genetic and antigenic characterizations of the virus.

Human infection with swine influenza A virus (IAV) had not previously been detected in Denmark, but sporadic cases have been reported from other countries (1). We report the identification of a case of zoonotic swine influenza infection in Denmark during a low-activity influenza season.

The variant IAV was detected by the National Influenza Center at Statens Serum Institut (Copenhagen, Denmark), as part of routine surveillance. A sputum sample was collected on January 21, 2021, in Zealand, Denmark, from a female patient in her 70s with various concurrent conditions, including a chronic respiratory disease, who was admitted to hospital after 2 days of moderate influenza-like symptoms: fever (39°C), coughing, sore throat, and difficulty breathing. The patient sample was positive for IAV in analyses at the local hospital microbiology laboratory; remaining sample material was submitted to the National Influenza Center, which confirmed it positive for influenza A(H1N1)pdm09 (Appendix, https://wwwnc.cdc.gov/EID/article/27/12/21-1361-App1.pdf).

We performed whole genome sequencing on the virus (2), and named it A/Denmark/1/2021 (vH1N1), and submitted to GISAID (https://www.gisaid.org; accession no. EPI_ISL_909652). BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and phylogenetic analyses revealed that all segments except the nonstructural gene belonged to influenza A(H1N1)pdm09 clade 1A3.3.2 (3), which is most similar (97%–98% nt identity) to viruses collected from swine in France and Germany in 2014 and 2015 (Table; Figure). The nonstructural gene was most similar (95%) to Eurasian avian-like H1Nx swine influenza virus of clade 1C. No segments had a near-exact match to sequences in GenBank or GISAID, and all were distinct from the seasonal vaccine strain, A/Guangdong-Maonan/SWL1536/2019 (Table).

Because of the suspected swine origin of the case virus, we used whole-genome sequencing to retrospectively analyze 68 IAVs with a hemagglutinin (HA) gene belonging to clade 1A3.3.2 sampled from swine herds in Denmark during 2014 and 2015 (Table; Figure). The nonstructural gene was most similar (95%) to Eurasian avian-like H1Nx swine viruses of clade 1C. No segments had a near-exact match to sequences in GenBank or GISAID, and all were distinct from the seasonal vaccine strain, A/Guangdong-Maonan/SWL1536/2019 (Table).
This finding suggests that the virus from the human case originated from swine in Denmark. The patient and her husband reside in the countryside, <2 km from a medium-sized farm with finisher pigs. Because of coronavirus disease pandemic restrictions, she had not been in close contact with other persons or been close to the pig farm. Both the patient and her husband, who had no signs of illness, were vaccinated against seasonal influenza in October or November 2020. European General Data Protection Regulation (https://gdpr.eu) restrictions on reporting personally identifiable information prevent revealing additional information about the patient or the farm. Veterinary authorities in Denmark collected nose swab samples from 68 pigs at the neighboring farm on February 1, 2021, according to standard procedures. All samples tested negative by PCR for IAV. Because of the high prevalence of influenza-positive herds in Denmark, we could not be confident potential seropositive swine were infected by the virus in question, so we did not take blood samples. However, we therefore could not exclude previous virus circulation in the herd, because swabs were taken 11 days after virus detection in the patient. According to the Danish Meteorological Institute, the patient’s residence was downwind of the pig herd most days preceding clinical symptoms.

Most of the case virus genes were derived from influenza A(H1N1)pdm09, which has been circulating in the human population of Denmark since 2009. However, the HA gene is different from that of the strains currently circulating (4), and it is therefore difficult to predict the level of immunity in the human population against this virus. Antigenic characterization (5) showed no or very poor cross-reactivity to all reference antiserum used for analysis (Appendix Table 1), and the HA gene contained several more mutations at antigenic sites compared with the seasonal vaccine strain (Appendix Figure). Therefore, vaccine effectiveness of the 2020–2021 seasonal influenza vaccine against the variant virus has been assessed as low.

Neuraminidase inhibition tests showed no reduction of oseltamivir or zanamivir inhibition, and the viral genome contains no known antiviral mutations except the V27A mutation in the M2 gene, known from most other H1N1 viruses circulating in human and swine (6,7). We identified no amino acid changes presumed to be related to increased risk of human infection (8), but further in vitro and in vivo analyses are planned to explore this possibility.

Because national coronavirus disease pandemic restrictions limited interpersonal contact, there

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<th>Table. Percentage identity similarity between gene and protein segments of influenza virus isolate A/Denmark/1/2021 (vH1N1) from a patient in Denmark and reference viruses from GISAID*</th>
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*GISAID, https://www.gisaid.org. PB1/PB2, polymerase basic protein 1/2; PA, polymerase acidic protein; HA, hemagglutinin; NP, nucleoprotein; NA, neuraminidase; MP/M1/M2, matrix protein 1/2, NS/NS1, nonstructural protein; NEP, nuclear export protein
†GISAID accession no. EPI_ISL_504870.
‡GISAID accession no EPI_ISL_227813.
¶GISAID accession no. EPI_ISL_377080.
§GISAID accession no. EPI_ISL_1673688.
were only 46 confirmed influenza cases in Denmark during the 2020–2021 season, and transmission of the variant virus was considered negligible. The Danish Patient Authority did not identify any person-to-person swine influenza transmission, and no further public health response measures were enacted.

The effects of the most recent swine influenza pandemic and the extensive diversity and reassortment in swine influenza viruses indicate the obvious zoonotic potential of these viruses (9,10). Therefore, more attention should be given to routine detection and control of swine influenza viruses.

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Dr. Nissen is a postdoctoral researcher at the National Influenza Center, Statens Serum Institut, Copenhagen.
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Correction: Vol. 27, No. 10

The name of author Xiaohui Wang was misspelled in Emergomyces orientalis Emergomycosis Diagnosed by Metagenomic Next-Generation Sequencing (D. He et al.). The article has been corrected online (https://wwwnc.cdc.gov/eid/article/27/10/21-0769_article).
In Modern Epidemics: From the Spanish Flu to COVID-19, Salvador Macip presents an ambitiously comprehensive overview of human diseases. This updated version, translated into English by Julie Wark, provides an accessible introduction to the microbes that cause harm, the science behind treatment and prevention, and the challenges to successful disease control. The book begins, appropriately, with how bacteria have enabled humans to evolve and persist and then pivots to pathogenic microbes. Modern Epidemics is written with lay readers in mind, so diseases are grouped by relevance to humans—for instance microbes with bioterrorist potential—rather than by pathogen relatedness. Macip guides readers through tools for prevention and treatment, and current knowledge for neglected and emerging diseases, including coronaviruses.

Unsurprisingly, coronavirus disease (COVID-19) information has become rapidly outdated, including the death toll, but that chapter maintains relevance as a time capsule of the early pandemic. Some uncertainties Macip relates also have been resolved; for instance, the promise of the Moderna vaccine has been borne out. Still, some uncertainties remain, such as whether COVID-19 will persist as a seasonal disease, like influenza or a mild cold-like illness, or as isolated outbreaks, damaging but limited in scope.

Macip describes the impossibility of predicting future pandemics, which could emerge from diverse microbes or, as he states, “a supervirus that doesn’t even exist yet,” but I suspect such predictions could become possible. Understanding why pathogens evolve to cause harm represents a major focus in evolutionary biology. Indeed, recent research investigates why pathogens evolve transmission before symptoms (1), a trait responsible for considerable COVID-19 spread. This perspective narrows the possibilities by examining whether harmful traits are unlikely to emerge or simply have not evolved yet (2). Macip instead addresses the more immediate question of how to plan for the worst and incorporate data as it becomes available.

Modern Epidemics ends with an overview of major ongoing epidemics, including influenza, HIV, tuberculosis, and malaria. Macip explains the mechanics of producing influenza vaccines, including why producing vaccines against certain strains can be difficult; the surprising reason is that some viruses replicate agonizingly slowly inside chicken eggs. Despite impressive scientific gains, these epidemics continue to impose an enormous health burden; discussing them last underscores the substantial challenges that remain even after the COVID-19 pandemic.

Throughout Modern Epidemics, Macip avoids idolizing preeminent scientists, past and present. For example, he points out that immunization had precedent in other cultures long before it was supposedly discovered by Edward Jenner. Crucially, Macip’s narrative continues beyond the scientific discovery of effective treatments or preventative measures to outline the enormous political and economic barriers that persist despite scientific advances. Unfortunately, the comprehensiveness of Modern Epidemics precludes in-depth exploration of fascinating topics that arise during these narratives, such as the use of disease as a weapon of colonization and the economics of sustaining disease control. Nonetheless, Modern Epidemics serves as a broad-ranging introduction to the history, biology, and sociology of infectious diseases and will be useful to readers wishing to rapidly gain a sense of the field.

References

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In the business of space exploration, it is said that the best spacecraft is the one that is on the rocket. Similarly, the best standard operating procedure is the one that produces the desired outcome and can be, and is, followed. By applying universal human behaviors and making safety engaging, in Prepare and Protect: Safer Behaviors in Laboratories and Clinical Containment Settings, the author, Sean G. Kaufman, encourages the reader to think about and promote biosafety in new, significant ways.

Kaufman speaks to his readers in an approachable manner, taking us on an honest journey on which he asks us to rethink many preconceived ideas about what truly makes us safe or unsafe in the laboratory. Breaking down near misses, accidents, and disasters into their most basic components to see where things went wrong and why, Kaufman helps the reader begin to see patterns emerge. Memorable lessons include the impact of safety culture, the importance of meaningful risk assessment, human risk factors, and the ways biosafety professionals can best serve their programs.

The book begins with basic biosafety principles such as containment, risk mitigation, and the primary controls of safety, always presented with interesting and important discussion and nuance. For example, the author presents a proposal for clinical containment levels, thoughts on risk assessment as a living process, and the concept that it is critical to teach the workforce why they are asked to do something. We are also introduced to the intriguing subject of human risk factors, where we discover how our mental, physical, and emotional states, capabilities, institutional culture, and experiences all affect safety when working in the laboratory. This chapter is a pleasure, highlighted by stories about behavioral evolution and the dangers of appealing to authority.

With the same unique insight and fresh approaches, later chapters consider effective plans, emergency preparedness and response, and standard operating procedures to address these challenges. We also read about the critical impact on safety culture of accountable leadership. One important contribution of note is that personal protective equipment must be matched with a corresponding and effective standard operating procedure for doffing, exemplified by the beaking method of glove removal. Some chapters could possibly have been reordered or combined to feel more intuitive, but their conciseness makes each topic easier to process. First-person narratives included are often compelling and give insight into personal journeys of biosafety practitioners, complementing didactic content with real-world experience and insight.

The ongoing pandemic illustrates how the lessons in this book apply far beyond the lab, underscoring the extent to which we all contribute to biosafety. Aspiring, new, and experienced biosafety practitioners will enjoy this timely, informative, and engaging journey, one that can lead us to safer labs and better health for all. Above all, Kaufman reminds us to treat each other with kindness, respect, and dignity in our journeys towards safer outcomes.

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On March 11, 2020, the World Health Organization declared COVID-19 to be a pandemic. Shortly thereafter, reports of animals becoming infected with SARS-CoV-2 appeared. Although the pandemic is being driven by person-to-person transmission, transmission from people to animals of multiple species has been documented. According to the Centers for Disease Control and Prevention, the animals known to have been infected with SARS-CoV-2 include otters, mink, white-tailed deer, dogs, ferrets, and felids, including domestic cats, lions, pumas, and tigers.

An April 2020 US Department of Agriculture statement concerning a New York zoo’s lions and tigers showing clinical signs of respiratory illness was among the earliest such reports. Diagnostic samples taken from one tiger confirmed infection with SARS-CoV-2, and public health officials postulated that the source was exposure to a zoo employee positive for the virus. Stories about captive great cats with clinical signs of respiratory illness testing positive for SARS-CoV-2 have continued generating headlines from diverse locations around the globe.

Reports of animals becoming infected with SARS-CoV-2 through contact with humans may be in the spotlight, but humans are also disease vectors for numerous other pathogens. A 2014 literature review in PLoS One documents myriad cases in which humans transmitted influenza A virus, Mycobacterium tuberculosis, methicillin-resistant Staphylococcus aureus, and other pathogens to animals and stated

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that “transmission occurred in every continent except Antarctica therefore indicating a worldwide disease threat.” A report by Iatta et al. in the International Journal for Parasitology: Parasites and Wildlife states, “Infectious diseases by pathogens, including those of zoonotic concern, may act as a primary or contributory cause of threat to wildlife conservation and may represent a risk for human health, mainly for people working at, or visiting the zoological parks.”

That message is underscored by accounts of captive great cats becoming infected with SARS-CoV-2. More tigers now live in captivity than in their natural habitats, putting them at potential risk of acquiring infections from people. Actions that are based on One Health and that recognize that the health of humans, animals, and the environment is closely connected will be increasingly important for ensuring the survival of animals of keystone species, such as tigers, as well as in helping to disrupt the cycle of transmission for zoonotic pathogens, and in increasing understanding of One Health issues across disciplines and sectors.

This month’s cover image, The Tiger, is by German Expressionist artist Franz Marc. He was the son of a landscape painter, and he studied at the Academy of Fine Arts, Munich. In 1903 and 1907, he traveled to Paris, where he learned about Japanese woodcuts and the art of the Impressionists, Cubists, and Expressionists. Marc, along with Russian artist Wassily Kandinsky, founded the avant-garde group Der Blaue Reiter (The Blue Rider). Many of Marc’s works completed during his short life—he was killed during combat in World War I—vividly depict animals. The Brooklyn Museum notes that he “cultivated a dynamic Expressionist style that used rhythmic patterns of color and line to evoke movement.” In Marc’s own words, he wanted to “achieve a pantheistic empathy with the throbbing and racing of the blood in nature, in trees, in animals, in the air.”

The Lenbachhaus Museum, which houses the painting, notes that the “almost square image format is dominated by the mighty, crouching form of a tiger, which, with angular outlines as if carved out of stone, turns its beautifully shaped head back in a bold swing.” Marc used interlocking, bold blocks to form the tiger’s yellow and black body. The tiger’s diamond shaped eyes transfix the viewer. The landscape surrounding the tiger comprises angular, cubic forms that Marc imbues with rich, glowing shades of red, green, violet, and orange. The image bristles with tension and energy, as though its fractured components could suddenly coalesce and lunge snarling from the canvas.

The Lenbachhaus offers additional insight into Marc’s tiger: “The facets of his glowing yellow body join with the transparent, cubic formations of his surroundings to form an indissoluble unit in which there is no longer any distinction between organic and inorganic substances.” The viewer, captivated, gains some knowledge of the tiger’s perspective, perhaps feeling both discomfort and connection. Argentine writer Jorge Luis Borges concludes his mid-1940s essay, “A New Refutation of Time,” with the following lines that both evoke the One Health perspective on the interconnectedness of humans, animals, and nature and that, in recalling Marc’s notion of pantheistic empathy, could serve as a caption for The Tiger: “Time is the substance from which I am made. Time is a river which carries me along, but I am the river; it is a tiger that devours me, but I am the tiger; it is a fire that consumes me, but I am the fire.”

Bibliography

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Upcoming Issue

- Severe Acute Respiratory Syndrome Coronavirus 2 and Respiratory Virus Sentinel Surveillance, California, USA, May 10, 2020–June 12, 2021
- Outbreak of Mucormycosis in Coronavirus Disease Patients, Pune, India
- Using the Acute Flaccid Paralysis Surveillance System to Identify Cases of Acute Flaccid Myelitis, Australia, 2000–2018
- Potential Association of Legionnaires’ Disease with Hot Spring Water, Hot Springs National Park and Hot Springs, Arkansas, USA, 2018–2019
- Extensively Drug-Resistant, Carbapenemase-Producing Pseudomonas aeruginosa and Medical Tourism from United States to Mexico, 2018–2019
- SARS-CoV-2 Infections, Including Coronavirus Disease Vaccine Breakthrough Infections, Associated with Large Public Gatherings, Massachusetts, USA
- Global Genome Diversity and Recombination in Mycoplasma pneumoniae
- Transmission Dynamics of Large Coronavirus Disease Outbreak in Homeless Shelter, Chicago, Illinois, USA, 2020
- Mask Effectiveness for Preventing Secondary Cases of COVID-19, Johnson County, Iowa, USA
- Effect of Hepatitis E Virus RNA Universal Blood Donor Screening, Catalonia, Spain, 2017–2020
- Coronavirus Disease Case Definitions, Diagnostic Testing Criteria, and Surveillance in 25 Countries with Highest Reported Case Counts
- Systematic Genomic and Clinical Analysis of Severe Acute Respiratory Syndrome Coronavirus 2 Reinfections and Recurrences Involving the Same Strain, Madrid, Spain
- Emergence of SARS-CoV-2 Delta Variant, Benin, West Africa, May–July 2021
- SARS-CoV-2 Shedding in Semen and Oligozoospermia of Patient with Severe Coronavirus Disease 11 Weeks after Infection
- Use of Private Sector Workforce Respiratory Disease Short-Term Disability Claims to Assess SARS-CoV-2, Mexico, 2020
- Melioidosis Manifesting as Chronic Femoral Osteomyelitis in Patient, Ghana
- Coronavirus Disease Spread during Summer Vacation, Israel, 2020
- Low Seroprevalence among Undetected COVID-19 Cases, Faroe Islands, November 2020
- Use of Incoming Travelers Arriving at Hong Kong for Genomic Surveillance of SARS-CoV-2
- Effectiveness of International Travel Controls for Delaying Local Outbreaks of COVID-19
- Postmortem Antigen-Detecting Rapid Diagnostic Tests to Predict Infectivity of SARS-CoV-2–Associated Deaths
- Atezolizumab Treatment for Progressive Multifocal Leukoencephalopathy
- Unexpectedly High Prevalence of Hepatitis C Virus Infection, Southern Lao People’s Democratic Republic

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Article Title
Clinical Characteristics of Corynebacterium Bacteremia Caused by Different Species, Japan, 2014–2020

CME Questions

1. Your patient is a 67-year-old man with acute myeloid leukemia and positive blood culture for Corynebacterium. On the basis of the retrospective medical record review by Yamamuro and colleagues, which one of the following statements about proportion of true bacteremia and differences in clinical characteristics of patients with bacteremia from C. striatum, C. jeikeium, and other Corynebacterium species is correct?
   A. Proportions of true bacteremia cases caused by C. striatum and C. jeikeium were significantly higher than for other Corynebacterium species
   B. Of the 115 cases evaluated, more than three quarters represented true bacteremia
   C. The most common underlying disease was diabetes mellitus
   D. Among cases with true bacteremia, central venous port infection was the most common infective focus

2. According to the retrospective medical record review by Yamamuro and colleagues, which one of the following statements about differences in mortality and antimicrobial susceptibility in patients with bacteremia from C. striatum, C. jeikeium, and other Corynebacterium species is correct?
   A. Survival in patients with C. jeikeium bacteremia was significantly worse than in patients with bacteremia from other Corynebacterium species
   B. 90-day mortality rate was 34% for C. striatum bacteremia
   C. Most tested strains were resistant to minocycline
   D. C. striatum and C. jeikeium were more susceptible than other species to meropenem

3. On the basis of the retrospective medical record review by Yamamuro and colleagues, which one of the following statements about clinical implications of differences in clinical characteristics of patients with bacteremia from C. striatum, C. jeikeium, and other Corynebacterium species is correct?
   A. C. striatum and C. jeikeium detected in blood cultures are generally contaminants and need not be evaluated further
   B. C. striatum and C. jeikeium are less likely than other species to form biofilms
   C. Antibiotic sensitivity in this study differed substantially from that found in previous studies
   D. The authors emphasize the need to actively identifying coryneform in specimens, even if unsterile (e.g., sputum or urine), especially in suspected cases of Corynebacterium bacteremia
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Article Title
Trends in Incidence and Clinical Outcomes of Clostridioides difficile Infection, Hong Kong

CME Questions

1. You are advising a large hospital regarding anticipated trends in Clostridioides difficile infection (CDI). According to the updated territory-wide survey study in Hong Kong by Guo and colleagues, which of the following statements about disease burden, incidence, and clinical outcomes of CDI among hospitalized patients in Hong Kong is correct?
   A. Three-quarters of CDI cases were healthcare-associated (HA-CDI), and one-quarter was community-associated (CA-CDI)
   B. CDI incidence increased significantly from 2006 to 2017 but plateaued in 2018 and 2019
   C. 30-day mortality rates remained stable from 2015 to 2019 whereas 60-day recurrence rates decreased
   D. Patients with HA-CDI were significantly younger than patients with CA-CDI and had similar mortality

2. According to the updated territory-wide survey study in Hong Kong by Guo and colleagues, which of the following statements about antibiotic usage and other CDI-associated risk factors and clinical outcomes among hospitalized patients in Hong Kong is correct?
   A. CDI incidence trend was significantly correlated with overall antibiotic use ($r = 0.865; p<0.0001$), with decreasing incidence since an antibiotic stewardship program began in 2017
   B. On multivariate logistic regression analysis, the main predictors for death in 30 days were use of H2 antagonists and comorbid stroke
   C. 42% of patients had taken high-risk antibiotics within 8 weeks before CDI diagnosis
   D. Tetracycline use within 8 weeks before CDI diagnosis decreased from 2015 to 2019

3. According to the updated territory-wide survey study in Hong Kong by Guo and colleagues, which of the following statements about clinical and public health implications of the epidemiologic pattern of CDI, CDI-associated risk factors, and clinical outcomes among hospitalized patients in Hong Kong is correct?
   A. The best explanation for changes in C. difficile epidemiology was changes in patient comorbidities
   B. Sulphonamides and carbapenems are high-risk for CDI
   C. The decrease in 30-day mortality rates was attributed solely to improved effectiveness of CDI treatment and management
   D. Ribotypes 002 and 017, both virulent strains with high antibiotic resistance, may have been positively selected in the past because of excessive antibiotic use
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