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September 2018

Paul Klee (1879–1940), *Tropische Dämmerung (Tropical Twilight)*, 1921. Oil on white primer on paper on cardboard; 13.5 in × 9.1 in/33.5 cm × 23 cm. Fondation Beyeler, Riehen/Basel, Switzerland; Beyeler Collection; Photo: Robert Bayer

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On the Cover

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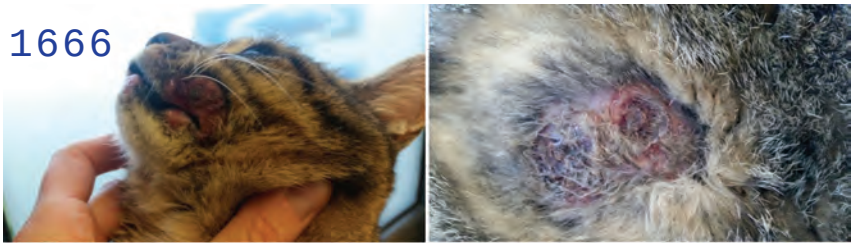

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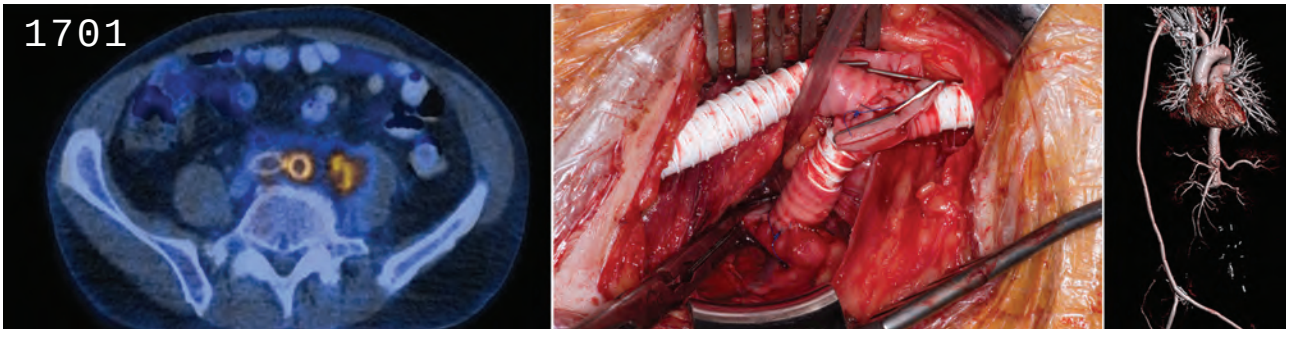

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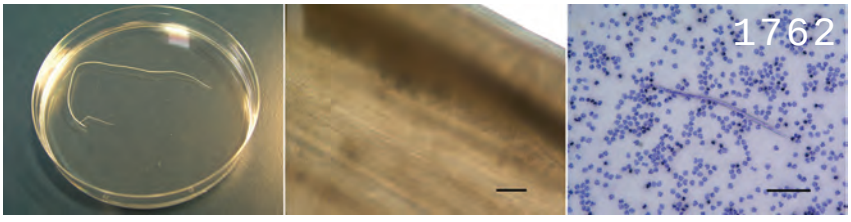

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The author list was incorrect in Death from Transfusion-Transmitted Anaplasmosis, New York, USA, 2017 (R. Goel et al.), and a name was missing from the acknowledgments.

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Several corrections to the text were needed in Phenotypic and Genotypic Characterization of *Enterobacteriaceae* Producing Oxacillinase-48–Like Carbapenemases, United States (J.D. Lutgring et al.).

ICEID Abstracts

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World Rabies Day is September 28

Every nine minutes, someone dies from this deadly, yet preventable disease.

Join the fight to end human deaths from canine rabies by 2030.

Vaccinate your dog against rabies.

The Rabies Crisis by the Numbers

40%

40% of the people bitten by dogs globally are children.

150

Rabies occurs in more than 150 countries.

70%

Vaccinating just 70% of dogs will protect humans in at-risk areas.

Globally, more than 95% of human rabies deaths occur after bites from rabid dogs. Most of these cases are in sub-Saharan Africa and Asia. CDC, in collaboration with the Global Alliance for Rabies Control and Mission Rabies, is helping countries around the world tackle rabies through education and mass dog rabies vaccination campaigns. To learn more, go to www.cdc.gov/rabies.

Ethics of Infection Control Measures for Carriers of Antimicrobial Drug–Resistant Organisms

Babette Rump, Aura Timen, Marlies Hulscher, Marcel Verweij

Many countries have implemented infection control measures directed at carriers of multidrug-resistant organisms. To explore the ethical implications of these measures, we analyzed 227 consultations about multidrug resistance and compared them with the literature on communicable disease in general. We found that control measures aimed at carriers have a range of negative implications. Although moral dilemmas seem similar to those encountered while implementing control measures for other infectious diseases, 4 distinct features stand out for carriage of multidrug-resistant organisms: carriage presents itself as a state of being; carriage has limited relevance for the health of the carrier; carriage has little relevance outside healthcare settings; and antimicrobial resistance is a slowly evolving threat on which individual carriers have limited effect. These features are of ethical relevance because they influence the way we traditionally think about infectious disease control and urge us to pay more attention to the personal experience of the individual carrier.

Antimicrobial resistance (AMR) is one of the most serious health threats of the 21st century. It challenges effective treatment of infectious diseases, now and in the future. AMR may imply that infections that used to be relatively harmless will pose a severe threat to patients in the future (*1*). Many countries have implemented measures to control AMR, including proper use of antimicrobial drugs in humans, minimization of antimicrobial drug use in animals, and prevention of further transmission of resistant microbes within the healthcare system (*1–5*). AMR raises a range of ethical questions (*6–12*). We explored ethical issues that arise in relation to carriage of antimicrobial drug–resistant organisms (hereafter called carriage).

AMR control measures are directed at carriers. The types of control measures vary by microorganism and depend on resistance pattern, virulence, and mode of transmission. Measures can include control precautions taken during patient care, such as use of personal protective equipment; cleaning and disinfection of the care environment; dedicated single-patient use of rooms and equipment; eradication treatment, if applicable; and, in exceptional cases, exclusion of the carrier from work or joint facilities. The actual control measures recommended by health authorities vary among countries. Countries in northern Europe, for instance, have implemented far-reaching infection control interventions that include preemptive use of contact precautions at the time of admission until the patient is proven culture negative and closure of hospital units to new admissions when applicable. Countries in southern Europe and North America follow a less aggressive approach, emphasizing contact precautions after detection of multidrug-resistant organisms (*1–4*).

Control measures may effectively control transmission of multidrug-resistant organisms, but negative effects on the health and well-being of carriers have been reported from countries that follow stringent multidrug-resistant organism policies and from countries that have a less aggressive approach (*13–16*). These negative effects make AMR control measures, apart from a technical and medical challenge, also an ethical issue. Our aim with this study was to examine the ethical context of multidrug-resistant organism carriage: what are the negative implications for carriers, and what is the ethical relevance?

Methods

We analyzed 227 consultations/inquiries associated with multidrug-resistant organisms registered from January 1, 2008, through January 16, 2016, by the Centre for Infectious Disease Control in the Netherlands (Table 1; Figure). We looked for potentially negative implications on freedom, well-being, and other ethical values and assessed the respects in which the ethically relevant features of carriage differ from those of infectious disease in general. The Netherlands follows a strict multidrug-resistant organism search-and-destroy policy (Table 2) (*2*,*17*,*18*). Estimated prevalence rates for multidrug-resistant organisms in the Netherlands are low

Author affiliations: The Netherlands National Institute for Public Health and the Environment, Bilthoven, the Netherlands (B. Rump, A. Timen); Radboud University Medical Center, Nijmegen, the Netherlands (M. Hulscher); Wageningen University, Wageningen, the Netherlands (M. Verweij)

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Table 1. Detailed information from 227 consultations about antimicrobial-resistant organisms, Centre for Infectious Disease Control, Bilthoven, the Netherlands, January 1, 2008–January 16, 2016*

Characteristic	No. (%)
Type of multidrug-resistant organism	
Methicillin-resistant *Staphylococcus aureus*	177 (78)
Vancomycin-resistant *Enterococci*	18 (8)
Extended-spectrum β-lactamase	9 (4)
Klebsiella pneumoniae carbapenemase-producing *Enterobactericeae*	5 (2)
Unknown	18 (8)
Setting	
Long-term care facilities	61 (27)
Paramedical facilities	23 (10)
Home-care facilities	14 (6)
Rehabilitation centers	5 (2)
Carriage among healthcare workers	50 (22)
Social interaction of healthcare workers	32 (14)
Other	42 (19)

*In the Netherlands, 25 regional Public Health Services (PHS) are in charge of communicable disease control. Healthcare institutions such as hospitals and nursing homes have a responsibility to detect, monitor, and control outbreaks within their facility and report these to the PHS. The PHS assists healthcare institutions and professionals and provides advice on the basis of national guidelines. In turn, the Centre for Infectious Disease Control of the National Institute of Public Health and the Environment (RIVM) acts as national public health authority; it develops and publishes national guidelines and offers support in outbreak management including a 24-hour consultation helpdesk for PHS and other health professionals. The center is consulted by PHS professionals >1,000 times/y about a variety of cases of notifiable diseases, outbreaks, and incidents that occur in the community (*15*,*17*,*18*). Since 2008, all consultations have been anonymously registered in a database. During the 8-year study period, RIVM registered 227 consultations associated with carriage of multidrug-resistant organisms that needed national guidance.

(online Technical Appendix, https://wwwnc.cdc.gov/EID/article/24/9/17-1644-Techapp1.pdf) (*2*,*19*–*21*).

Results

Negative Implications of Control Measures for Carriers

Problems with Access to Healthcare

A clear implication of AMR control measures involves problems with access to healthcare. During their consultations, several carriers asked about being faced with postponement of planned surgery, about cancellation of admission to rehabilitation, and about being denied access to dental clinics. A nursing home, for instance, wanted to deprioritize a person at the top of the waiting list because this person was carrying a multidrug-resistant organism. A medical daycare center refused to admit a child because of persistent carriage.

Restrictions within Healthcare Facilities

Another distinct implication of AMR control measures involves restrictions within healthcare facilities. Several consultations involved questions about carriers of methicillin-resistant *Staphylococcus aureus* (MRSA) in care facilities in which elderly carriers were banned from organized social activities or not allowed to dine at the same table with fellow residents. In medical daycare facilities, children who were carriers were banned from group activities or kept away from their peers, and in a psychiatric institution, a group of patients was placed in a closed ward because of carriage. Other inquiries concerned privileges that carriers received; for instance, carriers in nursing homes were allocated a single room or a private bathroom.

Negative Implications for Daily Life

The control measures also affected daily life. One inquiry concerned a MRSA-positive child who faced restrictions after returning to school because a classmate was a cystic fibrosis patient for whom acquiring a MRSA infection would constitute a health risk. Another inquiry was about adoption of a child with special health needs; the family had already adopted their first child with a previous diagnosis of persistent MRSA carriage, and they hesitated to adopt a second child because the MRSA would most likely be transmitted to that child, bringing extra MRSA-associated health risks. Also, parents of a healthy MRSA toddler were confronted with a daycare center caregiver who refused to attend to their child for fear of transmitting MRSA to her newborn baby at home. Some inquiries concerned interaction with animals; for instance, a family struggled with persistent MRSA carriage and 1 of their children was denied access to a medical daycare center. They were advised to relocate or abandon their cats, which were thought to be the source of reinfection.

Negative Implications for Carriers Who Work in Healthcare

Control measures can also have negative implications for those who work in healthcare. We found cases of healthcare workers (HCWs) who were restricted at work, banned from work, and faced income loss. For example, a nurse who was a carrier was assigned administrative tasks instead of patient care, thereby missing out on the substantial financial benefits that come along with performing patient care during night and weekend shifts. A temporary employee's contract was not renewed because of past carriage, and a fifth-year medical student discontinued training because of a chronic MRSA infection. HCWs were also pressed to

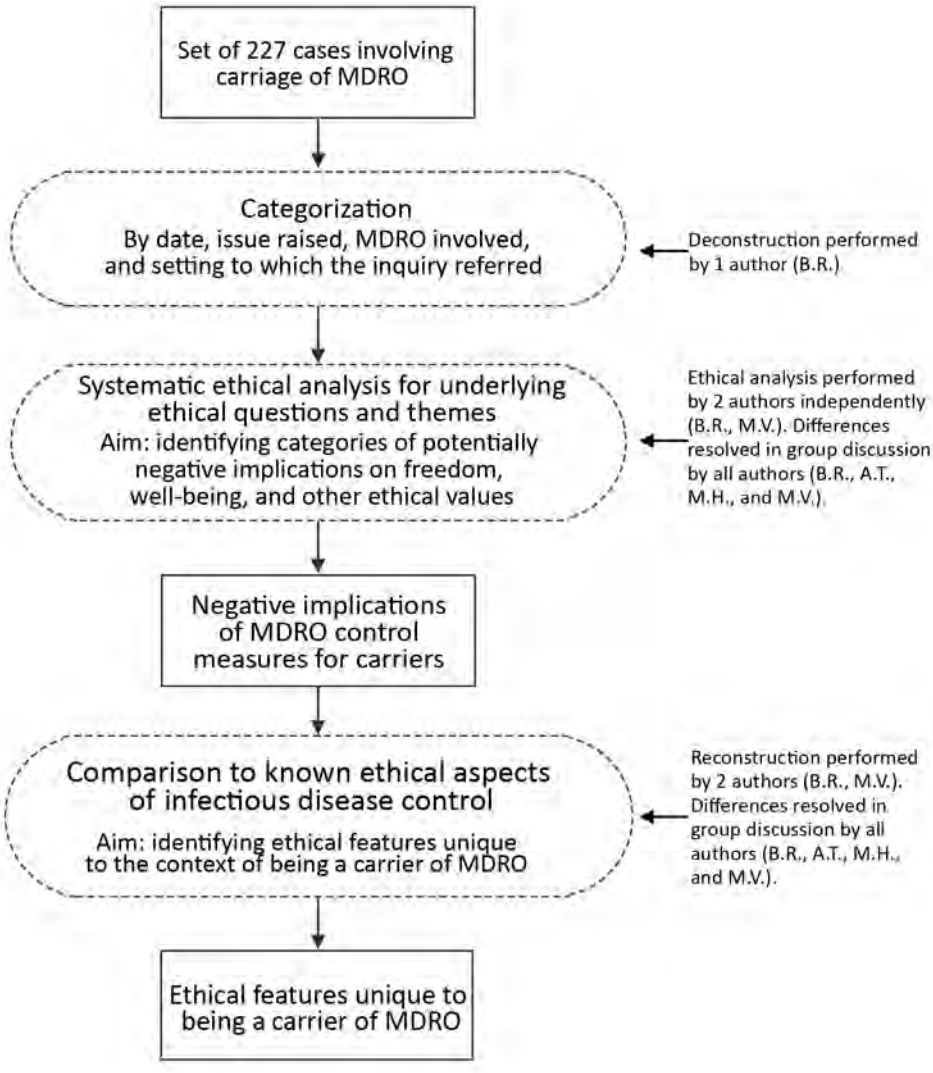

Figure. Methods used in study of ethics of infection control measures for carriers of antimicrobial-resistant organisms, the Netherlands, January 1, 2008–January 16, 2016. MDRO, multidrug-resistant organism.

cooperate with testing and treatment. A temporary healthcare employee was asked to show proof of being MRSA negative, and MRSA-positive nurses were pressed to cooperate with intensive eradication treatment consisting of daily scrubbing of the skin and taking of oral antimicrobial drugs. In several instances, MRSA-negative HCWs were excluded from healthcare work because in their private life they cared for a MRSA-positive child or parent.

Negative Implications for Close Contacts of HCWs

Infection control measures for HCWs can also affect their family members and other contacts. For example, HCWs with MRSA were asked to disclose the names of their close contacts outside the hospital. Contacts needed to cooperate with MRSA screening and, if test results were positive, undergo eradication treatment. In some instances, such measures had far-reaching consequences for family members. For instance, in a single-income household, young children were subjected to very intensive MRSA eradication in order for the main breadwinner to be able to secure employment. In another case, contact screening started by the employer of a nurse who was a carrier included screening of the nurse's children. One child was physically handicapped and visited a medical daycare center. When results indicated that he was a carrier, he was denied access to this medical daycare center for several months.

The negative implications for carriers of multidrug-resistant organisms were not only defined by the outcome of the control measures advised in the policies but also were further enhanced by focus on collective benefits with less emphasis on harm for carriers (*1*) and by strong concerns with communication and disclosure when applying the policy (*2*). Several inquiries resulted in implementation of control measures that were more stringent than those prescribed by national policies.

Table 2. Indications for routine screening for multidrug-resistant organisms, the Netherlands*

Healthcare setting	Indication†
Hospital	Patients at high risk of carrying an MDRO (e.g., patients transferred from a hospital in a foreign country or patients working in animal husbandry)
	Patients at high risk of acquiring infection with an MDRO
	Patients with signs of clinical infection with an MDRO
	Patients for whom empiric treatment failed
	Patients with recurrent infection
	Family members of hospital patient known to carry an MDRO
	Personnel with unprotected exposure to a person known to carry MRSA
General practice	Patients for whom empiric treatment failed
	Patients with recurrent infection
Nursing home/care facility	Patients for whom empiric treatment failed
	Patients with recurrent infection
	Patient with unprotected exposure (e.g., shared a room, shared medical equipment) to a person with MRSA or carbapenemase-producing *Enterobacteriaceae*
	Personnel with unprotected exposure to a person known to carry MRSA
Home	Personnel with unprotected exposure to a person known to carry MRSA

*MDRO, multidrug-resistant organism; MRSA, methicillin-resistant *Staphylococcus aureus*.
†As advised by the Werkgroep Infectie Preventie guideline on measures against transmission of highly resistant microorganisms in hospitals (*2*,*19*,*20*).

Negative Implications because of Overemphasis on Collective Benefits

Inquiries reflected a strong focus on the benefits of AMR control measures and ignoring of the potential harm for carriers. Several inquiries reported control measures that went beyond the already stringent national policies. For example, in 2015–2016, a large influx of war refugees from Syria to the Netherlands caused some hospitals to demand that their employees refrain from volunteer work with refugees outside their working hours because of the possibility that they could be exposed to a multidrug-resistant organism by doing such work. In addition, a pig farmer who had undergone heart valve surgery was advised not to go back to work on the farm because of the small risk of contracting livestock-associated MRSA, which would make follow-up visits more complicated to schedule for the hospital.

Negative Implications because of Concerns about Communication and Disclosure

Some inquiries reflected outcomes that were motivated by concerns about disclosure and communication rather than actual risk for transmission of the multidrug-resistant organisms. For instance, a MRSA-positive child was not allowed access to a medical daycare facility, not because of the risk to other children, which was considered to be small, but because the facility felt an obligation to inform all other parents. The parents of the carrier, however, insisted on nondisclosure for fear of stigma. Another inquiry concerned a nurse who lived on a livestock farm and was therefore at high risk of contracting MRSA, a risk that was well-known and had been accepted by her employer for years. When, by accident, the nurse was screened and carriage was confirmed, she was no longer allowed to work at that facility. This response was not motivated by the risk for transmission—the employer acknowledged that she presumably had been carrying MRSA for a long time and had never caused an outbreak—but because the institution was concerned about the consequences should MRSA carriage of a hospital employee become public.

Ethical Features Unique to Being a Multidrug-Resistant Organism Carrier

Inquiries concerned questions about AMR control measures that primarily aimed to reduce further transmission of antimicrobial-resistant pathogens. In doing so, these measures resulted in negative implications that raised moral dilemmas.

In the inquiries explored, the exact nature of the moral dilemmas remained implicit. However, for almost all cases, it could be assumed that the control measures had negative effects on the carrier's well-being, autonomy, and (health-associated) justice. Well-being was affected because carriers were limited in their opportunities to work or to engage in social contacts. Autonomy may have been at stake when carriers were requested to disclose their medical condition or when they were pressed to undergo tests and eradication therapy they might have preferred to avoid. Their sense of dignity may have been affected when carriers were stigmatized because of their condition. The various implications also seemed to be involve injustices: health inequity if carriers were excluded from certain medical treatment or faced a delay in care, and social injustice if they were excluded from (the benefits of) going to work.

Although challenging, the moral dilemmas at hand—and the values at stake—seem not fundamentally different from dilemmas that arise in infectious disease control in general (*22*–*25*). Health equity issues, for instance, occur in many contexts of infectious disease control. In Europe, while the 2014–2015 Ebola outbreak was occurring in West Africa, persons suspected of having Ebola virus disease were banned from hospital emergency rooms (*26*). Often at the heart of outbreak management are quarantine,

isolation, and social distancing measures, which clearly involve tensions with respect to autonomy and deprive persons from contact with their loved ones and otherwise undermine their quality of life (*25*). Restrictions to healthcare staff (e.g., a surgeon who seems to be a hepatitis B virus carrier) are well-accepted ways to prevent bloodborne nosocomial infections (*27*). However, 4 differences stood out, suggesting that there is something ethically noteworthy about carriage of multidrug-resistant organisms.

Relevance of Carriage for the Carriers

Patients in this study were asymptomatic carriers for whom carriage did not affect their health. Some might have had other health conditions, but they were not ill from the drug-resistant microorganism they carried. Thus, carriage differs from most communicable diseases, in which the health of the persons carrying the microorganism is threatened or affected by the infection. Ebola virus infection, for instance, forms an acute threat to the health of the patient, who is in immediate need of treatment and medication while threatening the health of others, including health personnel. Other infectious diseases can also involve asymptomatic carriage; moreover, multidrug-resistant organisms can certainly also cause infections and thus illness. In fact, the proactive screening and preemptive use of control measures that are common in the Netherlands probably caused an overrepresentation of inquiries concerning these "carriers without multidrug-resistant organism infection" (*2*,*19*,*20*). What remains ethically noteworthy and relevant for preemptive and reactive AMR control strategies is that, although all carriers are at risk for their carriage resulting in clinical infection, multidrug-resistant organisms primarily threaten a specific subgroup of vulnerable patients in hospital settings. The extent to which multidrug-resistant organisms contribute to death has been debated and seems to remain limited to those with severe illness and concurrent conditions (*28–30*). Studies addressing multidrug-resistant gram-negative infections, for instance, show substantial diversity in the outcomes. It can be concluded that mortality rates are higher among those infected by multidrug-resistant gram-negative bacteria; however, concurrent conditions and severity scores are more commonly identified as predictors of death (*28–30*). From a broader public health perspective, the health threat of multidrug-resistant organism carriage thus appears limited.

Healthcare-Associated Relevance

A noteworthy finding is that carriage became relevant almost exclusively in healthcare-associated settings. In schoolchildren, for example, carriage was problematic because a classmate had a chronic illness and needed regular hospital checkups. A MRSA-positive family member is only problematic in the context of work in healthcare. Again, most outbreaks of infectious diseases are problematic within healthcare-associated settings, because these outbreaks lead to high morbidity and mortality rates, putting pressure on limited resources and putting HCWs in direct danger of contracting disease. Control measures for most communicable diseases therefore aim to regulate these threats (*25*). Outbreaks of multidrug-resistant organism infections, however, do not cause high morbidity and mortality rates (*21*,*28–30*). Public health measures aim to prevent introduction and further transmission of multidrug-resistant organisms in (some) healthcare-associated settings (*2*). Whether a carrier is subject to control measures does not depend on the severity of the pathogen but only on the likelihood that the resistant pathogen will be transmitted to a healthcare setting where vulnerable patients are cared for.

Multidrug-Resistant Organism Carriage as a State of Being

A salient feature of the inquiries was that carriage could last for a long time, making implementation of control measures even more burdensome. Some persons were colonized for such a long period, some even starting at birth, that it could be argued that the resistant microorganism was now part of their regular flora. The inquiries showed that, after a person receives a diagnosis of being a carrier, the label persists. It was often very difficult to eradicate the bacteria; moreover, there was no standard for determining whether a person was no longer a carrier. From an ethical perspective, persistence is particularly salient because inevitably, within the open population but also in healthcare settings, there will be a substantial group of unidentified asymptomatic carriers. Therefore, the severe restrictions faced by known carriers may not only be burdensome and stigmatizing but may also be considered unfair.

The Carrier as a Nondefining Factor in a Slowly Evolving Threat

In all cases analyzed for this study, the individual carrier was a possible link in the chain of transmission but certainly was not a central factor in the emergence and spread of multidrug-resistant organisms. The long-term clinical effect of multidrug-resistant organisms may be high, but it was not obvious that imposing restrictions, either preemptive or reactive, on individual carriers played a crucial role in controlling and mitigating that effect. The immediate threat posed by individual carriers was limited, certainly if compared with the role of conditions caused by other microorganisms, such as Ebola virus disease or meningococcal meningitis, for which devastating effects become evident in days, weeks, or months (*25*).

Discussion

We have shown how multidrug-resistant organism control measures undermine the well-being of asymptomatic

carriers. Although set in a country at the highest end of the spectrum with regard to strict AMR control measures, this finding is relevant to countries with all types of policies. The unique ethical features of multidrug-resistant organism carriage challenge the way we think about infectious disease control.

Traditionally, epidemics have been portrayed as an enemy attack of foreign microbes on human life, describing the carrier as "patient" or "victim" (*25*,*31*). However, multidrug-resistant organism carriers are not ill from carriage and can remain colonized for a long time. Any role as victim results more from the control measures than from the pathogen.

AMR control measures that may seem reasonable at first can easily lead to stigmatization. Stigma is defined as a social process characterized by exclusion, rejection, or blame resulting from experience, perception, or anticipation of adverse social judgments (*32*). In infectious disease control, the line between reasonable precaution measures and stigmatization has always been thin (*32*–*35*), but when carriage resembles a state of being, with limited relevance outside healthcare, the line also becomes vague and ambiguous (*33*).

Still, the dilemma of multidrug-resistant organism carriage represents one of the universal ethical challenges of public health: balancing the protection of the public while respecting individual well-being. Various public health ethics frameworks to guide decision-making have been suggested in this trade-off (*24*,*36*–*38*). Those frameworks have in common that they, explicitly or implicitly, call for clarity about the goals of a program and evaluation of effectiveness and proportionality. Such clarity is indeed valuable, but for multidrug-resistant organism control measures, the ultimate goals are not obvious. Of course, control measures are meant to control further spread of AMR, yet at the same time, overall mortality rates caused by multidrug-resistant organisms are still low and limited to vulnerable patients. Moreover, AMR is not a single epidemic; rather, it is a complex problem that slowly evolves and continually reemerges. Types of microorganisms displaying resistance and resistance mechanisms are constantly evolving. How AMR will emerge and what implications it will have in the next decades has yet to be determined (*39*). Although the control of AMR is of utmost importance, it is not obvious that strict control measures imposed on carriers will make a big difference in the overall objective.

AMR resembles a "wicked problem," a policy challenge that is not solvable by traditional policy instruments and to which no singular solution exists (*8*,*9*). Our analysis shows that control measures can be highly burdensome to carriers and that the magnitude of burden depends largely on the carrier's personal situation. Tailoring control measures to individual carriers' needs and values may therefore offer a way to deal with the wicked complexity.

Rather than asking whether it is justified to impose strict control measures to prevent antimicrobial resistance transmission from carriers, we propose asking, "How can we best care for this person's carriage and well-being in ways that do not imply unacceptable risk (for transmission) for other patients?" This question essentially takes an individualistic and contextual approach, acknowledging that different carriers can have different needs and values. For instance, some carriers enjoy the privacy that comes with isolation, many dislike the solitude, and others are most concerned about the quality of care and are relatively indifferent to isolation.

The question touches on the idea of patient-centered care, which involves caring for patients (and their families) in ways that are meaningful and valuable to each patient (*40*). At the same time, the problem goes beyond the scope of healthcare. Often the primary needs of carriers are not so much healthcare needs but rather are protection of the possibility that they can live a good life according to their own personal values. From this perspective, frameworks that use a rich account of quality of life may be helpful for evaluating the justice of control measures (*41*–*45*).

The question also requires critical reflection on the assessment of the risks of possible transmission of AMR to others in this specific context, ruling out that control measures imposed on individual persons are (implicitly) justified by appeal to the general (long-term) public health threat of AMR. A specific level of risk may be acceptable in a hospital in a region where baseline prevalence is high yet problematic in one where prevalence is low.

Especially when strict control measures are justified, an individualistic approach can help lower the individual burden. A nurse carrying multidrug-resistant organisms can be given other tasks instead of being sent home, some carriers could be compensated for financial consequences, and others could be helped by provision of childcare or extra support at home. Relieving the burdens of control measures on carriers will often come with financial costs for society or healthcare institutions, but it would be unreasonable if burdens of public health measures are borne by carriers individually.

In summary, AMR is one of the most severe threats of this century and control measures are needed; however, these measures are highly burdensome for carriers and of only limited benefit to the overall problem. Tailoring measures to personal needs and values of carriers may offer a new way to prevent carriers' transmission of multidrug-resistant organisms while minimizing compromises to their well-being.

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About the Author

Dr. Rump is a medical doctor who works at the National Coordination Centre for Communicable Disease Control in the Netherlands. She specializes in prevention of infectious diseases and has a special interest in public health ethics. Her research focuses on the development and implementation of a normative framework for ethical decision-making, addressing special treatment of persons carrying multidrug-resistant organisms.

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Address for correspondence: Babette Rump or Aura Timen, National Coordination Centre for Communicable Disease Control, RIVM–Centre for Communicable Diseases, PO Box 1, 3720 BA, Bilthoven, the Netherlands; email: babette.rump@rivm.nl or aura.timen@rivm.nl

National Surveillance for *Clostridioides difficile* Infection, Sweden, 2009–2016

Kristina Rizzardi, Torbjörn Norén, Olov Aspevall, Barbro Mäkitalo, Michael Toepfer, Åsa Johansson, Thomas Åkerlund

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Release date: August 16, 2018; Expiration date: August 16, 2019

Learning Objectives

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

- Describe *Clostridioides difficile* infection (CDI) incidence rates and resistance from 2012 to 2016, according to the Swedish national surveillance program
- Determine distribution of *C. difficile* types, including known outbreaks from 2012 to 2016, according to the Swedish national surveillance program
- Recognize the impact of diagnostic methods on CDI incidence and other possible reasons for changes in incidence and resistance, according to the Swedish national surveillance program

CME Editor

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CME Author

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Authors

Disclosures: ***Kristina Rizzardi, PhD; Torbjörn Norén, MD, PhD; Olov Aspevall, MD, PhD; Barbro Mäkitalo, PhD; Åsa Johansson, BSc;*** *and* ***Thomas Åkerlund, PhD,*** *have disclosed no relevant financial relationships.* ***Michael Toepfer, MD,*** *has disclosed the following relevant financial relationships: employed by a commercial interest, Unilabs AB (clinical microbiologist).*

Author affiliations: Public Health Agency of Sweden, Solna, Sweden (K. Rizzardi, O. Aspevall, B. Mäkitalo, T. Åkerlund); Örebro University, Örebro, Sweden (T. Norén); Unilabs Clinical Microbiology, Skövde, Sweden (M. Toepfer); Växjö Hospital, Växjö, Sweden (Å. Johansson)

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We report results from a national surveillance program for *Clostridioides difficile* infection (CDI) in Sweden, where CDI incidence decreased by 22% and the proportion of multidrug-resistant isolates decreased by 80% during 2012–2016. Variation in incidence between counties also diminished during this period, which might be attributable to implementation of nucleic acid amplification testing as the

primary diagnostic tool for most laboratories. In contrast to other studies, our study did not indicate increased CDI incidence attributable the introduction of nucleic acid amplification testing. Our results also suggest that successful implementation of hygiene measures is the major cause of the observed incidence decrease. Despite substantial reductions in CDI incidence and prevalence of multidrug-resistant isolates, Sweden still has one of the highest CDI incidence levels in Europe. This finding is unexpected and warrants further investigation, given that Sweden has among the lowest levels of antimicrobial drug use.

In a 1995 study assessing incidence of *Clostridioides difficile* infection (CDI) in Sweden, 5,133 cases were reported, corresponding to an incidence of 58 cases/100,000 inhabitants (*1*). After the initial reports of outbreaks of CDI in Europe associated with PCR ribotype 027 (RT027) in 2005 (*2*), a second incidence study was conducted in 2007, which showed that CDI incidence had increased to 90 cases/100,000 inhabitants (8,276 new cases). Based on recommendations from the European Centre for Disease Prevention and Control, a national surveillance program for CDI was initiated in Sweden 2009, aiming to monitor the apparent nationwide increase in CDI cases, detect trends and outbreaks, and determine the baseline incidence of CDI in the catchment areas of local clinical laboratories (*3*). The program, conducted by the Public Health Agency of Sweden, includes voluntary laboratory reporting of all new and recurring CDI cases as well as epidemiologic typing and susceptibility testing of isolates from clinical laboratories.

Countries in Europe have large variations in CDI incidence rates and distribution of prevalent PCR ribotypes, and the highest incidence rates occur in the northern countries, even though these countries have a low prevalence of RT027 (*4*,*5*). In addition, across Europe, a weak negative correlation has been observed between CDI incidence rates and cephalosporin use (*5*). Here we summarize results of the national CDI surveillance program in Sweden during 2012–2016, including CDI incidence rates, distribution of *C. difficile* types, known CDI outbreaks, and the effect of changes in diagnostic methods on reported CDI incidence.

Methods

Voluntary Surveillance Program

We collected epidemiologic case data through a voluntary reporting system, in which local laboratories reported the total number of CDI cases each week, including information of patients who had prior episodes of CDI within the previous 8 weeks. A new CDI case was defined as a patient with CDI with no prior diagnosis of CDI within the previous 8-week period. The reporting, which started during week 43 in 2009, also included catchment area (i.e., county) and sex and age of the patient. Initially, 16 of 28 laboratories reported CDI data, and by the end of 2011, all laboratories had joined the surveillance program. We collected denominator data by using a separate questionnaire, distributed yearly, which included the total number of tests and the number of positive samples per laboratory.

We performed epidemiologic typing and antimicrobial susceptibility testing twice a year on isolates collected during weeks 11 and 39. We chose these weeks arbitrarily because there was no reason to assume seasonal variation of CDI. We asked the local clinical laboratories to culture samples from all suspected CDI case-patients and to test the fecal samples and bacterial cultures by using that laboratory's standard diagnostic algorithm. To ensure that all isolates were identified during the study weeks, all culture-positive *C. difficile* isolates (including toxin-negative and toxin-positive according to the local laboratory's standard test algorithm) were sent to the Public Health Agency of Sweden for PCR ribotyping and antimicrobial susceptibility testing. Approximately 4% of all yearly cases are analyzed by this program. The laboratories also sent information stating their current diagnostic method and the test results for each isolate sent for testing.

PCR Ribotyping

From week 1 in 2009 through week 11 in 2012, PCR ribotyping was gel-based, as previously described by Stubbs et al. (*6*), with minor modifications (*7*). From week 39 in 2012, we performed PCR ribotyping with capillary gel electrophoresis and analyzed results with BioNumerics 7.5 (Applied Maths, Sint-Martens-Latem, Belgium) (*8*). We conducted identification on the basis of the Cardiff–European Centre for Disease Prevention and Control strain collection and other known types. We gave new types the prefix "x" followed by a chronological number until the strain was typed by the reference laboratory at Leiden University Medical Centre (Leiden, the Netherlands).

Antimicrobial Susceptibility Testing

We performed susceptibility testing by using the antimicrobial drugs recommended for treatment (i.e., metronidazole and vancomycin) and common antimicrobial drugs known to increase risk for acquiring CDI (i.e., moxifloxacin, clindamycin, and erythromycin). We tested all isolates by using Etest on *Brucella* agar, as previously described (*9*). The breakpoints for resistant isolates were epidemiologic cutoff values according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST): metronidazole, >2 mg/L; vancomycin, >2 mg/L; moxifloxacin, >4 mg/L; clindamycin, >16 mg/L; and erythromycin, >2 mg/L.

Statistical Methods

We analyzed county-level PCR ribotype diversity by using the Simpson reciprocal index, 1/D. We analyzed how switching diagnostic method affected positivity rates by using the χ^2 test. We compared ecologic MIC distributions of *C. difficile* isolates in Sweden to EUCAST distributions by using the Wilcoxon rank-sum test.

Results and Discussion

Incidence of CDI in Sweden during 2012–2016

In 2012, a total of 10,820 cases of CDI were reported, of which 8,104 (75%) were new cases and 2,716 (25%) were recurrent cases. Recurrent cases represent a maximum estimate because the data included a few double samples from the same patient. The incidence of new cases was 11.8/10,000 patient-days and 85/100,000 inhabitants, results that were almost unchanged compared with those reported in 2007 (11.9/10,000 patient-days and 90/100,000 inhabitants). In 2016, the incidence of new cases had decreased to 10.1/10,000 patient-days (a 15% decrease) and 66/100,000 inhabitants (a 22% decrease) (Figure 1, panel A and B). The decrease occurred in most counties and resulted in less geographic variation in incidence (range 4.0–18.7/10,000 patient-days in 2012 compared with 7.1–15.5/10,000 patient-days in 2016 [online Technical Appendix Figure, https://wwwnc.cdc.gov/EID/article/24/9/17-1658-Techapp1.pdf]).

CDI incidence decreased in all age groups except for 5–14 years; the largest reductions came in the age groups 0–4 years (25%), 45–64 years (23%), and ≥85 years (23%) (Figure 2, panel A). The incidence was reduced similarly in male and female inhabitants over time, although higher incidence occurred in male inhabitants 5–14 and ≥75 years of age during the entire period (Figure 2, panel B). For female inhabitants 15–64 years of age, higher incidence also occurred, 33% higher among the 15–44 years age group and 17% higher among the 45–64 years age group (Figure 2, panel B).

PCR Ribotype Distribution

The distribution of the most common PCR ribotypes in Sweden has, with a few exceptions, been relatively constant since 2009 (online Technical Appendix Table) and is comparable to that observed in other countries of Northern Europe (*4*). RT014 was the most common type throughout the whole period, except in 2011, when it was the second most common after RT020. Types frequently associated with multidrug resistance, such as RT012, RT078, RT046, and RT017, were among the 10 most common types in the first years of the surveillance program. In conjunction with the 7% reduction of incidence rate during 2014–2015, with the exception of RT078, all of the previously common multidrug-resistant (MDR) types were no longer among the 10 most common ribotypes. RT078 prevalence was ≈3% in Europe during 2012–2013; this ribotype is also common in pigs and calves (*4*,*10*). RT046 is predominant in scouring piglets in central parts of Sweden (*11*).

The distribution of PCR ribotypes was more variable between counties and over time. For example, Östergötland and Uppsala had relatively high levels of RT012 and RT231 in 2012 (Figure 3, panel A and B); these types are associated with outbreaks (*9*,*12*). By 2016, these types had diminished, and CDI incidence in these counties had decreased (to 35% in Östergötland and 52% in Uppsala) (online Technical Appendix Figure). An increase occurred in PCR ribotype diversity over time (Figure 3, panel A and B). Similarly, a study in England indicated that ribotype diversity increased as outbreak-prone types decreased (*13*). These results (i.e., the disappearance of major types, increase in type diversity, and decrease in incidence) suggest that hospitals adopted improved infection control during the study period. In contrast, no change in ribotype diversity was observed in the county of Västernorrland despite an increased incidence during 2012–2015 (Figure 3, panel C).

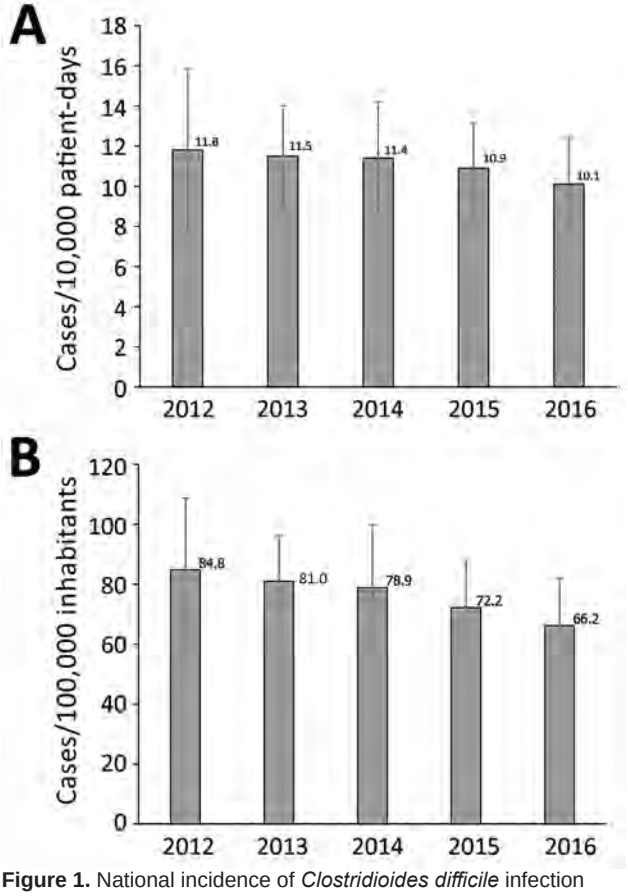

Figure 1. National incidence of *Clostridioides difficile* infection (CDI), Sweden, 2012–2016. A) CDI cases/10,000 patient-days. B) CDI cases/100,000 inhabitants. Error bars indicate SD of the mean county incidence for each year.

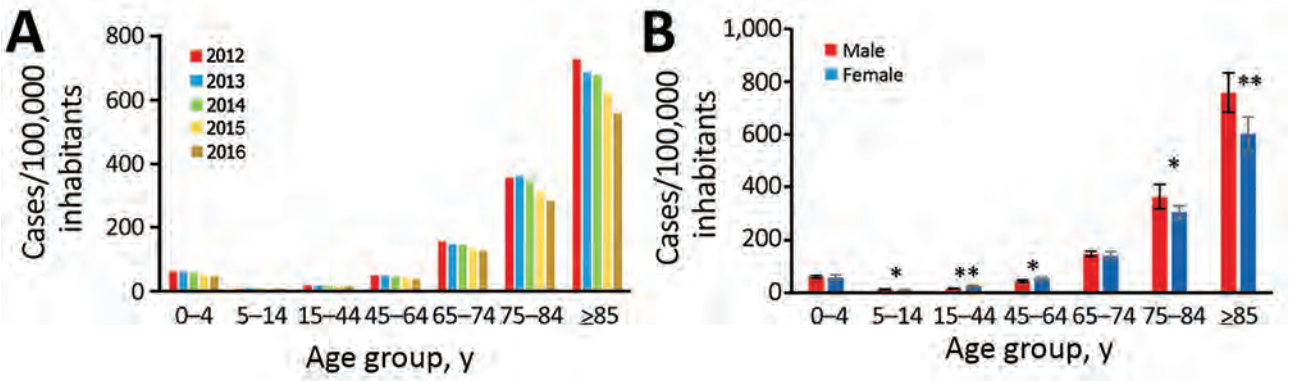

Figure 2. National incidence of new *Clostridioides difficile* infection (CDI) cases, Sweden, 2012–2016. A) Incidence by age group and year; B) incidence by age group and sex. Error bars indicate SD. *p<0.05; **p<0.01 (both by t-test).

Jämtland was another county that showed strong incidence variation: an increase during 2012–2014 and then a decrease in 2016 (online Technical Appendix Figure 1). In Jämtland, we observed a change in ribotype diversity, from high diversity in 2012 to low diversity during 2014–2015, then back to high diversity in 2016 (Figure 3, panel D). However, no clustering of MDR PCR ribotypes was evident in Västernorrland or Jämtland. Possible explanations for the incidence levels might include polyclonal outbreaks, changes in diagnostics, and changes in sampling procedures. All 4 counties have changed diagnostic methods from enzyme immunoassay for toxin A and B (as

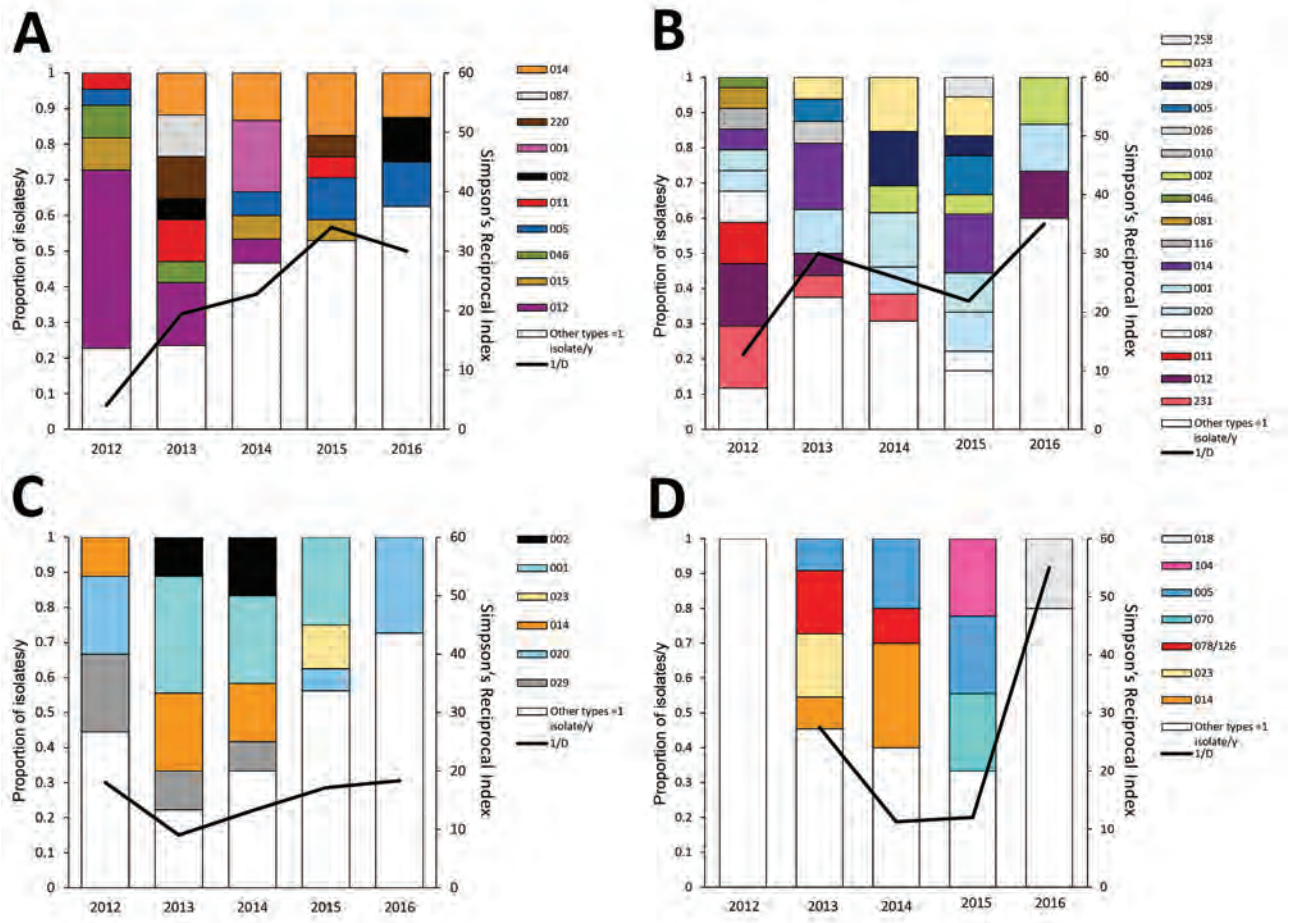

Figure 3. Variation in PCR ribotype distribution in 4 counties with large changes in *Clostridioides difficile* infection incidence rates, Sweden, 2012–2016. A) Östergötland; B) Uppsala; C) Västernorrland; D) Jämtland. 1/D, Simpson's reciprocal index 1/D.

standalone test) to generally more sensitive nucleic acid amplification tests (NAATs) (also as standalone test); Uppsala and Jämtland changed in 2012 and Östergötland and Västernorrland in 2015.

Outbreaks of *C. difficile*

A geographic clustering of MDR isolates might be explained by a clonal outbreak that is not always apparent in clinical practice or in infrequent surveillance programs. One insidious outbreak caused by MDR RT046 was detected in 2011 in the Eksjö catchment area in Jönköping County (*14*), where an incidence of 22 CDI cases/10,000 patient-days surfaced in the national surveillance. The outbreak isolate was predominant in 46% of cases, and an excess virulence was observed by a 30-day mortality rate of 30% and a >40% recurrence rate compared with other ribotypes (T. Norén, unpub. data). Because the geographic clustering of RT046 in Jönköping was obvious already in 2008 (*3*), the impact of this outbreak cannot be fully understood. Antimicrobial drug stewardship and improved hygiene were implemented stepwise during the initial 10 months, but the outbreak was not controlled until chlorine disinfection was introduced after this period. In addition to RT046, significant ($p<0.001$) geographic clustering of RT231 was detected in 2008 in the counties of Stockholm and Uppsala (*3*), and this type spread between several hospitals during an extended period until it finally diminished (*8*).

Another outbreak, caused by toxin A–negative MDR RT017, was detected late in 2012 at Ystad Hospital in Skåne County. Considerable clinical impact occurred, similar to outbreaks with this type in other countries in Europe (*15*,*16*). During a 6-month period, 27 patients experienced severe CDI with this ribotype, and 10 died in spite of treatment. In November 2013, a hospital outbreak of RT027 CDI started at Växjö Hospital in Kronoberg County and was discovered when isolates from several fulminant cases were ribotyped. During August 2013–April 2016, a total of 41 patients had RT027 CDI diagnosed; 6 patients died, resulting in a 15% 30-day mortality rate for this strain. The strain was traced back to a patient that had been abroad, but whether his case was the actual index case was unclear. Because of the limited periods of strain collection in the national surveillance program, the smaller outbreaks of RT017 and RT027 CDI could only be traced retrospectively, and the outbreak alert in these cases was prompted by clinical awareness of clustering of severe cases.

Increased local incidence of CDI can be polyclonal, like in Jämtland County, where a sudden increase from ≈2–4 cases/week to 10–20 cases/week occurred during a few weeks of the 2013–14 winter season. Typing revealed 5–6 different susceptible PCR ribotypes, and although no clear evidence of transmission could be found, the sudden increase contributed to a substantially higher incidence in the county in 2013 and 2014 (online Technical Appendix Figure 1). Increases of CDI in these scenarios might occur as a result of changes in diagnostic performance, in antimicrobial drug use, overcrowded wards, or a general decline in hygiene precautions, as opposed to introduction of a single virulent type.

Sampling and Diagnostic Algorithms

We found a positive correlation between sampling rate and CDI incidence per 100,000 inhabitants per county for all 6 years (Figure 4, panel A), consistent with findings in

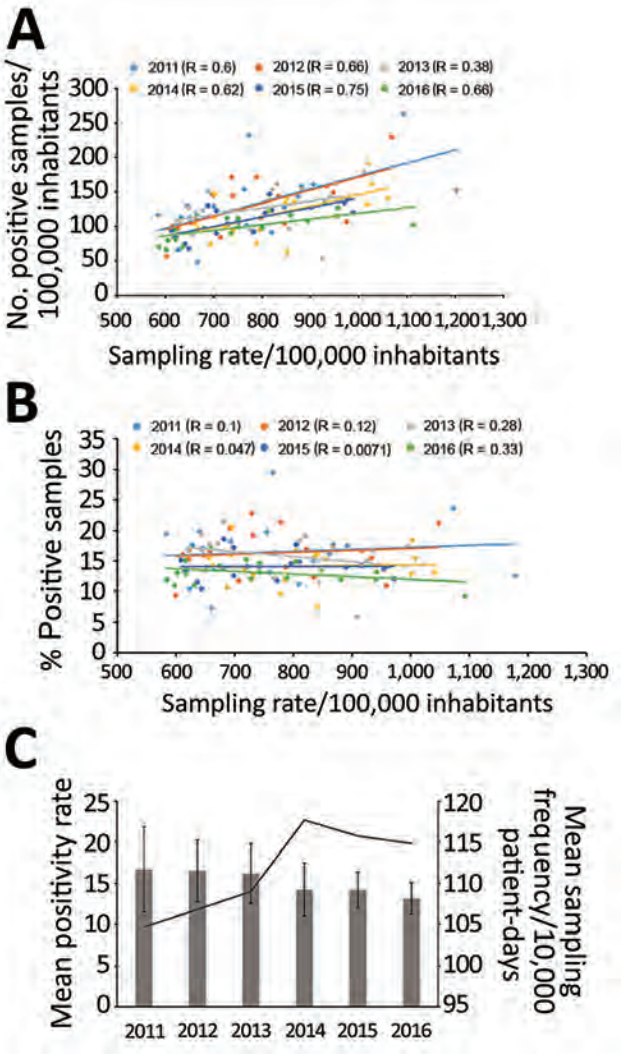

Figure 4. Correlation between *Clostridioides difficile* infection (CDI) cases and sampling rates, Sweden, 2009–2016. A) Correlation between number of positive CDI cases/100,000 inhabitants per county and sampling rates. B) Correlation between percentage of CDI cases and sampling rates. Dots indicate values per county; lines indicate regression analyses (R values as indicated). C) Mean positivity rate (bars) and mean sampling frequency (line), by year. Error bars show interlaboratory SD in positivity rates.

Table. Comparison of CDI positivity rates for clinical laboratories that switched methods during the 2009–2016 study period, Sweden*

Change in testing algorithm	Year algorithm was switched	Mean no. positive samples (mean positivity, %)		p value†	Change in positivity after switch, %	Local CDI‡ incidence change 2012–2016, %
		2 y before switch	2 y after switch			
EIA to NAAT						
Laboratory 1	2011	698 (21)	672 (19)	0.004	−9	−52
Laboratory 2	2011	176 (13)	355 (16)	<0.001	+24	−31
Laboratory 3	2011	101 (12)	168 (16)	<0.001	+36	−9
Laboratory 4	2011	635 (22)	506 (19)	<0.001	−13	+6
Laboratory 5	2012	322 (15)	312 (17)	0.013	+14	−27
Laboratory 6	2013	416 (22)	377 (18)	<0.001	−18	−34
Laboratory 7	2013	346 (16)	300 (12)	<0.001	−27	−28§
Laboratory 8	2013	176 (11)	272 (14)	<0.001	+25	−28§
Total		359 (18)	370 (17)	<0.001	−7	
Cytotox to NAAT						
Laboratory 9¶	2012	124 (7)	353 (20)	<0.001	+68	+67
Laboratory 10	2013	1,398 (13)	1233(11)	<0.001	−18	−9§
Total		761 (12)	793 (12)	0.358	−2	
Cytotox to EIA + GDH						
Laboratory 11	2014	294 (17)	273 (13)	<0.001	−22	−35
NAAT to EIA + NAAT						
Laboratory 12	2014	388 (19)	290 (14)	<0.001	−27	−44
EIA to EIA + NAAT						
Laboratory 13	2014	677 (17)	420 (13)	<0.001	−21	−35

*CDI, *Clostridioides difficile* infection; EIA, enzyme immunoassay; GDH, glutamate dehydrogenase assay; NAAT, nucleic acid amplification testing.
†By χ^2 test.
‡CDI incidence/100,000 population. Local incidence data from before 2012 not available.
§Data from >1 laboratory is included in local CDI incidence rates.
¶This laboratory used a nonstandardized cell cytotoxicity assay.

other studies (*17*,*18*). Indications for sampling and laboratory testing might differ between regions, and an extensive sampling might lead to lower positivity rates. However, sampling rates did not largely affect the diagnostic positivity rates, suggesting that the indications for sampling were similar among counties (Figure 4, panel B). The positivity rate and the intercounty variation in positivity rate decreased gradually during 2012–2016 (Figure 4, panel C), and, because many laboratories changed diagnostic methods during the period, the reduction in intercounty variation is most likely attributable to optimization of diagnostic algorithms and methods.

The number of laboratories using NAAT as a standalone method for CDI diagnostics increased from 6/28 in 2011 to 16/26 in 2016. Switching to NAATs as a standalone test has been associated with higher diagnostic sensitivity and up to a 67% increase in incidence (*19*–*21*), but we did not find any elevated positivity rate attributable to the increased use of NAAT. On the contrary, the average positivity rates 2 years after a method switch from enzyme immunoassay to NAAT decreased by 7% (Table). Moreover, we observed no consistent associations between method switch, positivity rate, and incidence. One explanation might be that most laboratories already had high sensitivity in the testing algorithms. Also, improved infection control and the elimination of certain PCR ribotypes that clustered in geographic areas during the study period most likely contributed to the lower positivity and incidence rates. Only 1 laboratory (laboratory 9, which serves Västerbotten County) showed a major increase in positivity and incidence rate after adopting NAAT after switching in 2013. The most likely explanation for this increase is that the laboratory used an unusual diagnostic method prior to the switch, including pretreatment of feces with alcohol followed by cell-cytotoxicity assay. A suboptimal diagnostic method might explain why this county had the lowest incidence rates in Sweden 2012 (online Technical Appendix Figure 1).

Antimicrobial Resistance

All 3,321 isolates collected during 2009–2016 within the national surveillance program have been tested for antimicrobial susceptibility to vancomycin, metronidazole, erythromycin, clindamycin, and moxifloxacin. Only 1 isolate (RT027) was resistant to metronidazole (MIC 4 mg/L), and no isolate was resistant to vancomycin. The proportion of isolates resistant to erythromycin, clindamycin, and moxifloxacin was reduced during 2009–2016 (Figure 5, panel A). The highest proportion of resistant isolates was observed in 2012, when 13% of isolates were MDR (resistant to moxifloxacin, clindamycin, and erythromycin). During 2012–2016, the proportion of MDR isolates was reduced by 80%; moxifloxacin-resistant isolates were reduced by 26%, clindamycin-resistant isolates by 51%, and erythromycin-resistant isolates by 46%. During 2009–2012, from 94% to 97% of the MDR isolates belonged to 4 PCR ribotypes (RT012, RT017, RT046, and RT231). In 2016, the same ribotypes accounted for only 30% of all MDR isolates (Figure 5, panel B).

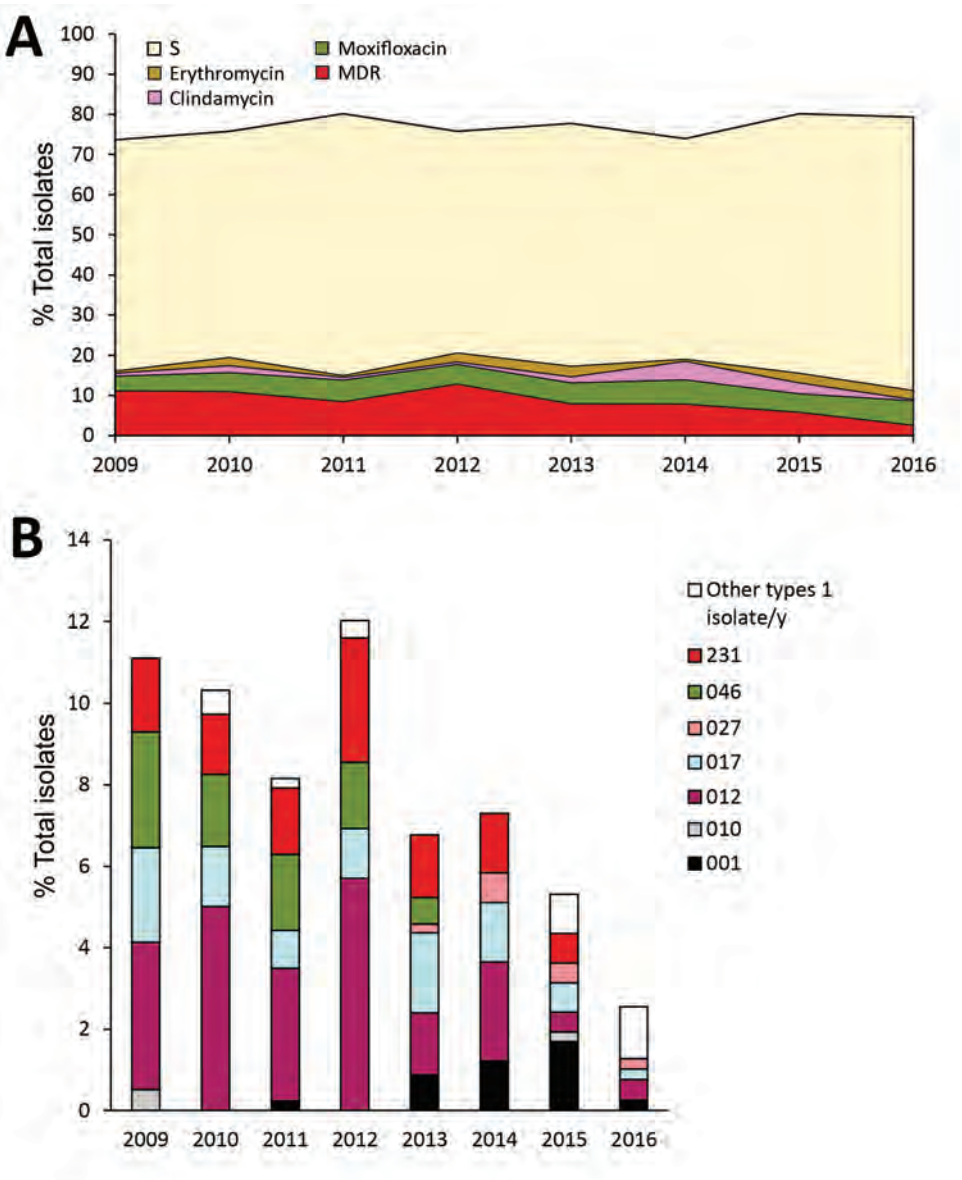

Figure 5. Resistance of *Clostridioides difficile* to indicator antimicrobial drugs, Sweden, 2009–2016. A) Percentage of isolates resistant and sensitive to indicator antimicrobial drugs erythromycin, clindamycin, and moxifloxacin. B) PCR ribotype distribution of MDR isolates. MDR, multidrug-resistant (i.e., resistant to erythromycin, clindamycin, and moxifloxacin); S, sensitive to erythromycin, clindamycin, and moxifloxacin.

The MIC distributions of isolates collected in Sweden during 2009–2016 showed significant differences to those from EUCAST for all antimicrobial drugs tested except for clindamycin. The collection from Sweden had a greater proportion of isolates with lower MICs than did the EUCAST collection ($p = <0.001$ by Wilcoxon rank-sum test) (online Technical Appendix Figure 2).

Conclusions

During 2012–2016, a sustained decrease in incidence rates of CDI has occurred in Sweden, as well as a dramatic decrease in the proportion of MDR *C. difficile* isolates. Although decreased antimicrobial drug consumption or prudent use might be part of the explanation, we suggest that the major impact is attributable to improved hygiene measures in healthcare settings. This hypothesis is supported by 1) the fact that the volume of antimicrobial drugs typically associated with increased risk for acquiring CDI sold to hospitals, where CDI is predominant (*4*), was virtually unchanged during the study period (*22*); 2) a substantial reduction in CDI cases that occurred among elderly patients, who are known to be hospitalized to a greater extent; and 3) the apparent disappearance of geographic clusters of specific *C. difficile* PCR ribotypes, indicative of reduced nosocomial spread. However, because CDI cases are not classified into community- and healthcare-associated CDI, we cannot entirely rule out the possibility that the observed incidence reduction occurred mainly in the community, where antimicrobial drug sales have decreased more compared with sales to inpatient facilities (*22*).

The surveillance system in Sweden has several limitations (e.g., the reporting is not mandatory, diagnostic methods vary across the country and over time, and isolates are collected only twice per year). However, the compliance of reporting has been rather high, probably because of the open reporting of information on geographic differences in incidence and clusters of *C. difficile* types. This reporting has in turn led to increased awareness of local epidemiology that is useful for tailoring hospital hygiene measures and antimicrobial stewardship policies.

Despite the 22% decrease in CDI incidence during 2012–2016, Sweden still has a comparatively high CDI incidence compared with other countries in Europe. Because only a few outbreaks have been reported and diversity of types is high in northern Europe, including Sweden (*4*), the high incidence is probably not explained by nationwide outbreaks but is more likely attributable to increased clinical awareness, contributing to correct diagnoses and treatment. Sampling rates for CDI in Sweden are also high (*17*), an average of 116 samples/10,000 patient-days in 2016, a factor that is correlated with higher incidence (*19*–*21*). Introduction of highly sensitive methods such as standalone NAAT has been correlated with higher CDI incidence (*17*), but the small effect on positivity rates after adopting NAAT that we report suggests that previous diagnostic algorithms were on par with NAAT methods. A high incidence might also be related to more subtle differences in the population, such as immunity or susceptibility to CDI, a possibility that warrants further research.

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About the Author

Dr. Rizzardi is a research scientist and analyst at the Public Health Agency of Sweden. Her research interests include the epidemiology and molecular typing of *Clostridioides difficile*.

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Address for correspondence: Thomas Åkerlund, Public Health Agency of Sweden, Unit for Surveillance of Bacterial Pathogens, Nobels väg 18, Solna, Sweden; email: thomas.akerlund@folkhalsomyndigheten.se

Travel-Associated Zika Cases and Threat of Local Transmission during Global Outbreak, California, USA

Charsey Cole Porse, Sharon Messenger, Duc J. Vugia, Wendy Jilek, Maria Salas, James Watt, Vicki Kramer

Zika and associated microcephaly among newborns were reported in Brazil during 2015. Zika has since spread across the Americas, and travel-associated cases were reported throughout the United States. We reviewed travel-associated Zika cases in California to assess the potential threat of local Zika virus transmission, given the regional spread of *Aedes aegypti* and *Ae. albopictus* mosquitoes. During November 2015–September 2017, a total of 588 travel-associated Zika cases were reported in California, including 139 infections in pregnant women, 10 congenital infections, and 8 sexually transmitted infections. Most case-patients reported travel to Mexico and Central America, and many returned during a period when they could have been viremic. By September 2017, *Ae. aegypti* mosquitoes had spread to 124 locations in California, and *Ae. albopictus* mosquitoes had spread to 53 locations. Continued human and mosquito surveillance and public health education are valuable tools in preventing and detecting Zika virus infections and local transmission in California.

The first human cases of Zika virus infection reported from the Americas were in May 2015 from Brazil (*1*). In the span of less than a year, Zika virus spread across South America, Central America, the Caribbean, and parts of Mexico. As observed with other mosquitoborne diseases, such as dengue and chikungunya, which have spread through Central and South America and the Caribbean, travel-associated cases of Zika were reported throughout the United States, and local transmission of Zika virus was eventually detected in Florida and Texas (*2*,*3*). Because California has established and expanding infestations of *Aedes aegypti* and *Ae. albopictus* mosquitoes, the main vectors of Zika virus, and is near Mexico, to which Zika virus is endemic, the risk for autochthonous transmission of Zika virus is a concern (*4*). During 2011–2015, *Ae. aegypti* and *Ae. albopictus* mosquitoes were detected in 85 cities and census-designated places in 12 counties of California (*5*).

In California, patient testing and evaluation focused on assessment of infection in pregnant women and symptomatic patients, and assessment of potential viremia in these patients in relation to proximity to known *Aedes* mosquito infestations. To describe travel-associated Zika cases and better assess the potential threat of local Zika transmission in California, we reviewed all Zika cases reported to the California Department of Public Health (CDPH) during November 2015–September 2017. We also summarized surveillance for *Ae. aegypti* and *Ae. albopictus* mosquitoes in California and laboratory testing for Zika virus during this time.

Methods

Zika cases were reported to CDPH by the 61 local health departments in California, either through the electronic California Reportable Disease Information Exchange (https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/CalREDIE.aspx) or through paper case report forms. Cases reviewed by CDPH during November 2015–September 2017 were analyzed for type of Zika disease or infection, as defined by the 2016 Council of State and Territorial Epidemiologists (CSTE)/Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA) criteria and classified as confirmed or probable (*6*). Variables examined included sex, age, race/ethnicity, country where exposure likely took place, duration of travel, symptoms, symptom onset date, and pregnancy status and outcomes.

We analyzed data by using SAS for Windows version 9.4 (SAS Institute Inc., Cary, NC, USA). For Zika case-patients with a travel duration of <6 months, we compared duration of time in Zika-affected areas between pregnant and all other case-patients by using the Kruskal-Wallis test

Author affiliations: California Department of Public Health, Sacramento, California, USA (C.C. Porse, W. Jilek, V. Kramer); California Department of Public Health, Richmond, California, USA (S. Messenger, D.J. Vugia, M. Salas, J. Watt)

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for 2 groups (unequal variances) to retrospectively assess time at risk between these 2 groups.

California has a network of local vector control agencies that monitors distribution and abundance of *Aedes* spp. and other mosquito populations. Mosquito surveillance typically includes trapping and identifying mosquitoes. Surveillance might be augmented by submitting mosquito specimens, especially specimens collected in and around residences or workplaces of case-patients, to the Davis Arbovirus Research and Training Laboratory at the University of California (Davis, CA, USA) for Zika virus (*7*), dengue virus (DENV) (*8*), and chikungunya virus (Davis Arbovirus Research and Training Laboratory at the University of California, unpub. data) testing by multiplex quantitative reverse transcription PCR (RT-PCR), as described by CDC (*9*). Mosquitoes submitted during West Nile virus (WNV) season (June 1–October 15) are also tested for WNV, St. Louis encephalitis virus, and western equine encephalitis virus (*10*). Agencies enter mosquito surveillance data into the California Vectorborne Disease Surveillance Gateway Database (https://gateway.calsurv.org/), which is used to generate statewide data for mapping of *Aedes* mosquito locations. We used a geographic information system (ArcGIS Desktop version 10.5; Esri, Redlands, CA, USA) to generate maps that enabled spatial and temporal mapping of *Aedes* mosquito populations in relation to presumed places of residence of presumed viremic Zika case-patients. We generated latitude and longitude data by using the Gateway Database for mosquitoes and determined case-patient place of residence by using the California Reportable Disease Information Exchange.

Testing of humans for Zika virus was performed by the CDPH Viral and Rickettsial Disease Laboratory (VRDL), CDC, local public health laboratories, commercial laboratories, and blood banks. Testing for Zika virus infection was completed for appropriate tissue, serum, or urine specimens by using Zika virus nucleic acid or serologic tests. We analyzed symptomology and pregnancy status of those tested, volume of testing at the CDPH VRDL, types of tests conducted, and time from symptom onset to specimen collection date. For purposes of local transmission risk assessment, a potentially viremic patient was defined as a Zika-positive case-patient with symptom onset ≤7 days before or any time after return from travel to their place of residence.

Results

Descriptive Statistics

During November 2015–September 2017, a total of 588 travel-associated Zika cases were reported in California, including 139 infections in pregnant women, 10 congenital infections, and 8 sexually transmitted infections. Sixty-two case-patients were <18 years of age. On the basis of the 2016 CSTE surveillance case definition for Zika, 410 cases met the confirmed criteria and 178 were probable. Of these, 466 case-patients had noncongenital Zika disease with symptoms meeting the 2016 CSTE case definition for noncongenital Zika (≥1 of the following: fever, rash, arthralgia, or conjunctivitis); 112 had a symptomatic noncongenital Zika infections, 6 had congenital Zika disease with Zika-associated birth defects (birth defects reported include those detected in infants infected with Zika virus before, during, or shortly after birth, including microcephaly, calcium deposits in the brain indicating possible brain damage, excess fluid in the brain cavities and surrounding the brain, absent or poorly formed brain structures, abnormal eye development, or other problems resulting from damage to the brain that affects nerves, muscles, and bones, such as clubfoot or inflexible joints, and confirmed hearing loss); and 4 had congenital Zika infections with no Zika-associated birth defects (*6*).

A total of 66% (391/588) of case-patients were female; median age of case-patients was 35 years (range <1–89 years). Of persons with reported ethnicity, 69% (306/443) were Latino/Latina. For the 139 women pregnant at the time of diagnosis, median age was 27 years (range 14–44 years), and 78% (87/111) of those with reported ethnicity were Latina.

Of 570 case-patients who contracted Zika virus while traveling outside California, most case-patients reported travel to Mexico (36.4%), Central America (34.3%), or the Caribbean (13.1%). The top 10 countries and territories for travel were Mexico (36.4%), Nicaragua (9.6%), Guatemala (8.4%), El Salvador (7.0%), Dominican Republic (4.4%), Puerto Rico (4.4%), Honduras (3.9%), Costa Rica (3.7%), Jamaica (2.5%), and Colombia (1.8%). The timeline for travel-associated Zika cases reported in California mirrored the spread of the outbreak across the Americas (Figure 1); the number of case-patients with travel to Mexico increased substantially starting in June 2016 as the number of Zika cases reported in Mexico steadily increased.

Of 570 case-patients who traveled, 79 (13.9%) lived in their country of exposure for ≥6 months before coming to California, where they were subsequently tested for Zika virus. When we excluded these 79 persons, women who were pregnant at the time of Zika diagnosis had a significantly ($p = 0.03$) longer travel duration (median 14 days [range 1–153 days]) than all other Zika case-patients (median 11 days [range 1–137 days]).

For 466 case-patients with symptoms, rash was the most common (89.0%, 415), followed by arthralgia (62.5%, 291), fever (60.1%, 280), myalgia (36.9%, 172), and conjunctivitis (35.0%, 161). A rash without any other symptom was seen in 49 (10.5%) case-patients. For those case-patients with >1 symptom, the most common combination of symptoms, reported by 13% of case-patients, was rash, arthralgia, and

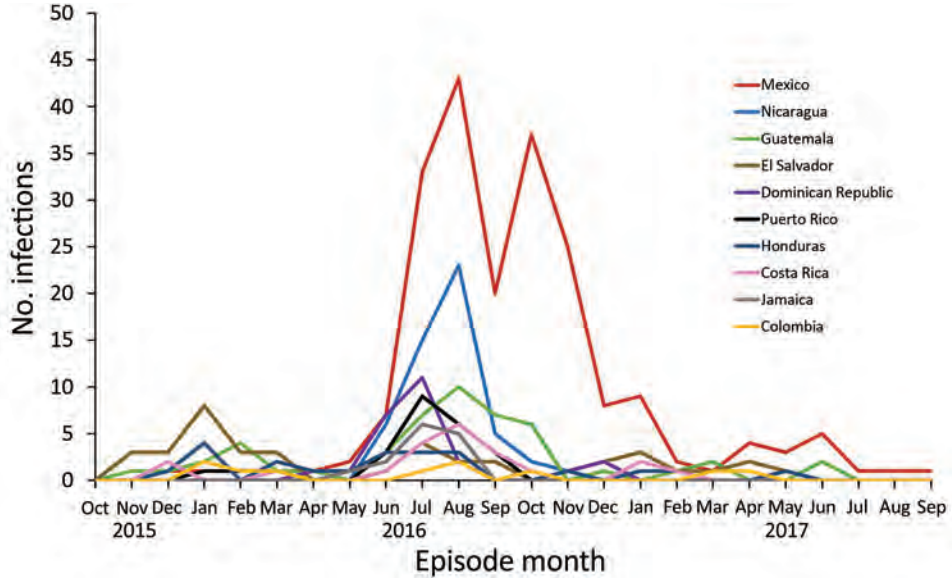

Figure 1. Number of human Zika virus infections in residents, by month and year of onset and country of travel (top 10 countries shown), California, USA, October 1, 2015–September 1, 2017. Month was determined by date of symptom onset for symptomatic persons or specimen collection date for asymptomatic persons.

fever. Seven case-patients were hospitalized for a median of 3 (range 1–8) days. On the basis of symptom onset date, the number of Zika cases reported in California in 2016 increased from June through August and then decreased through November (Figure 2).

Of 139 women who were pregnant at the time of Zika diagnosis, 120 had completed their pregnancies by September 1, 2017: 114 with live births and 6 with fetal losses. Fourteen women were still pregnant, and the status of 5 women was unknown. For live births, 90 (78.9%) infants were tested for Zika virus at or shortly after birth; 84 (73.7% of live births) infants showed negative results for Zika virus by nucleic acid and IgM tests, and 6 (5.3% of live births) showed positive results. Of the remaining 24 live births, 7 infants were not tested, and the testing status of 17 infants was unknown. In addition to the 6 congenitally infected infants that were born to Zika virus–positive mothers, 4 additional infants whose mothers were exposed to Zika virus but showed negative results by nucleic acid or IgM tests were positive for Zika virus.

Eight infants were born in California with Zika-associated birth defects. Of these infants, 2 were negative and 6 were positive for Zika virus by PCR and IgM test. Both Zika virus–negative infants had mothers who were positive for Zika virus, and 3 of the Zika virus–positive infants had mothers who had negative results for Zika virus.

Mosquito and Human Case Surveillance

During January 1, 2016–September 1, 2017, we detected 78 new locations for *Ae. aegypti* mosquitoes and 25 new locations for *Ae. albopictus* mosquitoes, for a total of 133 cities or census-designated places for *Ae. aegypti* mosquitoes and 56 for *Ae. albopictus* mosquitoes, an increase of 142% for *Ae. aegypti* mosquitoes and 81% for *Ae. albopictus* mosquitoes in 20 months. In 2017, *Ae. aegypti* mosquitoes were detected in 12 counties and *Ae. albopictus* mosquitoes in 5 counties, including 2 new counties containing *Ae. aegypti* mosquitoes in the Central Valley (*11*).

As of September 1, 2017, a total of 13,499 *Ae. aegypti* mosquitoes and 2,719 *Ae. albopictus* mosquitoes had been tested by Davis Arbovirus Research and Training for Zika virus, chikungunya virus, and DENV. None of these mosquitoes were positive for these arboviruses, although 5 pools of *Ae. aegypti* mosquitoes and 1 pool of *Ae. albopictus* mosquitoes were positive for WNV. Of the 588 case-patients reported who had Zika virus infections, 435 (74.6%) were likely viremic while in California. Of those viremic case-patients, 279 (64.1%) were also residents of California counties where *Ae. aegypti* or other *Aedes* spp. mosquitoes have been detected; their co-location was more common in southern California (Figure 3).

Laboratory Testing

Although the VRDL performed most (58.7%; 345/588) testing for Zika-positive cases in California, commercial laboratories accounted for 17.5% (103/588), local health departments for 13.1% (77/588), and CDC for 9.5% (56/588). Seven Zika cases reported in California were identified through blood bank screening. Most testing at VRDL was performed for asymptomatic pregnant women (7,795 asymptomatic pregnant women/11,603 total patients; 67.2%). Eighty (1.0%) of these asymptomatic pregnant women were positive for Zika virus by quantitative RT-PCR (1 woman) or IgM test and plaque-reduction neutralization test (PRNT) (79 women).

Of the 120 completed pregnancies for women who were infected with Zika virus while pregnant, 45 placental tissues (including placenta, membrane, and umbilical

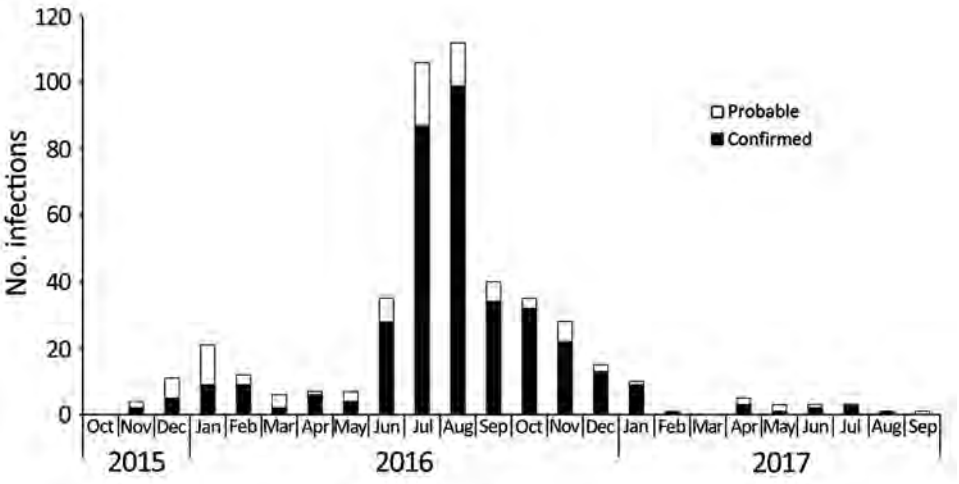

Figure 2. Confirmed and probable symptomatic Zika virus infections, by symptom onset month and year, California, USA, October 2015–September 2017.

cord) were sent to CDC for testing. Zika virus was detected by RT-PCR in placental tissues of 8 women. Detection of Zika virus in the placental tissue provided confirmatory testing for 5 of these women (3 were already confirmed by serum PRNT).

Of the 410 confirmed Zika cases reported in California, 319 (77.8%) case-patients had Zika virus detected by nucleic acid tests in serum, urine, or placental tissue, and the other 91 were confirmed by detection of neutralizing antibodies to Zika virus (and not DENV). For symptomatic case-patients, the median time from illness onset to specimen collection was 5 days (range 1–194 days). For cases confirmed by serum or urine nucleic acid tests, the time to collection was shorter, with a median of 3 days (range 1–33 days), than for PRNT, with a median of 16 days (range 1–194 days).

Discussion

Since the global Zika outbreak began in South America in 2015, many travel-associated Zika cases have been documented in California, including infections in pregnant women, congenital infections, and sexually transmitted infections. With the establishment and continuing spread of *Ae. aegypti* and *Ae. albopictus* mosquitoes in California, prevention of local transmission of Zika virus has been and continues to be a public health priority. In working to identify possible local transmission, CDPH used the data for travel-associated Zika cases described in this article to develop our Zika testing prioritization. Although CDC recommended specific criteria for travel-associated Zika virus testing, different criteria were needed when testing persons without travel history, especially when the number of confirmed Zika cases was increasing in California and local Zika virus transmission was reported in Florida (*2*). The goal of such testing was to identify anyone who potentially had Zika virus, without testing large numbers of persons at low risk.

CDPH subsequently provided criteria for local health departments in California to consider in evaluating whether suspected persons without travel history should be considered for Zika virus testing, including factors that could increase risk for local transmission, as well as signs and symptoms most suggestive of Zika. For example, CDPH allowed that, for counties where *Aedes* mosquitoes have been detected, Zika virus testing could be offered to persons who live in an area containing *Aedes* spp. mosquitoes and who came to their healthcare provider with a maculopapular rash and 1 other symptom consistent with Zika (fever, arthralgia, or conjunctivitis), without an alternative explanation, such as a drug reaction or other infection. Rash was recommended as the primary criterion in this setting because nearly 90% of Zika case-patients had a rash. This allowance for Zika virus testing for persons with no travel or sexual exposure was used in some counties in California and identified several persons suspected of having Zika who were tested, all of whom showed negative results. This testing allowance would not be appropriate in areas that did not contain *Aedes* spp. mosquitoes and is being reconsidered as the number of Zika cases has decreased.

Although California health officials did not identify any episodes of local Zika virus transmission, our data indicate that large numbers of likely viremic travelers returned to areas containing *Ae. aegypti* and *Ae. albopictus* mosquitoes, especially in southern California, as has also been found for dengue and chikungunya (*4*). This overlap of viremic travelers and *Aedes* spp. mosquito vectors potentially increases the risk for local transmission and will continue to be a public health concern requiring ongoing mosquito and human case surveillance. CDPH works closely with local health departments and vector control agencies to prepare for the potential of a locally transmitted outbreak. The close coordination of mosquito control programs in California with programs of local health departments, the common use of air conditioning or window

screens by residents, and the variable distribution of *Aedes* spp. mosquitoes in some affected counties in California would likely limit the extent of a local outbreak should it occur. Nonetheless, knowledge of co-located vector mosquitoes and infected returned travelers is needed to aid in rapid investigation of any suspected locally transmitted case(s) and to limit potential spread.

Mosquito seasonality influences risk for local transmission, and although *Aedes* spp. mosquitoes can be found year-round in California, they are most abundant from June through November, typically peaking in September and October (*5*). Large numbers of potentially viremic case-patients returned to California during June–November 2016 (Figure 2), corresponding with the season of high *Aedes* spp. mosquito activity in California. This seasonality also reflects *Ae. aegypti* mosquito activity in northern Mexico, where *Ae. aegypti* mosquitoes are abundant from August through November.

Similar to the chikungunya outbreak in the Americas that began in 2013 and rapidly peaked in most locations before decreasing (*12*), the number of Zika cases is now decreasing. This decrease in Zika cases has been observed both in countries reporting local transmission and in the number of infected returned travelers reported in the United States and in California (*13*). Although the level of Zika virus transmission has decreased, many countries, including Mexico, have continued to report moderate levels of local Zika virus transmission (*14*). Given the large number of travelers between Mexico and California, it is critical that Zika prevention messaging, surveillance, and outreach continue, especially as it pertains to women traveling while pregnant.

The large volume of testing for asymptomatic pregnant women reinforces that potential Zika virus exposure incidents were occurring in high numbers even with extensive provider education and public health messaging in California and nationally. Women who were pregnant at the time of their Zika diagnosis had a longer duration of travel in their exposure country than all other case-patients. Because most infected pregnant women were Latina, it is possible that many of them had traveled to visit family and therefore had longer stays. Given the health risk to pregnant women and their fetuses, this finding is of great concern. We need to ensure that English- and Spanish-language public health messaging about risks of travel or travel of sexual partners to Zika-affected countries continues to reach pregnant women and their healthcare providers. Although a decrease in reported travel-associated Zika cases was observed in California in March 2017, we did not detect a decrease in

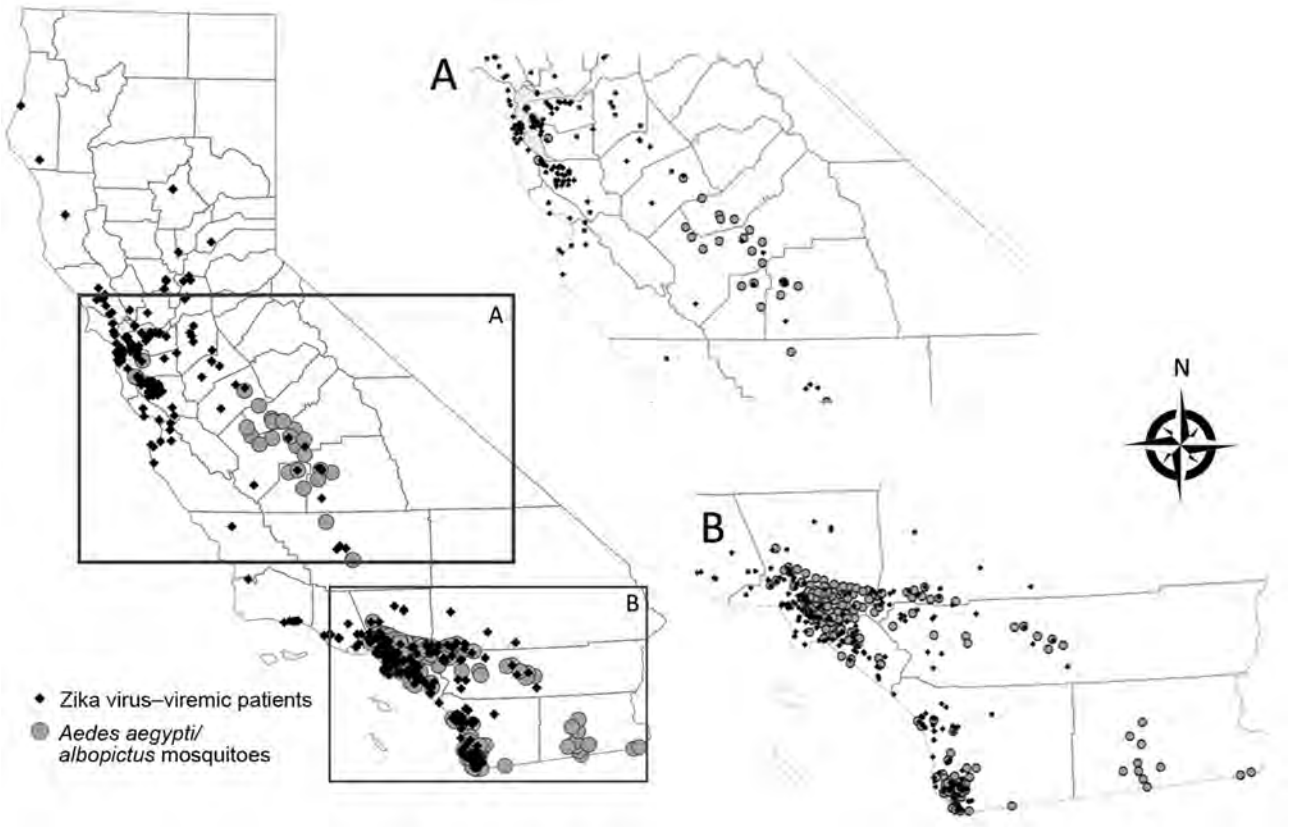

Figure 3. Locations where *Aedes* spp. mosquitoes were detected and residences of possibly viremic case-patients infected with Zika virus, central (A) and southern (B) California, USA, October 2015–September 2017. Insets show larger views of corresponding region.

specimens submitted for asymptomatic pregnant women to VRDL until August 2017.

Laboratory testing for Zika virus has proved challenging throughout the outbreak. Results of assays were difficult to interpret because serologic cross-reactivity with other flaviviruses, especially DENV, was common (*15*). Detection of neutralizing antibodies against Zika virus and DENV was observed for 178 probable Zika case-patients reported in California. Thus, the specific flavivirus of the infection in these case-patients could not be determined. In addition, Zika IgM has been reported to persist in serum, making timing of infection difficult to determine (*16*). The discordant testing results observed in the mother/infant pairs were equally challenging, suggesting that a negative test result could rarely rule out a Zika virus infection. All these factors, in addition to the difficulty of determining the date of exposure for many case-patients, especially for women who lived in the area of exposure for an extended time, made the interpretation of negative results problematic and created challenges for ensuring that affected infants received appropriate follow-up care.

Our study and data interpretation have several limitations. First, the data included only case-patients who were positive for Zika virus, not case-patients who were negative but had been potentially exposed to Zika virus. Analysis of such persons who were negative for this virus but had potentially been exposed would be helpful to further delineate risk and discriminate symptoms. However, negative results, particularly from commercial laboratories, often have limited associated clinical and demographic data. Second, some dengue cases might be misclassified as Zika cases because of cross-reactivity and nonspecific binding in available serologic assays. Given the large percentage of case-patients in California with previous exposure to flaviviruses, especially DENV, there is potential for false-positive interpretation of PRNT results. All case-patients with neutralizing antibodies against DENV and Zika virus were classified on the basis of the CSTE case definition as having Zika because of the higher risk during pregnancy from exposure to Zika virus. In addition, low pretest probability, especially in asymptomatic persons, increases the risk for misclassification because of type I errors (false-positive results). Third, an estimated 80% of persons infected with Zika virus are asymptomatic (*17*), making it difficult to determine when, where, and how many potentially viremic persons are returning to California. Fourth, there is a clear testing bias toward pregnant and reproductive-age women, which skews demographic data.

Although Zika virus transmission and Zika case numbers have decreased across the Americas, we expect to see continued, limited, local transmission in some affected countries. Thus, there is still a risk for pregnant women and all those who travel to these countries, and it is necessary that prevention messaging remains targeted and operative. Healthcare providers should continue to be suspicious of returning travelers with rash, fever, conjunctivitis, or arthralgia, particularly when other diagnoses have been ruled out. The expansion of *Ae. aegypti* and *Ae. albopictus* mosquitoes into 12 counties in California, especially along the southern border region, increases the risk for local Zika transmission in California. The large percentage of potentially viremic travelers returning to areas that contain *Aedes* spp. mosquitoes, in addition to an unknown number of returned travelers who are asymptomatically infected but not detected, makes the risk for local transmission a continuing threat, albeit low, in California. Zika has complicated disease manifestations and transmission dynamics, such as sexual and congenital transmission, which are not typically observed for other arboviruses. It is vital that we apply the public health lessons learned during the Zika outbreak to prepare for complexities that might arise during future epidemics of emerging and reemerging arboviruses.

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About the Author

Dr. Porse is an epidemiologist in the Vector-Borne Disease Section, Infectious Diseases Branch, Division of Communicable Disease, California Department of Public Health, Sacramento, CA. Her research interests include epidemiology, mosquitoborne diseases, and public health.

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Address for correspondence: Charsey Cole Porse, California Department of Public Health, 1616 Capitol Ave, MS-7307, Sacramento CA 95814, USA; email: charsey.porse@cdph.ca.gov

Distinguishing Japanese Spotted Fever and Scrub Typhus, Central Japan, 2004–2015

Eiichiro Sando, Motoi Suzuki, Shungo Katoh, Hiromi Fujita, Masakatsu Taira, Makito Yaegashi, Koya Ariyoshi

Japanese spotted fever (JSF) and scrub typhus (ST) are endemic to Japan and share similar clinical features. To document the clinical and epidemiologic characteristics that distinguish these 2 rickettsial diseases, during 2004–2015 we recruited 31 JSF patients, 188 ST patients, and 97 non-rickettsial disease patients from the Southern Boso Peninsula of Japan. JSF occurred during April–October and ST during November–December. Patients with JSF and ST were significantly older and more likely to reside in wooded areas than were patients with nonrickettsial diseases. Spatial analyses revealed that JSF and ST clusters rarely overlapped. Clinical findings more frequently observed in JSF than in ST patients were purpura, palmar/plantar rash, hyponatremia, organ damage, and delayed defervescence after treatment. Although their clinical features are similar, JSF and ST differ in seasonality, geographic distribution, physical signs, and severity. Because a considerable percentage of patients did not notice rash and eschar, many rickettsial diseases might be underdiagnosed in Japan.

Two rickettsial diseases are endemic to Japan, scrub typhus (ST) and Japanese spotted fever (JSF). ST, which is also called tsutsugamushi disease (*1*), was first reported in central Japan in 1878 (*2*). ST is caused by the mite-borne pathogen *Orientia tsutsugamushi*. According to the national surveillance data of notifiable diseases in Japan, during 2004–2015, the number of reported ST cases was nearly constant; each year on average, 396 ST cases and 2 deaths (case-fatality rate 0.5%) were reported (*3*). ST was originally believed to be confined to the Asia–Pacific region; however, ST has recently been reported in Kenya (*4*) and southern Chile (*5*). In 1984, JSF was identified in western Japan (*6*). JSF is caused by the tickborne pathogen *Rickettsia japonica* (*7*). Except for a case reported in South Korea (*8*), JSF is endemic nearly exclusively to the central and western portions of Japan (*3*). Recently, the number of reported JSF cases in this region increased, from 66 in 2004 to 215 in 2015, and the case-fatality rate increased from 1.5% to 2.3% (*3*); thus, JSF is a public health concern. Although JSF and ST have been reported in several prefectures in Japan, the areas of endemicity rarely overlap at the district level (*3*). One of the rare districts to which both JSF and ST are endemic is the southern Boso Peninsula, Chiba Prefecture, in central Japan.

The typical signs and symptoms of JSF and ST are similar (e.g., fever, rash, and eschar), although in patients with ST, the frequency of rash varies from 14% to 93% and of eschar from 8% to 93% (*9*–*13*). For a few patients with JSF and ST, severe conditions develop (*14*–*17*). However, clinical information regarding JSF has been limited by lack of an appropriate case definition, lack of in-depth information, and studies involving small sample sizes (*14*,*16*). The clinical features observed in patients with JSF and ST are not comparable across studies because of the different enrollment criteria and nonstandardized case definitions. To clarify the clinical and epidemiologic characteristics of JSF and ST patients by using stringent laboratory confirmation methods and to identify the factors that distinguish the 2 diseases, we conducted a multicenter study in the southern Boso Peninsula in central Japan, an area of high JSF and ST endemicity. The study was approved by the institutional review boards of the Kameda Medical Center and the Awa Regional Medical Center.

Methods

Study Design and Setting

The southern Boso Peninsula is a predominantly rural mountainous region with a long coastline facing the Pacific Ocean and Tokyo Bay. According to the census, the total population in 2015 was 350,000 and 35.4% of the residents were ≥65 years of age. We conducted prospective and retrospective case series reviews at 3 medical facilities: Kameda Medical Center (865 acute beds), Awa Regional

Author affiliations: Nagasaki University, Nagasaki, Japan (E. Sando, M. Suzuki, S. Katoh, K. Ariyoshi); Kameda Medical Center, Kamogawa, Japan (E. Sando, M. Yaegashi); Mahara Institute of Medical Acarology, Anan, Japan (H. Fujita); Chiba Prefectural Institute of Public Health, Chiba, Japan (M. Taira)

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Medical Center (149 beds), and Kameda Family Clinic–Tateyama (no beds).

Study Period and Entry Criteria

We prospectively enrolled patients from January 1, 2011, through December 31, 2015. We collected clinical, epidemiologic, and laboratory data from the patients who visited the study hospitals and exhibited signs and symptoms compatible with rickettsial disease. The patients were suspected to have rickettsial disease if they had any of the following clinical signs or symptoms without other apparent causes: fever, rash, eschar, respiratory symptoms, altered mental status, lymphadenopathy, neurologic abnormalities, systematic pain, chills/rigors, headache, or malaise. Using the same enrollment criteria, we also retrospectively collected data from patients who visited the study hospitals from January 1, 2004, through December 31, 2010, and who were not included in the prospective data collection. We used a standardized format to extract clinical and epidemiologic information from electronic medical records.

Laboratory Methods

All blood samples were sent to a commercial laboratory (SRL, Inc., Tokyo) for an indirect immunofluorescence assay (IFA) to identify the *O. tsutsugamushi* serotypes Kato, Karp, and Gilliam; the antigens were provided by Denka Seiken Co., Ltd. If JSF was suspected, the samples were sent to the Chiba Prefectural Institute of Public Health for IFA to identify the *O. tsutsugamushi* serotypes Kato, Karp, Gilliam, Irie/Kawasaki, Hirano/Kuroki, and *R. japonica* (YH strain). The blood samples collected during 2009 and 2010 were also sent to the Ohara Research Laboratory (Fukushima City, Japan), and samples collected during 2014 were sent to the Mahara Institute of Medical Acarology (Anan, Japan) for an indirect immunoperoxidase assay to identify 6 *O. tsutsugamushi* serotypes (the previously mentioned 5 serotypes plus the serotype Shimokoshi), *R. japonica* (Aoki strain), and *R. typhi* (*18*). The type-specific whole rickettsial particles were used as antigens in the IFA and immunoperoxidase assays. Serum samples were diluted from 1:40 to 1:40,960 for immunoperoxidase assays and from 1:10 (or 20) to 1:10,240 for IFA. The titer was expressed as the reciprocal of the highest dilution. Nested PCR assays were performed to identify the 56-kDa antigen of *O. tsutsugamushi* and the 17-kDa genus-common antigen of *R. japonica* from eschars at the Chiba Prefectural Institute of Public Health (Chiba, Japan) or Kameda Medical Center (Kamogawa, Japan) (*19*,*20*).

Case Definitions and Data Collection

A patient's rickettsial status was defined as confirmed if the PCR result from the eschar was positive for any rickettsiae or if a ≥4-fold increase in the IgM or IgG titer of IFA or immunoperoxidase assay was observed in paired serum samples (i.e., acute and convalescent phases). A patient's status that did not fulfill the criteria for confirmed was defined as probable if the IgM titer of IFA or immunoperoxidase assay was ≥80 for JSF or ST. A patient's status was defined as possible if the clinical course was compatible with that of JSF or ST but the laboratory test results did not fulfill the criteria for either confirmed or probable. A patient was defined as having a nonrickettsial disease if a diagnosis of an infectious or noninfectious disease other than a rickettsial disease was confirmed. We excluded from analysis those patients who were classified as having possible cases or a diagnosis of murine typhus or concurrent JSF and ST infection.

Traditionally, in Japan, fever, rash, and eschar have been considered the triad of JSF and ST. We classified the triad into 3 categories: 1) "chief complaint" if any of the signs were the reason for the visit; 2) "upon history collection" if patients noticed the signs but had not complained until the physician asked; and 3) "physical exam" if the signs were objectively identified at the initial physical examination.

Statistical Analyses

The clinical and epidemiologic characteristics of the patients were summarized and compared according to the 3 categories (i.e., JSF, ST, and nonrickettsial diseases). We used χ^2 or Fisher exact tests to compare characteristics of the patients by disease category. We computed odds ratios (ORs) with 95% CIs by using logistic regression models.

The patients' home addresses were geocoded and plotted on maps by using ArcGIS version 10.4.1 (Esri, Redlands, CA, USA). We calculated the population density and land use percentage within a radius of 500 m based on the census data and compared the 3 categories by using the Mann-Whitney U test. The Kulldorff scan statistics tool (SaTScan version 9.4.4) was used to identify the geographic clusters of JSF and ST (*21*). All tests were 2-tailed, and $p<0.05$ was considered statistically significant. All clinical data analyses were performed by using STATA version 13.0 (StataCorp LLC, College Station, Texas, USA).

Results

Laboratory Confirmation

A total of 661 patients were enrolled in the study: 303 by prospective and 358 by retrospective data collection. Overall, 42% of the patients were female, and the mean age was 60 years. Of the 50 patients whose eschars were tested by nested PCR, 8 were positive for *R. japonica* DNA and 29 were positive for *O. tsutsugamushi* DNA. The *O. tsutsugamushi* serotypes were identified in 22 patients; 16 were the Irie/Kawasaki type and 6 were the

Hirano/Kuroki type. All patients were tested by an IFA at least 1 time, and paired blood samples were available for 304 (46%) patients. The median time from acute-phase sample collection to convalescent-phase sample collection was 14 days (interquartile range 11–17 days). Of the 304 patients, JSF was confirmed for 33 and ST was confirmed for 155. Of the 357 patients whose convalescent-phase samples were unavailable, none had probable JSF and 35 had probable ST. Three patients who did not fulfill the serologic criteria for having a rickettsial disease but whose eschar was positive for *O. tsutsugamushi* DNA were confirmed as having ST. Two patients fulfilled the criteria for having both JSF and ST, and 1 patient was confirmed to have murine typhus. Overall, our analysis included 31 patients with JSF, 188 patients with ST, and 97 patients with nonrickettsial diseases (Figure 1). The final diagnoses of the nonrickettsial diseases are shown in online Technical Appendix Table 1 (https://wwwnc.cdc.gov/EID/article/24/9/17-1436-Techapp1.pdf).

Seasonal and Geographic Distributions

The seasonal distributions of JSF, ST, and nonrickettsial diseases are shown in Figure 2. All patients with JSF visited a medical facility during April–October; the numbers peaked slightly in July. Most (91%) patients with ST visited a medical facility in either November or December. No seasonal trend was observed for nonrickettsial diseases.

The geographic distributions are shown in Figure 3. We identified 1 JSF cluster ($p<0.001$) and 2 ST clusters ($p = 0.013$ and $p = 0.041$), and these clusters rarely overlapped. Patients with JSF and ST resided in less populated areas (population densities within a 500-m radius were 255/km^2 and 295/km^2, respectively) than patients with nonrickettsial diseases (904/km^2; $p<0.001$). Patients with JSF and ST more frequently resided in wooded areas (proportions in forested area within a 500-m radius were 51% and 43%, respectively) than patients with nonrickettsial diseases (17%; $p<0.001$).

Demographic and Clinical Features

The baseline characteristics of the patients are summarized in Table 1. The proportion of female patients did not differ among the 3 groups. Patients with JSF and ST were older than patients with nonrickettsial diseases; among patients in the oldest age group, JSF occurred more frequently than ST. Patients with JSF and ST were more frequently exposed to the natural outdoor environment than were patients with nonrickettsial diseases.

Clinical characteristics of the patients are summarized in Table 2. The triad (i.e., fever, rash, and eschar) was commonly observed by physicians but not necessarily noticed by the patients. Fever was a primary sign; however, at the initial physical examination, body temperature was high in only 74% of patients with JSF and 73% with ST. Among patients who did not have a high body temperature at their initial physical examination, fever developed during hospitalization for 5 (71%) of 7 with JSF and 9 (38%) of 24 with ST. Although most patients had a rash, only 60% of patients with JSF and 44% with ST had noticed their rash. Moreover, only 45% of patients with JSF and 28% of patients with ST reported their rash. Most patients did not notice the presence of eschar.

During physical examination, patients with JSF had hypotension more frequently than patients with ST (OR 5.1, 95% CI 1.9–13.8), but no significant difference was observed in the frequency of tachycardia and tachypnea. Considerably higher proportions of patients with JSF and ST than with nonrickettsial diseases had a rash and eschar; the mean ± SD size of the eschar was smaller in patients with JSF (5.8 ± 2.1 mm) than in patients with ST (9.7 ± 5.6 mm; $p = 0.024$). Purpura, palmar/plantar rash, and lung involvement were more frequently observed in patients with JSF than in those with ST. Prevalence of lymphadenopathy did not differ among the groups.

Patients with JSF and ST were less likely than patients with nonrickettsial diseases to have leukocytosis and anemia but more likely to have elevated aspartate aminotransferase and lactate dehydrogenase levels, hyponatremia,

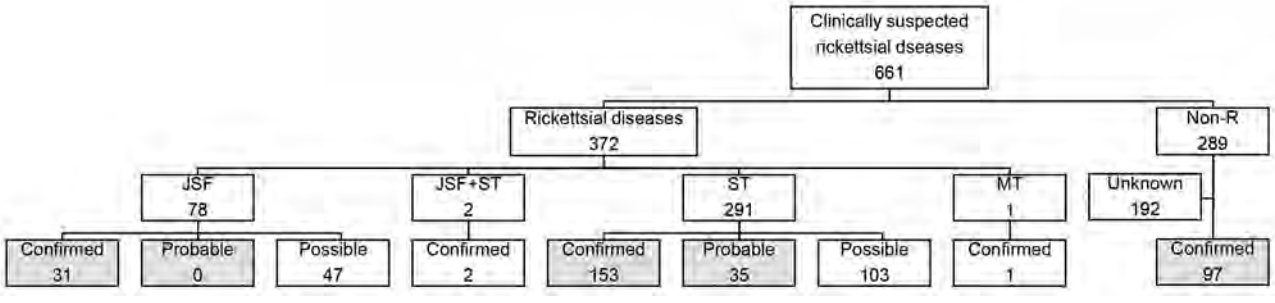

Figure 1. Numbers of patients with rickettsial or nonrickettsial diseases, Japan, 2004–2015. Of 43 patients tested by immunofluorescence and immunoperoxidase assays, 4 fulfilled the criteria for having confirmed JSF, 7 for confirmed ST, and 7 for probable ST. Gray shading indicates the cases included in the main analysis. JSF, Japanese spotted fever; MT, murine typhus; non-R, nonrickettsial diseases; ST, scrub typhus.

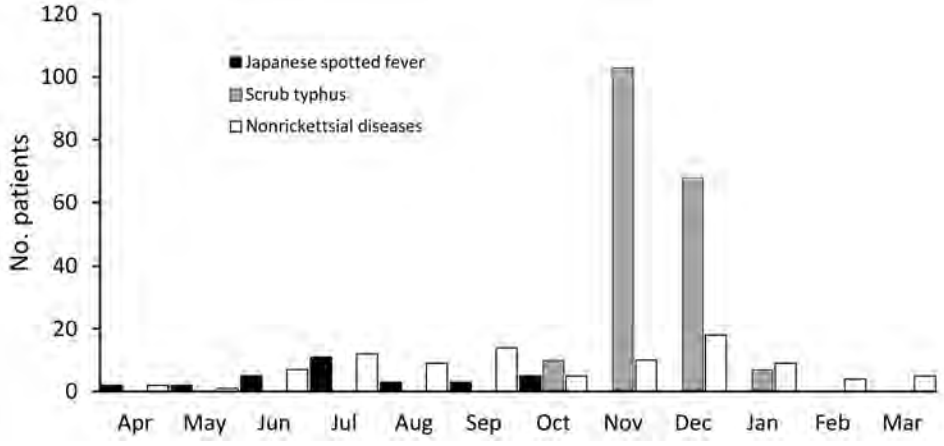

Figure 2. Number of patients with Japanese spotted fever, scrub typhus, and nonrickettsial diseases, central Japan, by month, 2004–2015.

and urine occult blood (Table 3). Patients with JSF were more likely than patients with ST to have low platelet counts; elevated bilirubin, creatinine kinase, blood urea nitrogen, and creatinine levels; hyponatremia; and high C-reactive protein.

Patients with JSF required hospitalization more frequently than did patients with ST; these associations did not change after we adjusted for age. Patients with JSF tended to visit a medical facility earlier than did patients in the other groups. JSF and ST were successfully treated in patients who received tetracycline; a 51-year-old patient receiving psychiatric care died of ST, but no patient died of JSF. The time to defervescence after treatment was longer for patients with JSF than for patients with ST.

Discussion

By using standardized laboratory definitions for diagnosis, we determined that the clinical and epidemiologic characteristics of JSF and ST in Japan differed by seasonality, geographic distribution, physical signs, and severity. JSF and ST showed distinct seasonal patterns. JSF occurred during April–October and peaked slightly in July, whereas most ST occurred during November–December. JSF and ST were distributed in less populated and more wooded areas, although their geographic clusters rarely overlapped. Patients with JSF were more likely than patients with ST to have purpura, palmar/plantar rash, and organ damage and to be hospitalized.

The different seasonal distribution of JSF and ST observed in our study can be explained by the ecology of the vectors (*3*,*22*) as follows: 1) *Haemaphysalis flava* and *H. longicornis* ticks, which transmit *R. japonica*, are active from spring until autumn in Chiba (*23*); 2) *Leptotorombidium scutellare* mites, which transmit the Irie/Kawasaki (and Hirano/Kuroki) serotypes of *O. tsutsugamushi*, are active in autumn and early winter (*24*) and unable to survive the winter; and 3) *L. pallidum* mites, which transmit the Karp and Gilliam serotypes of *O. tsutsugamushi*, are active from October through May (*24*). The difference in geographic distributions of JSF and ST may also be explained by the different distribution of the reservoirs in our study settings. Sika deer are wild hosts of ticks, and their distribution overlaps with that of ticks (*25*). The cluster of JSF identified in our study overlapped with the distribution of sika deer and Reeves's muntjacs, which are related to sika deer (*26*,*27*). In contrast, the field rat, which is the primary host of the *Leptotrombidium* mite, is spreading throughout this area, which may explain the wide distribution of ST. Although our data are limited, similar patterns (i.e., the clustering of JSF and relatively wide distribution of ST) were also observed in other prefectures (*28*,*29*). Further studies are needed to establish the temporal and geographic associations among the vectors, reservoirs, and rickettsial pathogens.

Although the clinical features of patients in this study with JSF and ST were similar, some clinical findings were characteristic of JSF. Patients with JSF more frequently had rashes on the palms/soles, purpura, and small eschars. Moreover, the following severe conditions occurred more frequently among patients with JSF than among those with ST: hypotension, low platelet counts, and increased creatinine levels. Rickettsiae invade and proliferate within vascular endothelial cells and cause a vasculitis-like systemic disease (*30*). In a study by Tai et al., cytokine and chemokine levels were higher in patients with JSF than in patients with ST, but no significant association was observed between cytokine levels and the clinical severity of disease (*17*). Although previous human and animal model studies have revealed the pathogenic mechanisms of severe rickettsial infections (*31*–*32*), the mechanisms of severe JSF remain not fully understood. Of note, the clinical severity of ST may differ according to the *Orientia* serotype. According to a systematic review, the mortality rate from ST substantially varied according to patients' age, co-occurring conditions, and regional *Rickettsia* strains (*33*). Our findings of ST in regions where Irie/Kawasaki type and Hirano/

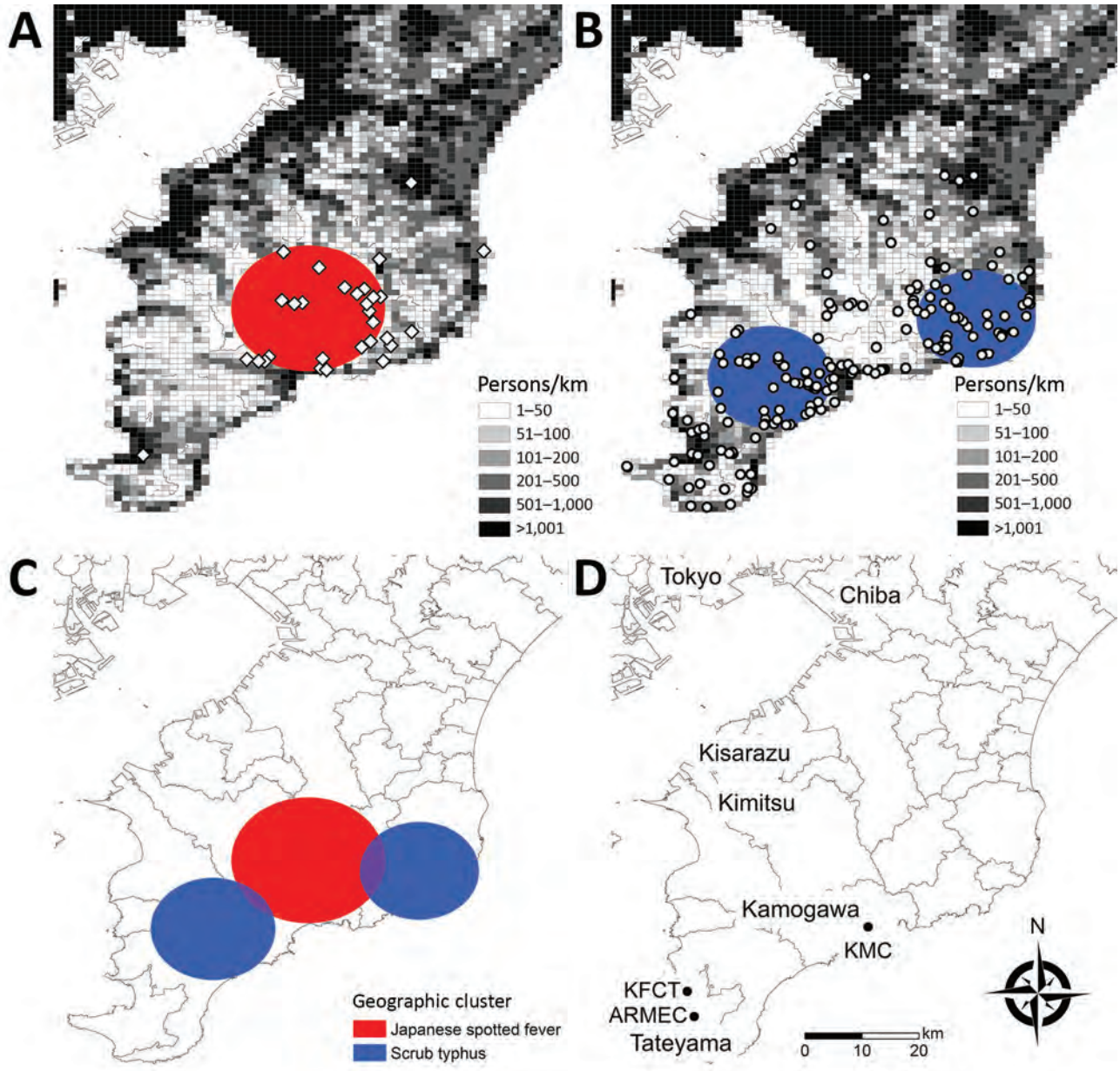

Figure 3. Geographic distribution and clusters of JSF and ST, Japan, 2004–2015. A) JSF; B) ST; C) geographic clusters of JSF and ST; D) locations of study facilities. White diamonds (JSF) and circles (ST) represent the locations of each patient's address. Shaded circles (red, JSF; blue, ST) represent statistically significant spatial clusters ($p<0.05$). The geographic distribution of the patients with nonrickettsial diseases, which were used for the cluster analysis as the reference, is shown in the Technical Appendix Figure (https://wwwnc.cdc.gov/EID/article/24/9/17-1436-Techapp1.pdf). ARMEC, Awa Regional Medical Center; JSF, Japanese spotted fever; KFCT, Kameda Family Clinic Tateyama; KMC, Kameda Medical Center; ST, scrub typhus.

Kuroki type are endemic may not be directly applicable to other settings in which other serotypes are endemic, such as Akita and Niigata in northern Japan.

Fever has been considered one of the typical signs of JSF and ST. Most patients in our study had a high body temperature during the clinical course of their illness; however, fever was not apparent at the time of initial physical examination for 26% of patients with JSF and 27% of patients with ST. Although rash and eschar were commonly observed by the physicians, more than half of the patients did not notice these signs. Consequently, 33% of the patients with JSF and 34% of the patients with ST received incorrect diagnoses during their first medical visit. Furthermore, fewer clinicians were aware of JSF than of ST (*34*). These findings indicate that a substantial number of rickettsial diseases may be underdiagnosed in Japan.

Table 1. Baseline characteristics of patients with JSF, ST, and nonrickettsial diseases, central Japan, 2004–2015*

Characteristic	JSF, no. (%), n = 31	ST, no. (%), n = 188	Non-R, no. (%), n = 97	JSF vs. non-R†		ST vs. non-R†		JSF vs. ST‡	
				OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Female sex	16 (52)	85 (45)	35 (36)	1.9 (0.8–4.3)	0.127	1.5 (0.9–2.4)	0.14	1.3 (0.6–2.8)	0.509
Age, y, mean (SD)	73 (10)	65 (15)	57 (20)						
Age group, y									
≤54	1 (3)	29 (15)	43 (44)	Reference		Reference		Reference	
55–64	7 (23)	48 (26)	14 (14)	21.5 (2.4–190.3)	0.006	5.1 (2.4–10.9)	<0.001	4.2 (0.5–36.1)	0.188
65–74	7 (23)	56 (30)	13 (13)	23.2 (2.6–205.9)	0.005	6.4 (3.0–13.7)	<0.001	3.6 (0.4–30.9)	0.239
>75	16 (52)	55 (29)	27 (28)	25.5 (3.2–203.3)	0.002	3.0 (1.6–5.8)	0.001	8.4 (1.1–66.8)	0.043
No exposure	1 (3)	18 (12)	32 (46)	Reference		Reference		Reference	
Living in/ stepped into mountainous areas	12 (40)	58 (38)	16 (23)	24.0 (2.9–201.2)	0.003	6.4 (2.9–14.3)	<0.001	3.7 (0.5–30.6)	0.162
Stepped into a bush	1 (3)	12 (8)	3 (4)	10.7 (0.5–217.2)	0.124	7.1 (1.8–28.6)	0.006	1.5 (0.1–26.4)	0.162
Farming	16 (53)	65 (42)	14 (20)	36.6 (4.4–303.4)	0.001	8.3 (3.6–18.7)	<0.001	4.4 (0.5–35.7)	0.162

*JSF, Japanese spotted fever; non-R, nonrickettsial diseases; OR, odds ratio; ST, scrub typhus.
†Non-R reference.
‡ST reference.

Because of the difficulties associated with locating patients to collect blood samples during the convalescent phase of illness, previous studies have relied on laboratory confirmation that uses acute-phase samples with variable cutoff IgM titers without considering local endemicity, which may have resulted in misclassification (*35*). In this study, we used the IFA or immunoperoxidase IgM titer of ≥80 as a cutoff for the diagnosis of

Table 2. Clinical characteristics of patients with JSF, ST, and nonrickettsial diseases, central Japan, 2004–2015*

Characteristic	JSF, no. (%), n = 31	ST, no. (%), n = 188	Non-R, no. (%), n = 97	JSF vs. non-R†		ST vs. non-R†		JSF vs. ST‡	
				OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Chief complaint									
Fever	26 (84)	135 (72)	78 (80)	1.3 (0.4–3.7)	0.668	0.6 (0.3–1.1)	0.115	2.0 (0.7–5.6)	0.165
Rash	14 (45)	52 (28)	17 (18)	3.9 (1.6–9.3)	0.003	1.8 (1.0–3.3)	0.06	2.2 (1.0–4.7)	0.053
Eschar	0 (0)	5 (3)	1 (1)	Not applicable	1.000§	2.6 (0.3–22.8)	0.382	Not applicable	1.000§
Headache	1 (3)	29 (15)	5 (5)	0.6 (0.1–5.5)	0.661	3.4 (1.3–9.0)	0.016	0.2 (0–1.4)	0.101
Fatigue	3 (10)	35 (19)	6 (6)	1.6 (0.4–6.9)	0.511	3.5 (1.4–8.6)	0.007	0.5 (0.1–1.6)	0.233
At history collection									
Fever	27 (87)	148 (82)	81 (84)	1.3 (0.4–4.1)	0.712	0.8 (0.4–1.6)	0.586	1.5 (0.5–4.6)	0.473
Rash	18 (60)	74 (44)	24 (26)	4.3 (1.8–10.1)	0.001	2.2 (1.3–3.8)	0.099	2.0 (0.9–4.3)	0.664
Eschar	1 (4)	20 (12)	5 (6)	0.6 (0.1–5.5)	0.664	2.1 (0.8–5.9)	0.141	0.3 (0–2.2)	0.232
Headache	4 (25)	75 (56)	27 (59)	0.2 (0.1–0.8)	0.026	0.9 (0.5–1.8)	0.748	0.3 (0.1–0.9)	0.026
Fatigue	17 (94)	97 (84)	32 (94)	1.1 (0.1–12.6)	0.962	0.3 (0.1–1.4)	0.138	3.3 (0.4–26.5)	0.256
Physical examination findings									
BT >37.5°C	23 (74)	132 (73)	53 (59)	2.0 (0.8–5.0)	0.132	1.9 (1.1–3.2)	0.02	1.1 (0.4–2.5)	0.883
Hypotension¶	8 (26)	12 (6)	5 (5)	6.4 (1.9–21.4)	0.003	1.3 (0.4–3.7)	0.679	5.1 (1.9–13.8)	0.001
Heart rate >120 bpm	2 (7)	13 (8)	6 (7)	0.9 (0.2–4.9)	0.942	1.2 (0.4–3.2)	0.777	0.8 (0.2–3.8)	0.793
Respiratory rate >20/min	13 (54)	40 (39)	23 (45)	1.4 (0.5–3.8)	0.464	0.8 (0.4–1.5)	0.457	1.9 (0.8–4.6)	0.174
Altered mental status	5 (16)	14 (7)	15 (15)	1.1 (0.3–3.2)	0.929	0.4 (0.2–1.0)	0.038	2.4 (0.8–7.2)	0.121
Rash	30 (100)	181 (96)	52 (57)	Not applicable	<0.001§	19.4 (8.2–45.9)	<0.001	Not applicable	0.597§
Localized	0	3 (2)	6 (12)	Not applicable	0.079§	0.1 (0–0.5)	0.004	Not applicable	1.000§
Purpura	11 (44)	4 (2)	7 (8)	8.9 (2.9– 26.8)	<0.001	0.2 (0.1–0.9)	0.028	36.1 (10.1–128.3)	<0.001
Palms/soles	21 (84)	13 (7)	4 (5)	101.1 (23.3– 438.4)	0.001	1.4 (0.5–4.6)	0.537	70.3 (21.0–235.3)	<0.001
Eschar	24 (89)	163 (87)	18 (22)	28.0 (7.6– 103.7)	<0.001	23.8 (12.1–46.8)	<0.001	1.2 (0.3–4.2)	0.801
Lung involv#	8 (26)	21 (11)	9 (9)	3.4 (1.2–9.8)	0.023	1.2 (0.5–2.8)	0.622	2.8 (1.1–7.0)	0.031

*BT, body temperature; involve, involvement; JSF, Japanese spotted fever; non-R, nonrickettsial diseases; OR, odds ratio; ST, scrub typhus.
†Non-R = reference.
‡ST = reference.
§Fisher exact tests.
¶Systolic blood pressure <90 mm Hg or vasopressor usage.
#Lung rales with pulmonary infiltrative shadow.

Table 3. Laboratory and treatment data for patients with JSF, ST, and nonrickettsial diseases, central Japan, 2004–2015*

Characteristic	JSF, no. (%), n = 31	ST, no. (%), n = 188	Non-R, no. (%), n = 97	JSF vs. non-R†		ST vs. non-R†		JSF vs. ST‡	
				OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Laboratory data									
Leukocytes >9,800/μL	5 (16)	23 (12)	42 (45)	0.2 (0.1–0.7)	0.006	0.2 (0.1–0.3)	<0.001	1.4 (0.5–3.9)	0.556
Hb <11 g/dL (F) or <13.5 g/dL (M)	5 (16)	29 (16)	46 (49)	0.2 (0.1–0.6)	0.002	0.2 (0.1–0.3)	<0.001	1.0 (0.4–2.9)	0.939
Platelets <130,000/μL	22 (71)	59 (32)	28 (30)	5.7 (2.3–13.9)	<0.001	1.1 (0.6–1.8)	0.806	5.3 (2.3–12.2)	<0.001
Albumin <3.4 g/dL	14 (61)	37 (29)	31 (55)	1.3 (0.5–3.4)	0.653	0.3 (0.2–0.6)	0.001	3.8 (1.5–9.6)	0.004
AST >33 IU/L	29 (94)	154 (83)	46 (50)	14.5 (3.3–64.3)	<0.001	4.8 (2.8–8.4)	<0.001	3.0 (0.7–13.3)	0.145
ALT >42 IU/L	16 (52)	100 (54)	42 (46)	1.3 (0.6–2.9)	0.566	1.4 (0.8–2.3)	0.204	0.9 (0.4–2.0)	0.824
LDH >229 IU/L	30 (97)	179 (97)	70 (78)	8.6 (1.1–66.8)	0.040	8.5 (3.3–22.1)	<0.001	1.0 (0.1–8.7)	0.996
Total bilirubin >1.0 mg/dL	9 (29)	13 (7)	15 (17)	2.0 (0.8–5.1)	0.166	0.4 (0.2–0.8)	0.016	5.2 (2.0–13.6)	0.001
Direct bilirubin >0.4 mg/dL	4 (22)	7 (5)	16 (28)	0.8 (0.2–2.6)	0.652	0.1 (0.1–0.4)	<0.001	5.2 (1.3–19.9)	0.017
Creatine kinase >150 IU/L	19 (66)	46 (29)	21 (28)	4.9 (2.0–12.2)	0.001	1.2 (0.6–1.9)	0.861	4.6 (2.0–10.7)	<0.001
BUN >22 mg/dL	15 (48)	35 (19)	20 (22)	3.4 (1.4–8.0)	0.006	0.8 (0.5–1.6)	0.58	4.0 (1.8–8.9)	0.001
Creatinine >1.2 mg/dL	11 (35)	22 (12)	7 (8)	6.7 (2.3–19.4)	<0.001	1.6 (0.7–4.0)	0.277	4.1 (1.7–9.6)	0.001
Sodium <135 mEq/L	24 (77)	71 (39)	16 (17)	16.3 (6.0–44.3)	<0.001	3.0 (1.6–5.6)	<0.001	5.4 (2.2–13.2)	<0.001
Chloride <98 mEq/L	17 (55)	37 (22)	15 (16)	6.2 (2.5–15.3)	<0.001	1.4 (0.7–2.7)	0.302	4.4 (2.0–9.7)	<0.001
CRP >10 mg/dL	16 (52)	32 (18)	34 (40)	1.6 (0.7–3.7)	0.266	0.3 (0.2–0.6)	<0.001	5.0 (2.2–11.1)	<0.001
Urine protein	27 (87)	116 (75)	34 (62)	4.2 (1.3–13.6)	0.018	1.8 (1.0–3.5)	0.068	2.3 (0.7–6.9)	0.148
Urine blood	29 (94)	122 (79)	31 (56)	11.2 (2.4–51.8)	0.002	2.9 (1.5–5.5)	0.002	3.9 (0.9–17.3)	0.071
Treatment and prognosis									
Duration of illness§ <5 d	16 (59)	74 (39)	24 (27)	4.0 (1.6–9.8)	0.002	1.8 (1.0–3.1)	0.039	2.2 (1.0–5.1)	0.054
Treatment: MINO/DOXY	31 (100)	180 (99)	42 (91)	Not applicable	0.144¶	17.1 (1.9–157.4)	0.012	Not applicable	1.000¶
Delayed defervescence#	11 (37)	17 (13)	30 (67)	0.3 (0.1–0.8)	0.012	0.1 (0–0.2)	<0.001	3.8 (1.5–9.3)	0.004
Hospitalization	28 (90)	104 (55)	80 (82)	2.0 (0.5–7.3)	0.302	0.3 (0.1–0.5)	<0.001	7.5 (2.2–25.7)	0.001
30-d mortality	0	1 (1)	2 (2)	Not applicable	1.000§	0.3 (0–3.0)	0.287	Not applicable	1.000¶

*ALT, alanine aminotransferase; AST, aspartate aminotransferase; BT, body temperature; BUN, blood urea nitrogen; CRP, C-reactive protein; DOXY, doxycycline; F, female patients; Hb, hemoglobin; JSF, Japanese spotted fever; LDH, lactate dehydrogenase; M, male patients; MINO, minocycline; non-R, nonrickettsial diseases; OR, odds ratio; ST, scrub typhus.
†Non-R = reference.
‡ST = reference.
§Duration from the onset of symptoms to the first diagnostic test.
¶Fisher exact tests.
#>3 d to decline of fever <37.3°C.

JSF and ST for patients for whom convalescent-phase samples were unavailable. To determine the optimum cutoff titer in our setting, we collected blood samples from patients with nonrickettsial diseases and confirmed that the highest IgM titer for *R. japonica* was <20 and that for *O. tsutsugamushi* was 10 (online Technical Appendix Table 2). Therefore, our diagnostic criteria must be very specific.

During the acute phase of the disease, sensitivity of the IFA is quite low; in our study, an elevated IgM titer by IFA was observed in the acute-phase samples of only 2 (6.5%) of 31 patients with JSF and 73 (38.8%) of 188 patients with ST. Hence, physicians may overlook these diseases if their diagnosis relies on IgM titer by IFA during the early phase. Furthermore, the ST serotypes affect the sensitivity of the IFA. In our study, of the 22 patients for whom serotype was identified, 16 serotypes were Irie/Kawasaki and 6 serotypes were Hirano/Kuroki. In Japan, health insurance covers IFAs for the standard serotypes Kato, Karp, and Gilliam only but not for serotypes Irie/Kawasaki and Hirano/Kuroki, which may not be cross-reactive to the standard serotypes (*22*). In our study population, use of IFAs to test for the standard serotypes could have led to underdiagnosis of ST for ≈5% of the patients because 2 patients with the Irie/Kawasaki and Hirano/Kuroki serotypes did not react to any of the standard serotypes.

Our study has limitations because we did not include the other 2 hospitals in the southern Boso Peninsula. However, our study sites are the only medical facilities in the district that have infectious disease specialists. Most patients with acute disease and fever in this district are expected to visit our clinic and hospitals. Thus, we believe that the effect of selection bias was minimal. Because our study is a combined prospective and retrospective case-series, the quality of the information may have differed between the prospectively and the retrospectively identified patients. However, we used an identical case definition throughout the study, and further analyses indicated that the clinical and epidemiologic characteristics did not differ between 2 groups (online Technical Appendix Table 3).

In conclusion, although JSF and ST share similar clinical features, in Japan the 2 diseases differ in seasonality, geographic distribution, physical signs, and severity. Patients with rickettsial diseases often do not notice their rash and eschar, and the sensitivity of the serologic test can be low during the acute phase of illness. A substantial number of rickettsial diseases may be underdiagnosed.

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About the Author

Dr. Sando is a general physician at Kameda Medical Center, Kamogawa, Japan, and a postgraduate at Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan. His primary research interests include rickettsial diseases such as ST and JSF.

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Address for correspondence: Motoi Suzuki, Nagasaki University, Department of Clinical Medicine, Institute of Tropical Medicine, Sakamoto 1-12-4, Nagasaki, 852-8523, Japan; email: mosuzuki@nagasaki-u.ac.jp

Systematic Review and Meta-analysis of Postexposure Prophylaxis for Crimean-Congo Hemorrhagic Fever Virus among Healthcare Workers

Önder Ergönül, Şiran Keske, Melis Gökçe Çeldir, İlayda Arjen Kara, Natalia Pshenichnaya, Gulzhan Abuova, Lucille Blumberg, Mehmet Gönen

We performed a systematic review and meta-analysis on the effectiveness of ribavirin use for the prevention of infection and death of healthcare workers exposed to patients with Crimean-Congo hemorrhagic fever virus (CCHFV) infection. Splashes with blood or bodily fluids (odds ratio [OR] 4.2), being a nurse or physician (OR 2.1), and treating patients who died from CCHFV infection (OR 3.8) were associated with healthcare workers acquiring CCHFV infection; 7% of the workers who received postexposure prophylaxis (PEP) with ribavirin and 89% of those who did not became infected. PEP with ribavirin reduced the odds of infection (OR 0.01, 95% CI 0–0.03), and ribavirin use ≤48 hours after symptom onset reduced the odds of death (OR 0.03, 95% CI 0–0.58). The odds of death increased 2.4-fold every day without ribavirin treatment. Ribavirin should be recommended as PEP and early treatment for workers at medium-to-high risk for CCHFV infection.

Crimean-Congo hemorrhagic fever (CCHF) virus (CCHFV) is listed as a highly infectious pathogen that could cause a public health emergency (http://www.who.int/medicines/ebola-treatment/WHO-list-of-top-emerging-diseases/en/). CCHFV infection has been reported from >30 countries in Africa, Asia, Europe, and the Middle East (*1*,*2*). CCHFV is a member of the genus *Orthonairovirus* in the family *Nairoviridae* that causes severe disease in humans; the reported case fatality rate is 3%–30% (*1*). Humans can become infected through the bites of ticks, by contact with patient blood or bodily fluids, or by contact with blood or tissues from viremic livestock. The risk for human-to-human transmission of CCHFV increases in parallel with the lack of preparedness (*3*).

Healthcare workers need to be well prepared against the emerging threat of CCHF outbreaks. The efficacy of postexposure prophylaxis (PEP) with ribavirin for high-risk exposures to CCHFV needs clear evidence. The relatively low secondary attack rates of CCHFV and ethics constraints make controlled, prospective efficacy trials for ribavirin PEP unlikely. In the absence of efficacy studies, a thorough examination and logical extrapolation of the existing data can be useful for developing recommendations. The efficacy of PEP for healthcare workers exposed to CCHF patients might be similar to that for healthcare workers with high-risk exposures to Lassa fever patients (*4*). A series of cases of healthcare workers infected with CCHFV has been reported (*5*–*10*). Integration of the details on the exposures and the outcomes of the infections from these published reports could provide the opportunity to generate powerful conclusions about the risk for infection and death among healthcare workers. We described the efficacy of PEP with ribavirin for CCHFV infection and early ribavirin use in CCHF treatment.

Methods

Search Strategy

We performed a systematic review of individual participant data (IPD) and reported data by using PRISMA-IPD (Preferred Reporting Items for Systematic Reviews and Meta-Analyses for IPD) guidelines (*11*). We searched PubMed, Google, ProMED, and conference proceedings by using the keywords “Crimea-Congo hemorrhagic fever,” “health care worker,” “nosocomial,” “CCHF,” and “health professional.” We included all published reports in peer-reviewed journals through September 3, 2017.

Author affiliations: Koç University, Istanbul, Turkey (Ö. Ergönül, M.G. Çeldir, İ.A. Kara, M. Gönen); American Hospital, Istanbul (Ş. Keske); Rostov State Medical University, Rostov-on-Don, Russia (N. Pshenichnaya); South-Kazakhstan State Pharmaceutical Academy, Shymkent, Kazakhstan (G. Abuova); National Institute for Communicable Diseases, Johannesburg, South Africa (L. Blumberg)

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Definitions and Outcome of Interest

We defined CCHFV exposure as visible contact or imperceptible contact (i.e., contact with patient aerosols) with a CCHF patient. Primary outcomes were infection with no symptoms, infection with symptoms, and death. The primary study objective was to assess the protective role of PEP and early ribavirin treatment. Early treatment was defined as treatment occurring ≤48 hours after the onset of symptoms.

Exposure Risk Groups

Healthcare workers were grouped into 3 categories with respect to their risks for CCHFV infection. The high-risk group consisted of healthcare workers who were directly exposed to blood or bodily fluids of a CCHFV-infected patient, such as through a needle stick or splash. Healthcare workers in this group were categorized as being without personal protective equipment (PPE) of any sort by default because a PPE breach had occurred. The moderate-risk group comprised healthcare workers without a known direct exposure to blood or bodily fluids of a CCHFV-infected patient but either handled patients who bleed or visibly produced other body fluids or were involved in aerosol-producing procedures (e.g., intubation, bronchoscopy, and resuscitation) without wearing an N95 mask. The low- or unknown-risk group consisted of healthcare workers who cared for CCHFV-infected patients who did not actively bleed or produce bodily fluids and did not participate in aerosol-producing procedures.

Inclusion and Exclusion Criteria

In this study, we included healthcare workers who were exposed to CCHFV through a defined transmission event who did and did not receive PEP, healthcare workers with laboratory-confirmed CCHFV infections who had a detailed exposure history and were closely followed by laboratory tests for their clinical outcomes, and symptomatic healthcare workers who did and did not receive ribavirin ≤48 hours after the onset of symptoms. In the reports from Albania (*12*,*13*), Pakistan (*14*,*15*), South Africa (*9*,*16*,*17*), and India (*18*,*19*), some of the cases were duplicates (included in >1 article). In these instances, we avoided duplicated data and selected the case information from the earlier publication for inclusion. We did not include seroprevalence studies, gray literature, or screening reports for tracing cases that did not have defined exposures; if the information was incomplete or lacking, we requested the information directly from the authors, and we did not include the articles if the data we needed were unavailable.

Data Collection

We entered IPD obtained from reports into a structured data sheet. We performed analyses using an integrated dataset in Stata version 11 (https://www.stata.com/). In our dataset, we included information on demographics, transmission, PEP, the course of infection, the number of days from onset of disease, and treatment. The dataset also included information on the predictors of infection and death. Study authors (Ö.E., Ş.K., M.G.Ç., and İ.A.K.) resolved discrepancies during discussions with local physicians.

Statistical Analysis

We followed the PRISMA-IPD statement guidelines (*11*) using R studio (https://www.rstudio.com/). We used a 2-stage approach for analyses. First, we analyzed the studies that were suitable for calculating an effect estimate (odds ratio [OR]). Then, we pooled all the participant studies, including single case reports, and calculated a common effect estimate (OR) and 95% CI. We used random effects models.

Bias Assessment

We performed an analysis for confounders with the integrated dataset. We used the χ^2 test for categorical data and *t*-test for continuous data and performed logistic regression to detect potential confounding predictors of infection and death. To predict infection, we included in our model the covariates PEP with ribavirin, being in the high-risk group, being a nurse or physician, and providing care for a CCHF patient who died. To predict death, we included in our model the covariates days from onset of symptoms to ribavirin treatment, being in the high-risk group, and being a nurse or physician. These were the most critical variables for predicting death. In statistical analyses, we used Stata version 11, and we considered p values <0.05 statistically significant. For meta-analysis, we used meta: General Package for Meta-Analysis version 4.9-1 (https://cran.r-project.org/web/packages/meta/index.html).

Results

We reviewed 1,224 published reports on CCHF, and 33 studies met our inclusion criteria (Figure 1). In the included studies, 175 healthcare workers from Turkey (*5*,*7*,*20*–*25*), Pakistan (*15*,*26*–*32*), Germany (*6*), Iran (*33*–*36*), India (*18*,*19*), South Africa (*9*), Russia (*8*), Tajikistan (*37*), United Arab Emirates (*38*), Mauritania (*39*), Kazakhstan (*40*), Sudan (*41*,*42*), Albania (*12*), and Spain (*2*) were exposed to patients infected with CCHFV (Table). We included all of the healthcare workers who were infected, but because of the lack of detailed exposure histories among those who were not infected, we excluded 47 healthcare workers from Tajikistan (*37*), 75 from Turkey (*5*,*10*), 40 from Germany (*6*), and 33 from Pakistan (*15*,*30*). The diagnoses were based on reverse transcription PCR results for 58 (57%) healthcare workers, ELISA for 47 (46%), both ELISA and reverse transcription PCR

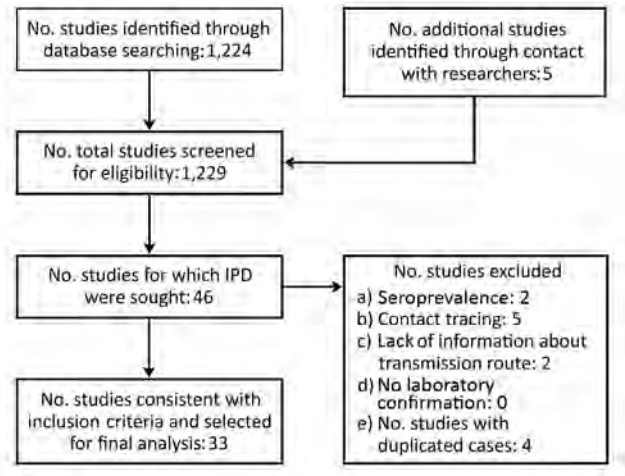

Figure 1. Identification and selection of studies included in a meta-analysis of the effectiveness of postexposure prophylaxis with ribavirin and early treatment with ribavirin among healthcare workers exposed to patients infected with Crimean-Congo hemorrhagic fever virus, 1976–2017. IPD, individual participant data.

for 26 (25%), immunofluorescence assay for 13 (12%), and complement fixation for 10 (10%).

The population of healthcare workers included in our study was 53% male and 47% female. The percentages of infected male and female healthcare workers did not differ (p = 0.828), and the percentage of symptomatic male (38%) and female (29%) healthcare workers who died was not significantly different (p = 0.413). Among symptomatic healthcare workers, the mean age was 33 (SD 8.5, range 20–61) years, and the case-fatality rate was 34%. The percentage of symptomatic cases did not differ from the percentage of asymptomatic cases (p = 0.545), and the case-fatality rate was not higher in symptomatic than asymptomatic healthcare workers (p = 0.674). Being a nurse or physician (p = 0.01) and caring for a CCHF patient who died (94% of infected healthcare workers vs. 80% of non-infected healthcare workers; p = 0.006) were factors more common among healthcare workers who were infected than those who were not.

We performed 2 meta-analyses: 1 on the effectiveness of PEP with ribavirin to prevent CCHFV infection (Figure 2, panel A) and 1 on the effectiveness of early ribavirin treatment after CCHF symptom onset to prevent death (Figure 2, panel B). In the first stage of the meta-analysis on PEP, the OR could be calculated for only 4 studies (OR 0.05, 95% CI 0.01–0.26); at the second stage, after pooling all healthcare worker study data, the OR was 0.01 (95% CI 0–0.03; Figure 2, panel A). The heterogeneity of these studies was not significant ($I^2 = 3\%$, $\Gamma^2 = 0.1157$; p = 0.38). During the first stage of the meta-analysis on early ribavirin use, the OR could be calculated for only 2 studies (OR 0.04, 95% CI 0–1.33); at the second stage of the analysis, after pooling all healthcare worker study data, the OR was 0.03 (95% CI 0–0.58; Figure 2, panel B). No heterogeneity was detected among these studies ($I^2 = 0\%$, $\Gamma^2 = 0$; p = 0.92).

In univariate analyses of healthcare workers exposed to CCHF patients, splashes with blood or bodily fluids (OR 4.2, 95% CI 2.04–9.7; p<0.001), being a nurse or physician (OR 2.1, 95% CI 1.13–4.1; p = 0.019), and caring for a patient who died (OR 3.8, 95% CI 1.38–10.46; p = 0.01) significantly increased the odds of a healthcare worker acquiring an infection. However, PEP with ribavirin significantly reduced the risk for CCHFV infection (OR 0.01, 95% CI 0.003–0.03; p<0.001). To control for confounders, we performed a multivariate analysis of the dataset. In multivariate analyses of exposed healthcare workers, PEP with ribavirin was found to significantly protect against CCHFV infection (OR 0.009, 95% CI 0.001–0.039; p<0.001). In a sensitivity analysis, the area under the receiver operating curve was 94%. In a multivariate analysis of symptomatic healthcare workers adjusted by risk group (high risk vs. others) and worker type (nurse or physician vs. others), the

Table. Characteristics and outcomes for healthcare workers exposed to patients with Crimean-Congo hemorrhagic fever virus infection, 1976–2017

	No. (%)					
Country (references)	Exposed, N = 175	High risk, n = 107	Moderate risk, n = 65	Low or no known risk, n = 3	Infected, n = 102	Died, n = 34
Turkey (*5*,*7*,*20*–*25*)	49 (28)	23 (22)	26 (40)	0	19 (19)	3 (9)
Pakistan (*15*,*26*–*32*)	45 (26)	21 (20)	24 (36)	0	18 (18)	6 (18)
Germany (*6*)	18 (10)	18 (17)	0	0	2 (2)	0
Iran (*33*–*36*)	12 (7)	10 (9)	1 (2)	1 (33)	12 (12)	3 (9)
India (*18*,*19*)	8 (5)	5 (5)	3 (5)	0	8 (8)	6 (18)
Russia (*8*)	8 (5)	6 (6)	0	2 (67)	8 (8)	0
South Africa (*9*)	8 (5)	3 (3)	5 (8)	0	8 (8)	2 (6)
Tajikistan (*37*)	7 (4)	7 (7)	0	0	7 (7)	2 (6)
United Arab Emirates (*38*)	5 (3)	1 (1)	4 (6)	0	5 (5)	2 (6)
Kazakhstan (*40*)	5 (3)	3 (3)	2 (3)	0	5 (5)	3 (9)
Mauritania (*39*)	5 (3)	5 (5)	0	0	5 (5)	5 (15)
Sudan (*41*,*42*)	3 (2)	2 (2)	1 (2)	0	3 (3)	2 (6)
Albania (*12*)	1 (1)	1 (1)	0	0	1 (1)	0
Spain (*2*)	1 (1)	(1)	0	0	1 (1)	0

risk for death increased 2.4-fold for every day of delay in the start of ribavirin treatment (OR 2.4, 95% CI 1.27–4.56; p = 0.005). Appropriate use of PPE and PEP with ribavirin predicted death completely; therefore, both of these factors were not included in the model. The sensitivity of this model, calculated by the area under the receiver operating curve, was 95%.

Of 175 healthcare workers exposed to CCHF patients, 55 (31%) received and 110 (63%) did not receive PEP with ribavirin (Figure 3). In the PEP arm, 7% acquired infection, and in the no PEP arm, 89% acquired infection (p<0.001; Figure 3). In the no PEP arm, 97 (99%) of 98 infected healthcare workers became symptomatic. None of the symptomatic healthcare workers who received ribavirin within 48 hours after the onset of symptoms died, whereas 42% of the symptomatic healthcare workers who did not receive ribavirin within 48 hours died (p<0.001; Figure 3). Among symptomatic healthcare workers who received ribavirin, the median time from onset of symptoms to ribavirin treatment was 5 days for those who died and 1.25 days for those who survived (p<0.001).

For 104 (59.4%) of 175 healthcare workers, the appropriateness of the PPE was assessed by the authors of the original report. The percentage who became infected was lower for those who used PPE appropriately (55%) than those who did not (70%), although this difference was not significant (p = 0.301). No fatal cases were reported among those who used PPE appropriately. In all reports, the PPE used included a mask, gloves, and a gown; in 1 study in Germany (*6*), the additional use of goggles was reported.

Discussion

We analyzed all published reports on healthcare workers who were exposed to CCHF patients and had a moderate-to-high risk of acquiring a CCHFV infection. These cases represent the case density in the 14 countries included, in parallel with previous reports (*43*,*44*). Most cases were from Turkey, Pakistan, and Iran. However, healthcare workers could acquire the infection from persons from other countries (*6*), and in 2017, a nurse in Spain acquired (*2*) a CCHFV infection from a patient with an autochthonous case.

We determined that PEP with ribavirin reduced CCHFV infection among healthcare workers and early ribavirin use reduced death among CCHFV-infected healthcare workers (Figure 2). In most case series and case reports, no healthcare workers died who received PEP with ribavirin (*5*–*8*,*10*), including those who received PEP soon after a high-risk incident (Figure 3).

Early use of ribavirin in the treatment of CCHFV infection has been reported as beneficial (*45*,*46*) and is considered to be beneficial, despite a controversial report (*47*). In the report in which authors disagreed with ribavirin use being beneficial, the authors did not account for the starting time of ribavirin treatment after symptom onset,

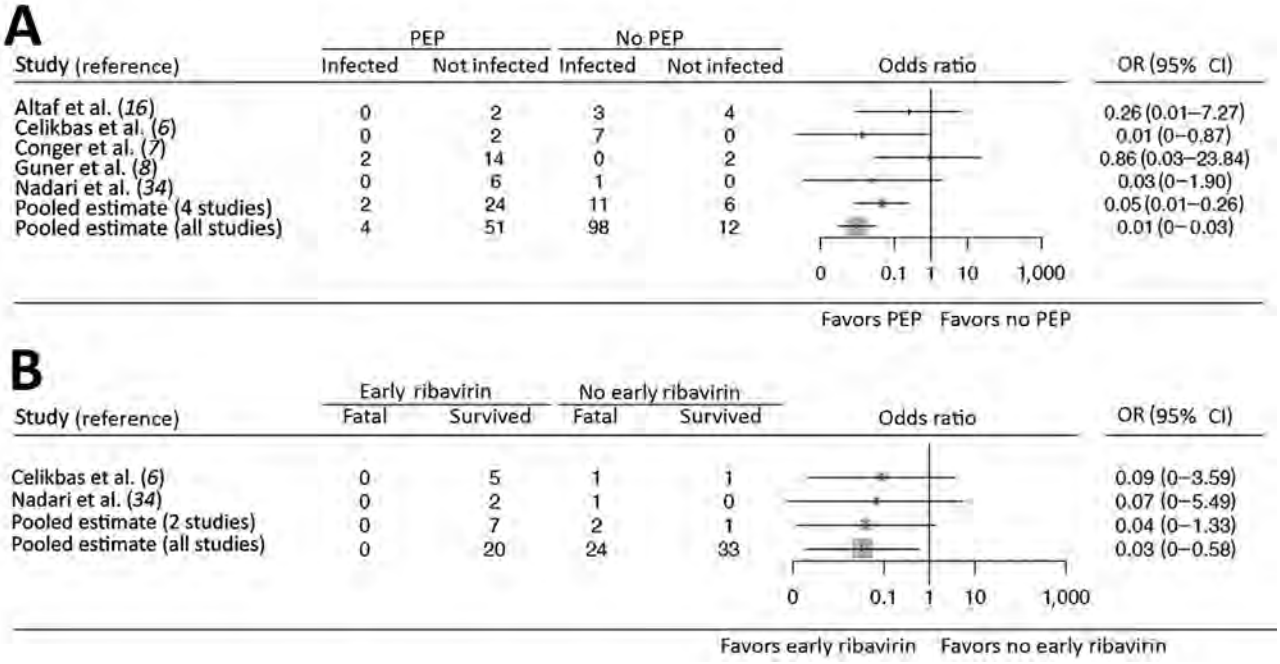

Figure 2. Effectiveness of PEP and early treatment with ribavirin among healthcare workers exposed to patients infected with Crimean-Congo hemorrhagic fever virus, 1976–2017. A) Two-step meta-analysis of the effectiveness of PEP with ribavirin for preventing Crimean-Congo hemorrhagic fever virus infection. We could determine the effect estimates for only 4 individual studies, and we included 33 reports in the final pooled estimate. B) Two-step meta-analysis on the effectiveness of early ribavirin use for preventing death caused by Crimean-Congo hemorrhagic fever virus infection. We could determine the effect estimate for only 2 individual studies, and we included 33 reports in the final pooled estimate. OR, odds ratio; PEP, postexposure prophylaxis.

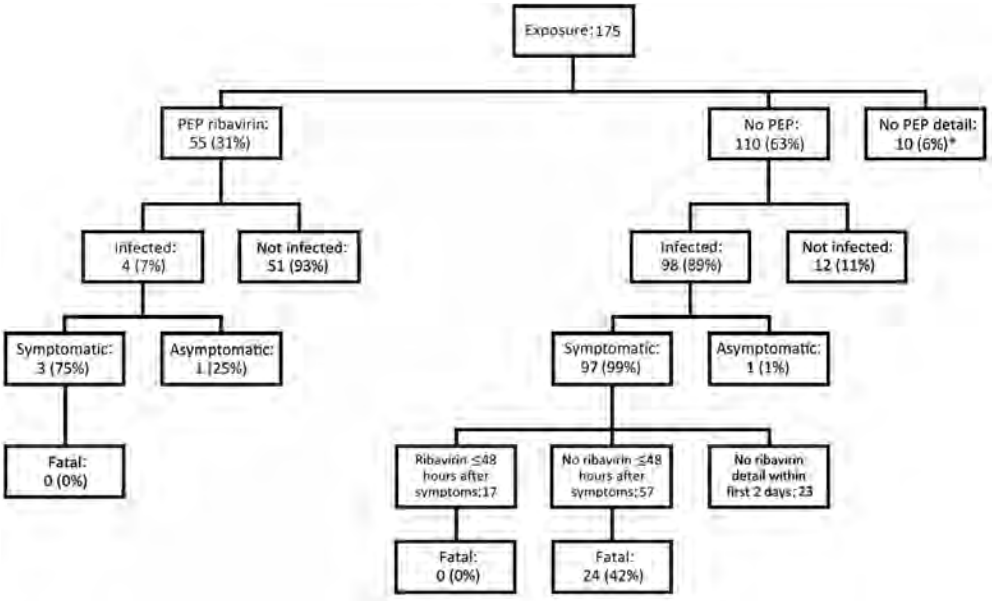

Figure 3. Flowchart of healthcare workers exposed to patients infected with Crimean-Congo hemorrhagic fever virus who did and did not receive PEP with ribavirin or early ribavirin treatment ≤48 hours after symptom onset, 1976–2017. *Healthcare workers for which PEP information was not included in the original report. PEP, postexposure prophylaxis.

even though this factor can significantly alter its efficacy. Close follow-up of infected healthcare workers provides an opportunity to determine the efficacy of early ribavirin use; assessing the quality of treatments given to exposed healthcare workers is much more feasible than assessing that of patients with suspected CCHF transferred from rural areas. In this study, we showed that every day of treatment delay increases the risk for death by 2.4-fold. Of note, none of the symptomatic healthcare workers who received ribavirin within 48 hours after the onset of symptoms died, whereas 42% of those who did not receive treatment in that time frame died ($p<0.001$; Figure 3). Late diagnosis of the source case can result in delayed PEP and treatment of healthcare workers with ribavirin (*21*,*30*).

Some centers have reported aerosol CCHFV transmission (*5*–*8*,*33*,*48*). A number of procedures (e.g., bronchoscopy, nasal tamponade, intubation, cardiopulmonary resuscitation) as well as patient bleeding can lead to the aerosolization of CCHFV. Persons near CCHFV patients during these activities should be considered at moderate risk for acquiring the infection. Awareness of transmission after percutaneous injuries is high, but healthcare workers with imperceptible exposures to aerosolized pathogens should also be considered for close follow-up. Our study findings indicate that PEP with ribavirin should be recommended for those with CCHFV exposures, similar to the recommendations for healthcare workers with Lassa virus exposures (*4*).

In this study, we included all published reports of detailed, laboratory-confirmed cases; we avoided duplicated cases and excluded screenings of healthcare workers with nonspecified risk (*5*,*6*,*15*,*28*,*49*,*50*). One limitation of this study is reporting bias; we did not include unreported cases. Because of medical and legal issues, some fatal cases involving healthcare workers who were not using PPE appropriately or who did not receive PEP might not have been reported. For example, 2 fatal cases involving healthcare workers who were not given ribavirin, 1 from Turkey (http://www.hurriyet.com.tr/gundem/kan-alirken-eline-igne-batan-kubra-kkka-dan-oldu-11861967) and 1 from Pakistan (https://www.samaa.tv/uncategorized/2016/07/congo-fever-cases-emerge-in-bahawalpur/), were not published in the literature, but their stories appeared in the media. Even though we received detailed information about these cases, we did not include them in our study because they were not published in peer-reviewed journals. These unreported fatal cases support the use of ribavirin for PEP and early treatment, as we recommend in this report.

Another limitation of this study was regarding the reporting of the appropriateness of the PPE used, which was reported for only ≈60% of the healthcare workers included. PPE use is not standardized; appropriate use varies substantially from country to country. For instance, in a study in Germany, the use of surgical masks instead of N95 masks during aerosol-generating procedures is considered inappropriate (*6*); however, this practice was considered appropriate in many other studies. Implementing standard use of PPE in healthcare settings is urgently needed. Our study shows that N95 masks should be used in high- and moderate-risk events, including those involving contact with patients who are bleeding or visibly generating bodily fluids or aerosols.

Our analyses were performed by using an integrated dataset that included all necessary detailed information about the demographics, transmission, PEP, course of the infection, days from onset of disease, and treatment. This dataset could be supplied to researchers in the field and used as a tool for future investigations.

In conclusion, our results indicate a significant beneficial effect of PEP with ribavirin after CCHFV exposure. This beneficial effect extended to early use of ribavirin for treatment of infected healthcare workers. Imperceptible contact with infectious particles and splashes of blood or bodily fluids from infected patients should all be considered and prevented. A universal standard of care that includes PPE and PEP and treatment with ribavirin should be implemented for healthcare workers at risk for CCHF.

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About the Author

Dr. Ergönül is professor of infectious disease and clinical microbiology at Koç University School of Medicine in Istanbul, Turkey. His primary research interests include emerging infections.

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Address for correspondence: Önder Ergönül, Koç University, School of Medicine, Infectious Diseases Department, Davutpaşa Caddesi, No. 4, 34010 Topkapi, Istanbul, Turkey; email: oergonul@ku.edu.tr

Event-Based Surveillance at Community and Healthcare Facilities, Vietnam, 2016–2017

Alexey Clara,[1] Trang T. Do,[1] Anh T.P. Dao, Phu D. Tran, Tan Q. Dang, Quang D. Tran, Nghia D. Ngu, Tu H. Ngo, Hung C. Phan, Thuy T.P. Nguyen, Anh T. Lai, Dung T. Nguyen, My K. Nguyen, Hieu T.M. Nguyen, Steven Becknell, Christina Bernadotte, Huyen T. Nguyen, Quoc C. Nguyen, Anthony W. Mounts, S. Arunmozhi Balajee

Surveillance and outbreak reporting systems in Vietnam required improvements to function effectively as early warning and response systems. Accordingly, the Ministry of Health of Vietnam, in collaboration with the US Centers for Disease Control and Prevention, launched a pilot project in 2016 focusing on community and hospital event–based surveillance. The pilot was implemented in 4 of Vietnam's 63 provinces. The pilot demonstrated that event-based surveillance resulted in early detection and reporting of outbreaks, improved collaboration between the healthcare facilities and preventive sectors of the ministry, and increased community participation in surveillance and reporting.

After several international outbreaks of infectious diseases, including severe acute respiratory syndrome in 2003, all World Health Organization (WHO) Member States, including Vietnam, agreed to comply with the revised International Health Regulations 2005 (IHR 2005) to ensure global health security (*1*). The IHR 2005 requires countries to develop early warning and response functions that can rapidly detect, report, and respond to—and thereby control—public health events. WHO defines early warning and response as "the organized mechanism to detect as early as possible any abnormal occurrence or any divergence from the usual or normally observed frequency of phenomena" (*2*). Two complementary types of surveillance form the foundation of a functional early warning and response: indicator-based surveillance (IBS) and event-based surveillance (EBS) (*2*,*3*).

In Vietnam, IBS is mandated by Circular 54, a Ministry of Health regulation, disseminated in 2015 (*4*,*5*). Circular 54 focuses primarily on reporting of case-based hospital admissions through an electronic system, the eCDS (electronic Communicable Disease Surveillance software). Several disease- or syndrome-specific sentinel surveillance programs complement eCDS, focusing on conditions such as dengue; hand, foot, and mouth disease; Japanese encephalitis virus; influenza-like illness; and severe acute respiratory infections.

WHO defines EBS as the organized collection, monitoring, assessment, and interpretation of mostly unstructured information from diverse ad hoc sources, including communities, schools, and media. Signals may represent unusual disease patterns that signify early signs of an outbreak or event (*2*,*6*). Both IBS and EBS generate signals, which might consist of reports of cases or deaths (individual or aggregated); potential human exposure to biological, chemical, or radiologic hazards; or occurrence of natural or human-made disasters. These signals, which are unfiltered reports, are first triaged and verified to confirm the occurrence of a true event that needs further investigation. Decision 134/QD-DP, issued in 2014 by Vietnam's Ministry of Health's General Department of Preventive Medicine (GDPM), describes national EBS procedures but is largely focused on signal identification through media scanning and omits collection of information from other sources, such as pharmacies, animal and agricultural sectors, community, workplaces, the private sector, and schools (*7*).

Author affiliations: Centers for Disease Control and Prevention, Atlanta, Georgia, USA (A. Clara, T.T. Do, A.T.P. Dao, S. Becknell, A.W. Mounts, S.A. Balajee); General Department of Preventive Medicine, Hanoi, Vietnam (P.D. Tran, T.Q. Dang, Q.D. Tran); National Institute of Hygiene and Epidemiology, Hanoi (N.D. Ngu, T.H. Ngo); Pasteur Institute, Ho Chi Minh City, Vietnam (H.C. Phan, T.T.P. Nguyen); Preventive Medicine Center, Nam Dinh, Vietnam (A.T. Lai); Preventive Medicine Center, Quang Ninh, Vietnam (D.T. Nguyen); Preventive Medicine Center, An Giang, Vietnam (M.K. Nguyen); Preventive Medicine Center, Ba Ria-Vung Tau, Vietnam (H.T.M. Nguyen); Program for Appropriate Technology in Health, Seattle, Washington, USA (C. Bernadotte, H.T. Nguyen, Q.C. Nguyen)

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[1]These authors contributed equally to this article.

Regional Institutes in each of Vietnam's 4 administrative health regions are responsible for implementing and overseeing surveillance and response. Within each region, Provincial Preventive Medicine Centers (PPMCs) lead these activities within their jurisdictions, involving the Regional Institutes for larger events. The PPMCs are supported by 2 lower administrative levels, the District Health Center (DHC) and Commune Health Station (CHS). The CHS is generally staffed by a medical professional and village health workers (VHWs), who are largely volunteers. The VHWs promote prenatal visits and vaccinations and in theory are responsible for reporting outbreaks from their communities. In addition, several community members called health collaborators assist VHWs in these tasks.

A 2014 assessment of Vietnam's surveillance and reporting structures by a joint Ministry of Health and US Centers for Disease Control and Prevention (CDC) team found that the existing surveillance was largely IBS with reliance on healthcare facility (HCF) reporting that was case-based. HCFs were not required to report unusual patterns of unknown diseases, resulting in delays in detection of outbreaks and events caused by emerging pathogens (*5*). In addition, the team found that VHWs were underutilized and not actively engaged with detection and reporting of suspected outbreaks from their communities. Finally, the team found no alert thresholds established for routinely reported HCF data for many endemic seasonal diseases, such as dengue or hand, foot, and mouth disease.

To complement and reinforce the surveillance system, the GDPM in collaboration with CDC launched an EBS pilot project in 2016 focusing on communities and HCFs, including hospitals. Community EBS entailed reporting symptoms and unusual patterns that do not require specialized healthcare training from the communities by VHWs, health collaborators, and key informants. HCF EBS required healthcare workers to recognize and report unusual occurrences or disease patterns, such as a surge in admissions or healthcare worker sickness after patient exposure with similar illness.

For phase 1 implementation, GDPM selected the National Institute of Hygiene and Epidemiology and the Pasteur Institute of Ho Chi Minh City, the 2 larger Regional Institutes, and worked with them to select 2 pilot provinces per region. Criteria used to select provinces included support from the local government; availability of personnel for response; and previous occurrence of diseases of high concern, such as avian influenza. For phase 2, the intention was to pilot in 2 remaining Regional Institutes, including 2 provinces within their jurisdictions. Phase 1 of the pilot was implemented in 4 of Vietnam's 63 provinces. We describe the steps of phase 1 implementation and its preliminary assessment results.

Methods

Establishing a Technical Working Group for EBS

The GDPM formed an EBS Technical Working Group (TWG) consisting of stakeholders from the Ministry of Health, including the 2 Regional Institutes, PATH (an international organization), CDC, WHO, and technical staff from the pilot province PPMC. In addition to guiding the EBS planning and preparations, the TWG served as the advisory group for implementation throughout the project. TWG members also served on an assessment team and later assisted in disseminating the assessment results to stakeholders.

EBS signals do not need to be disease specific. However, to reduce the background noise and to provide a framework for reporting, the TWG listed priority diseases and conditions that were important for early detection in Vietnam. Criteria for inclusion included diseases that 1) have large public health impact in the country, 2) are outbreak prone and pose a major public health threat, 3) have previously been prevalent and might reemerge, and 4) are slated for eradication or elimination. High-priority diseases identified were rabies, avian influenza, vaccine-preventable diseases, cholera, and emerging new diseases.

The TWG then drafted a list of signals that could serve as an early indication of the appearance of these priority diseases in the community. Community signals represented constellations of symptoms and patterns that do not require specialized healthcare training; signals aimed at HCF were based on unusual occurrences and/or disease patterns, such as surge in admissions.

The TWG drafted an Interim Technical Implementation Guideline and training materials (*8*). Other materials included posters and flyers to increase community awareness of the signals and need to report, notebooks for VHWs with printed signals and pages for notes, logbooks for recording signals, and a monitoring checklist for supervisory visits at each administrative level.

Training the Public Health Workforce in EBS

A training of trainers workshop was conducted for the Regional Institutes and pilot provinces. These participants became master trainers and led cascade trainings in each province down to the commune level. At each level, a trainer from a higher administrative level provided mentorship and support.

Resources for Implementation Support

In addition to external funding for training, each province received a one-time start-up grant for infrastructural improvements, including purchase of a limited number of computers for reporting, a one-time allowance for VHW

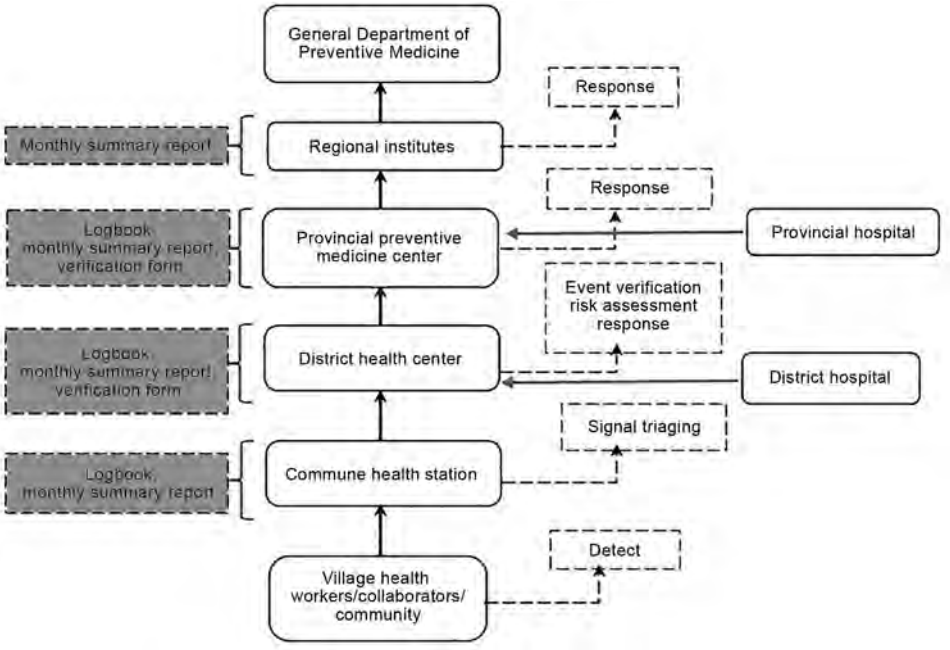

Figure 1. Existing surveillance and reporting system improved for event-based surveillance, Vietnam, September 2016–May 2017. Enhancements are shown in dashed boxes; the reporting tools at each level are shown in gray dashed boxes.

cellular phone minutes, and the printing and distribution of logbooks and communication materials. During the pilot phase, EBS district and provincial focal points received a small monthly honorarium for EBS oversight and support.

Enhancing Existing Information Flow and EBS Reporting

For EBS, the existing organizational structure and information flow from CHS to DHC to PPMC and to Regional Institutes was maintained with some enhancements (Figure 1), including 1) inclusion of VHWs at the CHS to identify and report signals; 2) addition of a triage step (the CHS decided which signals were "true" signals [rather than a spurious situation or nonthreatening rumor] before reporting these as events to DHCs); 3) training of DHCs and PPMCs in event verification and risk assessment; 4) distribution of logbooks for recording signals and events; 5) establishment of a requirement to immediately report events by phone call, in-person meeting, or email; and 6) training of healthcare providers to detect and immediately report signals to the correct public health unit.

Assessing the EBS Pilot

Approximately 9 months after launch, the TWG assessed the EBS pilot, with qualitative and quantitative methods, for timeliness of detection and reporting of events, as well as EBS acceptability and sustainability at all levels. This assessment included 1) a retrospective data collection table sent electronically to all districts to collect logbook time stamps for event notification and response, 2) questionnaires sent electronically to all levels with acceptability and sustainability related questions, and 3) key informant interviews and focus group discussions through field visits.

We used 3 criteria to select field visit sites. First, we assessed districts that were performing optimally and

Table 1. General characteristics of selected provinces in the pilot of event-based surveillance, Vietnam, September 2016–May 2017

	Province			
	North		South	
Demographic and administrative profile	Quang Ninh	Nam Dinh	Ba-Ria Vung Tau	An Giang
Demographics				
Population	1,211,300	1,850,600	1,072,600	2,158,300
Population density, persons/km^2	198	1119	539	610
Urban population rate, %	62.5	18	50.1	31.1
No. households	316,732	555,605	256,336	524,759
Administrative division no.				
Cities under provinces	4	1	2	2
District-level towns	2	0	0	1
Rural districts	8	9	6	8
Wards	67	20	24	21
Commune-level towns (townlets)	8	15	7	16
Commune Health Station	111	194	51	119

Figure 2. Provinces participating in event-based surveillance pilot project (stars), Vietnam, September 2016–May 2017.

suboptimally as defined by the metric signal incidence rate. Signal incidence rates were the number of signals detected from each district, adjusted by the district's population and the number of days engaged in signal reporting. We defined optimal performance as districts with a signal incidence rate higher than the 50th percentile and suboptimal performance districts as districts with a signal incidence rate of the 50th percentile or lower. Second, we selected districts that investigated public health events reported through EBS that could be useful case studies. Third, we selected sites that were willing to receive assessors.

We sent the time stamp data extraction form to all 43 pilot districts. Approximately 7,000 participants encompassing EBS focal points and volunteers at all levels of the workforce in all 4 provinces received the acceptability/sustainability survey. In each province, 2 districts and 2 CHSs per district were selected for site visits and key informant interviews/focus group discussions deployment.

Results

The EBS pilot covered 7% of the total population of Vietnam (9). The provinces represented both rural and urban areas (Table 1; Figure 2).

Resources and EBS Workforce

Twenty-four master trainers were trained in August 2016: two from each province and 16 GDPM and Regional Institute staff. A cascade training to lower administrative levels followed the master training. By October 2016, >7,000 persons in 4 provinces were trained to detect, record, and report signals and events, and 52 DHC staff were trained in basic risk assessment. Staff from every district, CHS, and public hospital within each province were trained, achieving 100% training coverage (Table 2).

At least 15,000 posters with community signals and reporting information were provided to CHSs (Figure 3). These posters were prominently displayed at public meeting places, CHS, village meetings, and other highly visible locations. In addition, 1,300 logbooks and 703,000 leaflets for the community were distributed (Table 3).

Table 2. Number of persons trained in the pilot provinces, Vietnam, September 2016–May 2017*

		North			South			
	National level,		Province		RI,	Province		
Type of training	GDPM	RI, NIHE	Quang Ninh	Nam Dinh	PI-HCMC	BRVT	An Giang	Total
Training of trainers	4	6	2	2	6	2	2	24
Cascade								
Hospital	NA	NA	17	13	NA	8	14	52
District	NA	NA	42	30	NA	24	33	129
CHS	NA	NA	186	229	NA	82	156	653
VHWs/HCs	NA	NA	1,768	3,801	NA	710	888	7167
Total	4	6	2,015	4,075	6	826	1,093	8,025

*BRVT, Ba Ria-Vung Tau; CHS, Commune Health Station; GDPM, General Department of Preventive Medicine, Vietnam Ministry of Health; NIHE, National Institute of Hygiene and Epidemiology, Hanoi, Vietnam; PI-HCMC, Pasteur Institute, Ho Chi Minh City, Vietnam; RI, Regional Institute; VHWs/HCs, village health workers/health collaborators; NA, not applicable.

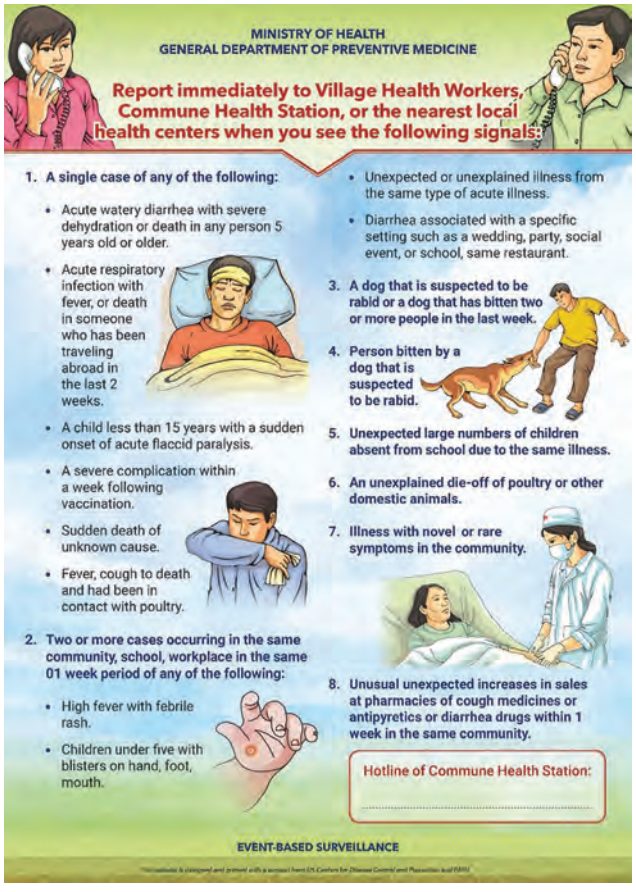

Figure 3. Poster displaying community-level signals for pilot of event-based surveillance, Vietnam, September 2016–May 2017.

EBS Pilot Assessment

As of July 1, 2017, we received 2,105 acceptability/sustainability surveys from 5 PPMC staff, 39 DHCs, 428 CHS, and 1,633 VHWs. Twenty-four (56%) of 43 districts returned the timeliness data extraction forms. We conducted 34 key informant interviews, and 32 focus group discussions, both totaling 160 participants (Figure 4).

During September 2016–May 2017, CHSs reported 2,520 signals to the districts (Figure 5). Quang Ninh province reported the largest number of signals. Of all 2,520 signals, 176 (7%) were verified as events by the districts and were responded to by the DHC or PPMC.

Although no preexisting timeline data were available for comparison, the pilot demonstrated that the mean times from detection to notification and detection to response were within 24 hours and 48 hours, respectively (Table 4) (*10*). We identified a case study illustrating the value of early event detection resulting in timely response (Figure 6). A trained VHW learned that diarrhea and vomiting developed in 2 persons who had attended a wedding party meal on September 25, 2016, at ≈13:00 hrs. The VHW called the CHS and reported the signal 30 minutes after learning of the episode. The CHSs EBS focal point visited the village and, after confirming the signal, immediately reported to the DHC EBS focal point, who joined the CHS team. The investigation found 93 other affected persons, 38 of whom were hospitalized. The DHC reported the event to the PPMC, which conducted a risk assessment classifying the event as high risk and launched a response the same day. The time to notification to the DHC was within 30 minutes, and the time to response was within 3 hours.

At the community level, signals were being recognized and reported from multiple sources. The most frequent EBS reporters were VHWs, teachers, community members, traditional healers, veterinarians, and representatives from industrial complexes (Figure 7). Reported events included multiple suspected avian influenza poultry die-offs and human outbreaks of chickenpox, mumps, and foodborne disease.

During the key informant interviews and focus group discussions, interviewees reported that the signal language should be further simplified, including alternatives for medical terms such "severe," "dehydration," and "complications."

Table 3. Resources provided to implement event-based surveillance in pilot provinces, Vietnam, September 2016–May 2017*

Resource	Province				Total
	North		South		
	Quang Ninh, no.	Nam Dinh, no.	BRVT, no.	An Giang, no.	
Computer + printer	15	11	9	12	47
Logbook					
For provincial level	2	2	2	2	8
For district level	26	22	16	22	86
For commune level	372	458	164	312	1,306
Communication materials					
Poster					
For community, displayed in public places	3,720	5,255	3,029	2,997	15,001
For HCFs at provincial level	60	40	100	60	260
For HCFs at district level	195	352	256	143	946
Other					
Leaflet for community	186,000	147,400	214,500	155,800	703,700
Plastic flyer holder	726	1,016	1,981	1,246	4,969
Handbook for VHWs/HCs	1,800	3,572	713	900	6,985

*BRVT, Ba Ria-Vung Tau; HCF, healthcare facility; VHW/HC, village health worker/health collaborator.

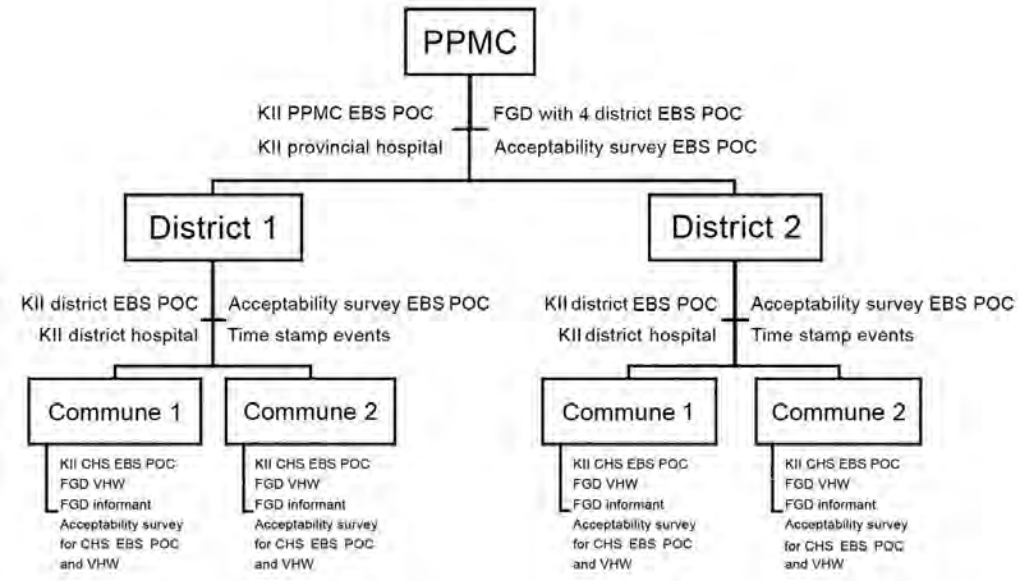

Figure 4. Assessment tools deployed at each site for assessment of an EBS pilot project, Vietnam, September 2016–May 2017. Acceptability survey and time stamp events tool were sent to all districts in the pilot provinces, not only to the sites selected for the FGD and KII site visits. CHS, commune health station; EBS, event-based surveillance; FGD, focus group discussion; FP, focal point; KII, key informant interview; POC, point of contact; PPMC, Provincial Preventive Medicine Center; VHW, village health worker.

Furthermore, some of the guideline language was deemed overly academic and needed to reflect everyday language. Most interviewees appreciated the illustrations in the posters and leaflets and noted their usefulness in areas that included ethnic minority populations that did not read Vietnamese.

A total of 82%–88% of VHW, CHS, and district respondents reported that EBS is very important in detecting public health events and helps to detect public health events earlier than before (Table 5). In addition, ≈85% of VHW and CHS respondents and 77% of district respondents said they were willing to continue participating in EBS. Data collected during field visits substantiated these results (data not shown).

Key motivating factors for participation expressed by the VHWs were a sense of service to the community, opportunities to increase community ties, and improvement in community trust. Some VHWs also said that the EBS project better defined their responsibilities. Staff reported that the EBS project increased communications between different levels of the public health system, which aided in early detection of events and outbreaks.

Discussion

The EBS pilot project builds on and expands the existing surveillance system in Vietnam to include community and HCF event-based surveillance. The pilot EBS implementation

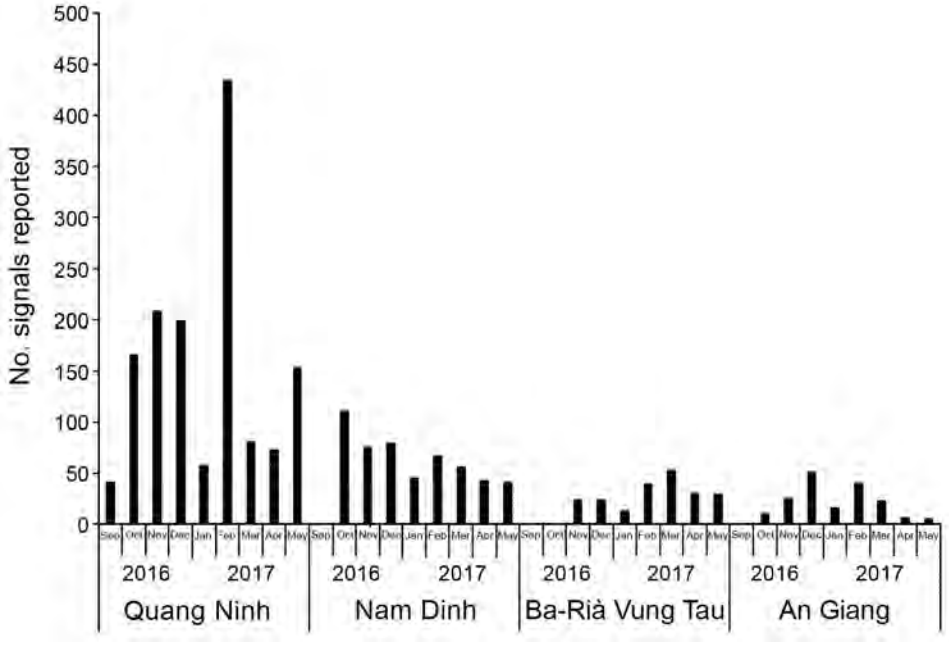

Figure 5. Number of signals reported to districts in the 4 event-based surveillance pilot provinces, Vietnam. Data were collected from the district monthly summary report during September 2016–May 2017.

Table 4. Time to notification and response during event-based surveillance pilot project, Vietnam, September 2016–May 2017

Type of event	No. events	Mean time to notification, h* (median [range])	Mean time to response, h† (median [range])
Suspected chickenpox	28	11 (12 [<1–24])	15.3 (12 [<1–48])
Hand, foot, and mouth disease	27	15 (12 [<1–171])	18 (12 [<1–171])
Suspected dengue	22	6.6 (2.5 [<1 –27])	36 (12 [5–318])
Avian influenza‡	14	3.4 (<1 [<1–12])	4.5 (1 [<1–15])
Foodborne disease	11	5 (<1 [<1 –24])	6.7 (<1 [<1–24])
Acute respiratory infection	10	9 (12 [1–12])	10 (12 [6–12])
Suspected mumps	9	9 (12 [<1–18])	18 (12 [<1–48])
Other	15	Not calculated	Not calculated
Total	136§	Not calculated	Not calculated

*Time from first detection to notification to the district level.
†Time from first detection to response.
‡Avian influenza in poultry, not human cases.
§From 176 events reported, 40 were excluded for timeliness analysis (incomplete, missing, incoherent or nonverified data).

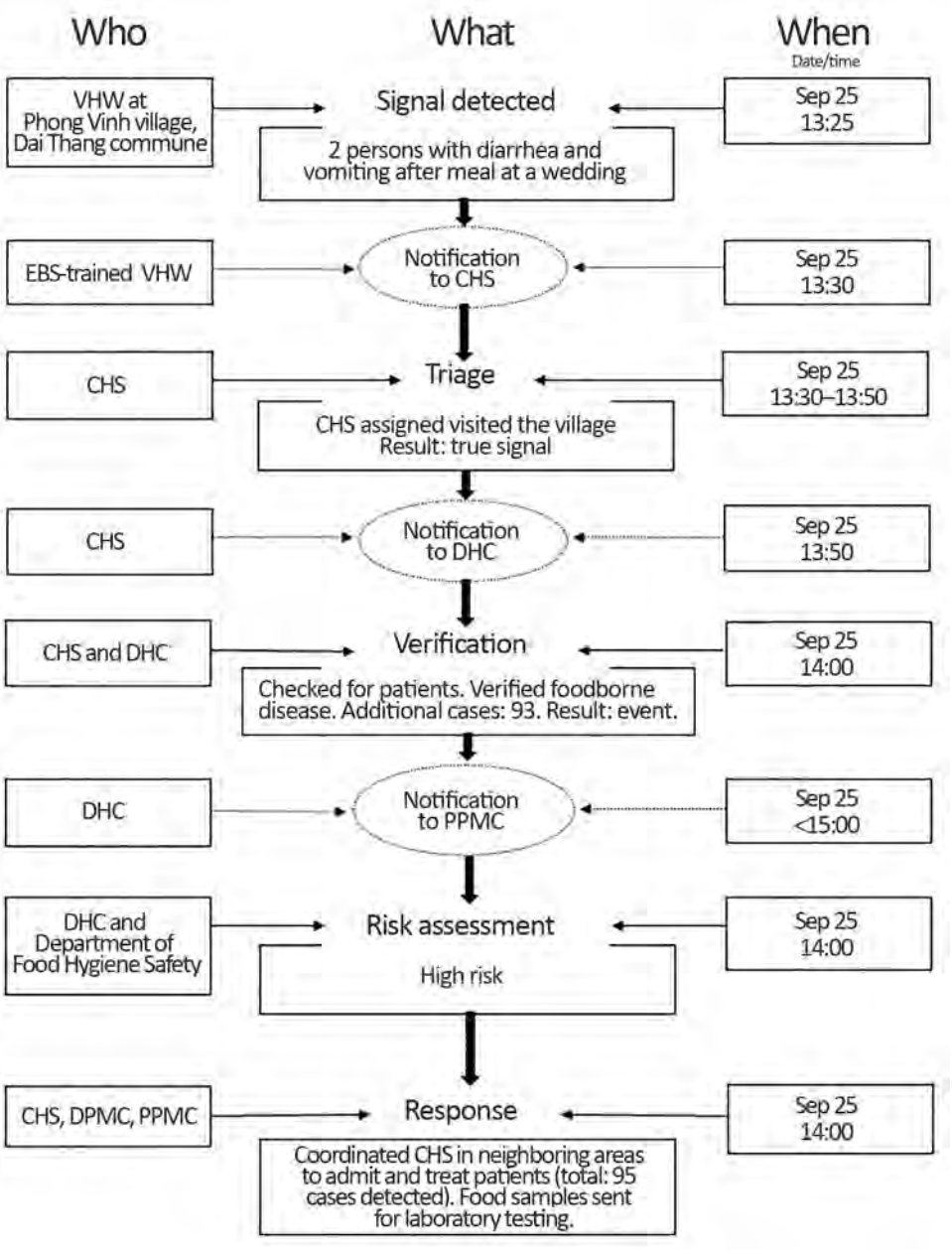

Figure 6. Case study of a cluster of food poisoning illustrating the value of EBS in early detection leading to rapid response, Dai Thang commune, Vu ban District, Nam Dinh Province, Vietnam, September 2016. CHS, Commune Health Station; DHC, District Health Center; DPMC, District Preventive Medicine Center; EBS, event-based surveillance; PPMC, Provincial Preventive Medicine Center; VHW, village health worker.

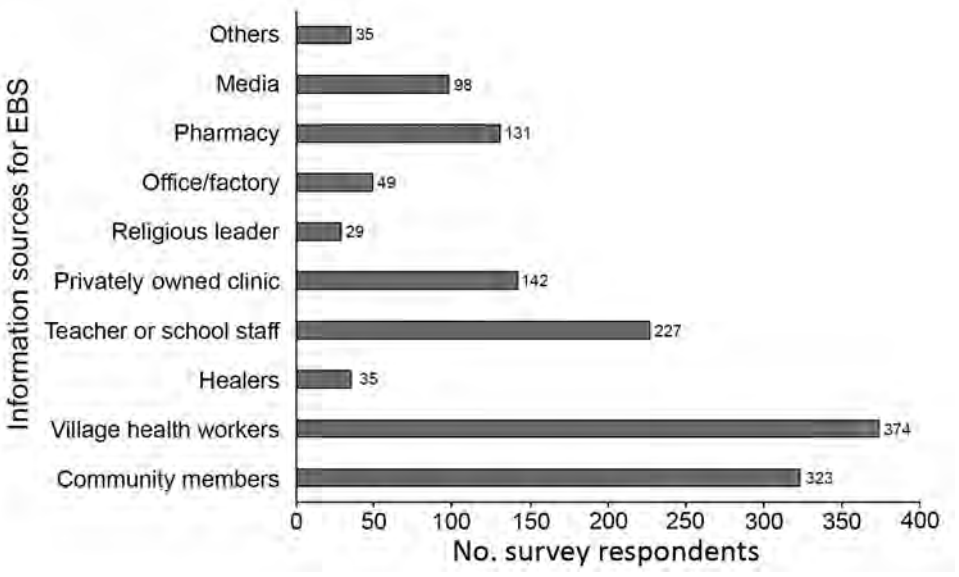

Figure 7. Sources contributing to signal detection and reporting through EBS at the community level in pilot provinces, Vietnam, September 2016–May 2017. Data were extracted from 428 acceptability survey questionnaires completed by Commune Health Station EBS focal points in July 2017. Each bar represents the number of survey respondents who identified the information source as contributing to EBS within the last 4 weeks. EBS, event-based surveillance.

in Vietnam demonstrated earlier detection and reporting of outbreaks, improved collaboration among HCFs, the preventive health and animal health sectors of the government, and increased participation of communities in surveillance and reporting. Thus, EBS implementation contributes to Vietnam's compliance with IHR 2005, thereby enhancing global health security.

The pilot initiative trained an existing network of VHWs and health collaborators to increase their awareness to look for and report signals as they appear in the community and to improve their understanding of patterns of disease that could signal the start of an outbreak. In most communes, the CHSs also recruited and trained additional community members as health collaborators through the current project. Most were persons with strong community ties, including money lenders, insurance agents, veterinary health staff, landlords, factory managers, community leaders, and others in a good position to directly observe community events. This wide participation broadened the sources of reporting and resulted in the reporting of numerous signals that otherwise would have been missed, such as school absenteeism reported by teachers and the resulting multiple detections of vaccine-preventable diseases (e.g., mumps and chickenpox). In contrast to reporting by clinicians from HCFs, VHWs recognized connections between cases in the community that doctors can miss, such as clusters among neighbors, co-workers, or persons with social connections.

The system did not rely on data reporting, aggregation, and analysis but rather used direct reporting methods to existing district and provincial authorities responsible for outbreak response. Based on the pilot implementation of EBS, it is plausible that focusing on patterns of occurrence in the community enabled outbreaks to be detected before they were large enough for HCFs to notice. Although all district and provincial public hospitals reported, no private hospitals and clinics participated in the EBS, making community-level participation critical to the detection process.

In the pilot districts, all events were detected and reported within 48 hours, and response was timely. Before EBS, such a rapid response by DHCs would not have been possible because ill persons would have to have been hospitalized to alert the system and, for certain diseases, traditional reporting often bypassed the CHSs. For example, foodborne illness events would first have to be reported to the Department of Food Safety and Hygiene, rather than the CHS, and ultimately to the DHC, resulting in delays. Similarly, animal events such as poultry die-offs or rabid dogs previously would have been reported to the Animal Health Department, and human health officials would not necessarily be alerted. During field visits, the DHC staff stated that because of the EBS pilot, multisectoral communication, such as between food safety and public health and human and animal health sectors, improved substantially.

Table 5. Acceptability and sustainability of survey results, EBS pilot project, Vietnam, June–July 2017*

Indicator	VHW, %, n = 1,633	CHS, %, n = 428	DHC, %, n = 39
Agree that EBS is very important in the detection of public health events	87.2	87.6	82.1
Agree that EBS helps detect public health events earlier than before	87.1	88	84.7
Willing to continue taking part in EBS	85.2	84.1	77
Agree that EBS should be continued	85.4	82.2	79.5

*The 5 provincial-level staff who received the survey responded. All agreed that EBS is important and should be continued. CHS, commune health station, community level; DHC, district health center, district level; EBS, event-based surveillance; VHW, village health workers.

Table 6. Revised signals for community and healthcare facilities in provinces participating in event-based surveillance pilot project, Vietnam, June 2017

Facility type	Signal
Community	1 child <15 y of age with • Sudden weakness of limbs • Fever, rash, respiratory infection, and possibly red eyes A single case severe enough to require hospital admission or causing death of any of the following: • ≥3 rice watery stools in 24 h in any person ≥5 y of age with dehydration • A new respiratory infection with fever in a person who has traveled abroad in the past 14 d • A new respiratory infection with fever after contact with live poultry • Illness within 14 d after vaccination • Illness never seen before or rare symptoms in the community ≥2 hospitalized persons and/or death with similar type of symptoms occurring in the same community, school, or workplace in the same 7-d period Unexpected large numbers of • Children absent from school because of the same illness in the same 7-d period • Sales at pharmacies of many people buying medicines for the same kind of illness • People sick with the similar type of symptoms at the same time • Deaths of poultry or other domestic animals A dog that is suspected to be rabid or • A sick dog that has bitten someone • Any dog that has bitten ≥2 persons in the past 7 d
Healthcare facility	Severe illness requiring hospital admission in healthcare workers after they cared for patients with similar symptoms ≥2 cases of severe acute respiratory infections within 7 d in the same community or household Large unexpected, sudden increases in admissions for any illness of the same type, including patients in intensive care units Severe, unusual, unexplainable illness, including failure to respond to standard treatment

The greatest challenge in quantifying EBS impact was lack of baseline outbreak data. Although Circular 54 requires outbreak reporting through eCDS, outbreak reports are not recorded even if detected, and therefore baseline data were not available. However, the absence of preexisting data demonstrates another important EBS contribution: the availability of data on outbreaks and events for planning public health interventions.

The assessment was an important part of the pilot and highlighted several problems that had to be rectified. Specifically, for some signals, wording needed to be simplified for VHWs, and the signal list itself needed to be more concise. In addition, for some diseases, such as hand, foot, and mouth disease, ongoing surveillance requires reporting of every case rather than clusters, creating some confusion. In some jurisdictions, leadership decided unilaterally to broaden signals to include single case reports, whereas the signal had been defined as a cluster, increasing the system's sensitivity, but with a very low specificity. This change resulted in only 7% of all signals becoming public health events. In the future, adherence to accepted signal definitions by the workforce can be maintained with continuous training and experience. Based on the assessment, the guidelines and training materials were revised and will undergo pilot testing before scale-up (Table 6).

Another challenge was the number of respondents to the online survey. The online acceptability survey was sent to the entire EBS workforce in the pilot provinces, but GDPM closed the survey after only 3 weeks. Thus, only a relatively small proportion (25%) of VHWs respondents were able to complete the survey, which might have limited the representativeness of some of the survey findings.

Despite the above limitations, experience gained through the pilot project in Vietnam might be useful for other countries looking to launch EBS. To that end, we recommend the following:

1. Early in the implementation process, form a TWG led and coordinated by the Ministry of Health and with participation from all stakeholders. A TWG facilitates coordination of technical and financial resources and a better understanding of the existing landscape of systems and actors, thereby reducing redundancies and improving buy-in from implementers.
2. Position EBS to fit within the existing legal framework for surveillance and reporting. The EBS TWG for this project researched the existing regulations around reporting and demonstrated how the program complemented the existing systems rather than something additional. The TWG also avoided introduction of new technologies and regulations whenever possible to minimize disruption.
3. Include focused training on risk assessment to help staff to prioritize events for investigation.
4. Provide repeated follow-up refresher training.
5. Build in resources for supportive monitoring visits and mentoring of district-level staff and below and include an evaluation process.

6. Engage community leaders early in the process to ensure uptake of the program.
7. Design pilot projects that can be scaled up.

Based on the experience gained by the initial EBS pilot project, the Vietnam Ministry of Health expanded the pilot to 2 new provinces in the central and highlands areas. The TWG revised training materials based on the findings of a final assessment and drafted with GDPM a decision letter to formally integrate EBS into the national surveillance system. The vice minister of health issued a mandate in March 2018 that directed all provinces to integrate event-based surveillance into the national surveillance strategy, ensuring sustainability of the CEBS program. The formalization of EBS as a Ministry of Health regulation will enable the provinces to seek funds in the provincial budget to support EBS. With the Ministry of Health mandate, revised EBS materials, and experience gained by launching an EBS pilot, Vietnam's surveillance system will soon function as an effective early warning and response system.

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About the Author

Dr. Balajee is the Associate Director for Global Health Sciences, Division of Viral Diseases, National Center for Immunization and Respiratory Diseases, CDC. Her primary research interests include strengthening capacities in resource-limited settings for early detection of events, rapid reporting, and appropriate response, in order to prevent the spread of infectious disease outbreaks.

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Address for correspondence: S. Arunmozhi Balajee, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop A27, Atlanta, GA 30329-4027, USA; email fir3@cdc.gov

Case Report and Genetic Sequence Analysis of *Candidatus* Borrelia kalaharica, Southern Africa

Katarina Stete,[1] Siegbert Rieg,[1] Gabriele Margos, Georg Häcker, Dirk Wagner, Winfried V. Kern, Volker Fingerle

Tickborne relapsing fever caused by *Borrelia* species is rarely reported in travelers returning from Africa. We report a case of a 71-year-old woman who sought treatment at University Medical Center in Freiburg, Germany, in 2015 with recurrent fever after traveling to southern Africa. We detected spirochetes in Giemsa-stained blood smears. Treatment with doxycycline for suspected tickborne relapsing fever was successful. Sequence analyses of several loci (16S rRNA, flagellin, *uvrA*) showed high similarity to the recently described *Candidatus* Borrelia kalaharica, which was found in a traveler returning from the same region earlier that year. We provide additional information regarding the genetic relationship of *Candidatus* B. kalaharica. Sequence information for an additional 6 housekeeping genes enables improved comparability to other borrelial species that cause relapsing fever. Our report underlines the importance and possible emergence of the only recently delineated pathogen in southern Africa.

An infection with *Borrelia* species bacteria causes relapsing fever (RF). It is transmitted by several arthropods, and dependent on the transmitting vector; louseborne relapsing fever (LBRF) is different from tickborne relapsing fever (TBRF) (*1*,*2*). The clinical picture of RF includes recurrent fever episodes accompanied by headache, hepatomegaly, splenomegaly, vomiting, conjunctivitis, myalgia, and arthralgia. It may be difficult to differentiate RF from other febrile illnesses, especially malaria. RF can be diagnosed by detection of spirochetes in blood smears or by PCR of EDTA-blood, and treatment is typically with penicillins or tetracyclines (*1*,*3*).

Whereas *B. recurrentis* is the cause of LBRF, which occurs mainly in the Horn of Africa, several *Borrelia* species may cause TBRF, which is found in many areas of the world. The endemic *Borrelia* species differ across geographic regions, and they have traditionally been divided into Old World and New World *Borrelia*. So far, ≈15 *Borrelia* species have been described to cause TBRF in humans worldwide (*1*). In Africa, TBRF has been traditionally attributed to *B. crocidurae* in western Africa, *B. hispanica* in northern Africa, and *B. duttonii* in eastern Africa (*1*,*4*).

Because microscopy is currently the standard method for diagnosis of TBRF in most countries in Africa, diagnosis does not usually include differentiation of species. With the advent of molecular diagnostic methods, scientists can identify species by sequencing different loci of *Borrelia* DNA from blood, such as the 16S rRNA gene, the flagellin gene (*flaB*), or the *glpQ* gene (*5*,*6*). Sequence analysis has challenged the assumption of strict division of species across Africa not only by the detection of geographic overlap of several *Borrelia* species, but also by detection of previously unknown species (*6*). Moreover, a *Borrelia* species found in ticks and in human blood in Tanzania showed more homology to New World *Borrelia* species than to the species known to be present in Africa (*7*–*9*). These findings were based on 16S rRNA and *flaB* partial sequences, which were deposited in GenBank as *B. duttonii* (accession nos. AB113315, AB105169, AB105132, AB057547, and AB105118). In 2015, a case of RF was described in a German tourist after traveling to the Kalahari Desert. The strain also showed greater genetic homology to New World *Borrelia* spp. and was proposed as a new species *Candidatus* B. kalaharica on the basis of the analysis of 16S rRNA, *flaB,* and *uvrA* genes (*10*).

Although RF is believed to be endemic to many areas in Africa, it is rarely diagnosed in travelers returning from these regions (*11*). In previous years, several cases of LBRF have been reported from several countries in Europe in migrants from eastern Africa (*2*,*3*,*12*–*16*). Reports on TBRF in travelers returning from Africa to Europe are limited to case reports. Most of these infections were acquired in West Africa (*17*–*23*), with single reports from other areas, such as Ethiopia and Morocco (*21*,*24*).

Author affiliations: Medical Center—University of Freiburg Faculty of Medicine, Freiburg, Germany (K. Stete, S. Rieg, G. Häcker, D. Wagner, W.V. Kern); National Reference Center for Borrelia, Oberschleißheim, Germany (G. Margos, V. Fingerle)

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[1]These authors contributed equally to this article.

We present a case of TBRF in a tourist from Germany returning from southern Africa and describe the results of a comprehensive molecular diagnostic analysis that underlines that *Candidatus* B. kalaharica represents a new species that is genetically distant from other RF group species and that it appears to be an emerging pathogen for humans that should be considered in the differential diagnosis of febrile patients. We obtained written informed consent from the patient for publication.

Materials and Methods

We performed slide microscopy after standard Giemsa staining of a thick and a thin blood smear. We obtained photographs from a 100× magnification objective using a Nikon Eclipse Ni microscope (Nikon Corporation, Tokyo, Japan).

We initiated in vitro cultures of infected blood using medium and conditions as previously described for RF species (*25*,*26*). We performed DNA extraction from EDTA blood using the Maxwell 16 FFS Nucleic Acid Extraction System Custom Kit (Promega, Mannheim, Germany) according to the manufacturer's instructions. We amplified fragments of the 16S rRNA, *glpQ* and *flaB* using primers and PCR conditions as described previously (*25*,*27*,*28*). We performed multilocus sequence analysis (MLSA) on housekeeping genes (*clpA, clpX, nifS, pepX, pyrG, rplB, recG,* and *uvrA*) as described (*29*; online Technical Appendix Table 1, https://wwwnc.cdc.gov/EID/article/24/9/17-1381-Techapp1.pdf). For PCR we ran HotStarTaq Mastermix (QIAGEN, Hilden, Germany) as touch-down protocol for the first 9 cycles with annealing temperatures of 55°C–48°C, decreasing 1°C each cycle, followed by 32 cycles at 48°C annealing temperature. The temperature profile was 95°C for 15 min for activation of Taq polymerase, 94°C for 30 s for denaturation, 30 s for annealing at the temperatures given previously, and 72°C for 60 s for elongation. A final step of elongation was at 72°C for 5 min, and then we held the samples at 12°C.

We used GATC Biotech AG (Konstanz, Germany) for sequencing, and performed sequence alignment, genetic distance analyses, and construction of phylogenetic trees in MEGA5 (*30*,*31*). We used BLAST (*32*) to compare the sequences we obtained (GenBank accession nos. KY560340–8) to sequences in GenBank (accession numbers in online Technical Appendix Tables 2–4) using standard settings. We conducted genetic distance analyses in MEGA5 (*31*) using the Kimura 2-parameter model (*30*). We inferred the evolutionary history by using the maximum likelihood method based on the general time-reversible model (*33*). We generated the initial trees for the heuristic search automatically by applying neighbor-joining and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach, and then selecting the topology with superior log likelihood value. We calculated node support values with 1,000 bootstrap repeats. We used discrete gamma distribution to model evolutionary rate differences among sites [+G]. The rate variation model allowed for some sites to be evolutionarily invariable [+I]. The trees are drawn to scale, with branch lengths measured in the number of substitutions per site. Codon positions included were 1st + 2nd + 3rd + Noncoding for *flaB* sequences and housekeeping gene sequences. We eliminated all positions containing gaps and missing data.

Results

Case Report

A 71-year old woman sought treatment for fever after a 4-week camping trip to South Africa, Namibia, Botswana, and Zimbabwe. The patient reported no malaria chemoprophylaxis, fresh water contact, or tick bites. Other than horseback riding, she could recall no direct contact with animals. Preexisting conditions were nonmetastatic breast cancer under treatment with exemestan and a history of penicillin allergy.

The patient reported fever episodes starting 3 days before returning to Germany. Malaria was ruled out at a local health unit in South Africa by thick smear microscopy. Three days after arriving in Germany, the patient came to our clinic with a history of fever but no other abnormal signs or symptoms. Leukocyte counts were normal; levels of C-reactive protein and procalcitonin were slightly elevated (Figure 1). A malaria thick blood smear, blood cultures, and a dengue nonstructural protein 1 antigen test showed negative results. The fever resolved spontaneously, and the patient was discharged and asked to return in case of recurrence of symptoms.

Seventeen days later, the patient returned with RF (temperature >39°C). She reported 2 episodes of fever lasting 2–3 days flanked by symptom-free intervals of ≈4 days (Figure 1). Leukocyte counts again were normal, and levels of C-reactive protein and procalcitonin were elevated. We detected no malaria parasites in a thick smear; however, we found multiple spirochetes compatible with *Borrelia* species (Figure 2). We made a presumptive diagnosis of TBRF on the basis of the travel route and with no evidence of body lice infestation. We started antimicrobial therapy with doxycycline (2 × 100) mg/d and close monitoring. We observed no signs of a Herxheimer reaction. PCR diagnostics of 16S rRNA confirmed the diagnosis of *Borrelia* infection. For further species differentiation, we sent a blood sample to the German National Reference Center for sequence analysis for *Borrelia*. An 11-day course of doxycycline led to an uneventful recovery with no recurrence of fever.

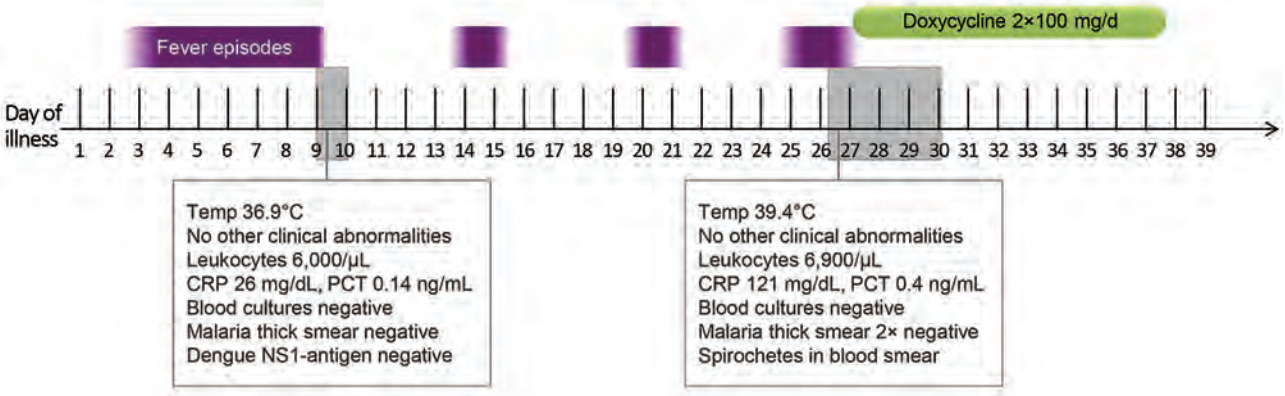

Figure 1. Timeline of the course of symptoms and treatment, including laboratory test results, for a patient with recurrent fever after traveling to southern Africa, 2015. Temp, temperature; CRP, C-reactive protein; PCT, procalcitonin.

Sequence Analysis and Phylogeny

To investigate the *Borrelia* species designation, we conducted BLAST searches using the 16S rRNA PCR fragment. Top hits included *Candidatus* B. kalaharica, *B. duttonii* strain VS4, *B. turicatae*, and *B. parkeri*. Genetic distance analyses using the 16S rRNA fragments in MEGA5 (*31*) revealed strains *Candidatus* B. kalaharica (*10*) and VS4 from Tanzania, an atypical RF strain present in the Old World (*8*), as closest matches (online Technical Appendix Table 2). Although designated *B. duttonii* in GenBank, VS4 was closely related to some strains found in the Mvumi region of Tanzania (*7*) which were shown to be more closely related to New World RF species than to *B. duttonii*. Genetic distance values obtained for the 16S rRNA fragment were 0.2% for *Candidatus* B. kalaharica and slightly higher for *B. parkeri*, *B. crocidurae*, and *B. turicatae* (0.4%) (online Technical Appendix Table 2).

When the sequence of a flagellin gene (*flaB*) fragment (252 bp) was used for genetic distance analysis, *Candidatus* B. kalaharica was again the most closely related strain, with genetic distance value = 0.000 (online Technical Appendix Table 3). Strains representing atypical *B. duttonii* (*7,8*) showed higher genetic distance values (strain TnB, 0.8%; strain EM14, 1.2%), whereas for other *Borrelia* species such as *B. anserina* BA2 (5.8%), *B. turicatae* (6.2%), and *B. parkeri* (6.2%) the values were even higher, indicating a close genetic relationship of the strain investigated here to *Ca*. B. kalaharica. This was also reflected in phylogenies (online Technical Appendix Figures 1, 2). In the 16S rRNA phylogeny, the DNA isolate investigated here formed a clade together with *Candidatus* B. kalaharica and VS4 from Mvumi, Tanzania (*8,10*). In the *flaB* phylogeny, our DNA isolate and *Candidatus* B. kalaharica formed a sister clade to strains from the Mvumi region in Tanzania (*7,8*), notably outside the clade containing Old World RF species such as *B. duttonii*, suggesting that they are divergent from *B. duttonii*.

We obtained similar results using 7 housekeeping loci (Figure 3; online Technical Appendix Tables 4, 5) and, in particular locus *uvrA*. For this locus, sequences of *Candidatus* B. kalaharica were available (Figure 3, panel A). Genetic distance analysis (online Technical Appendix

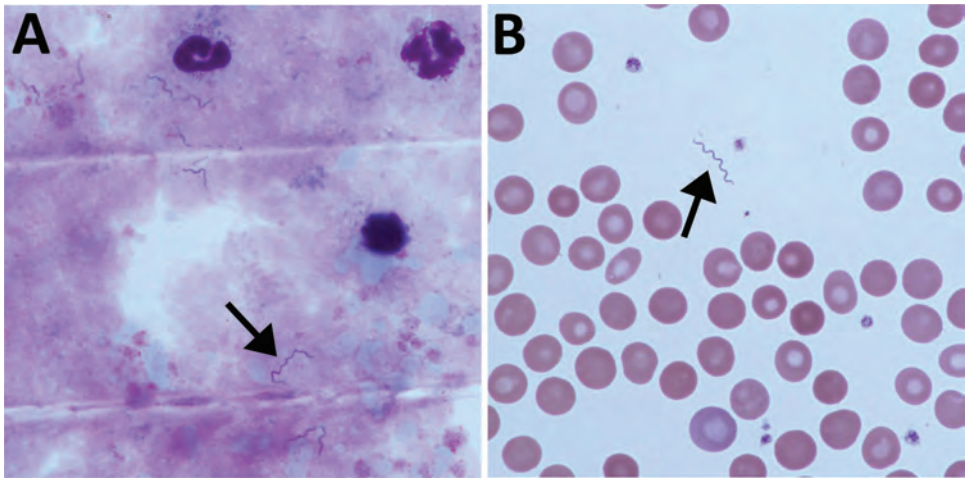

Figure 2. Microscopy of blood from a patient with recurrent fever episodes after traveling to southern Africa, 2015. Arrows indicate spirochetes. A) Thick smear specimen; B) thin smear specimen. Original magnification ×100.

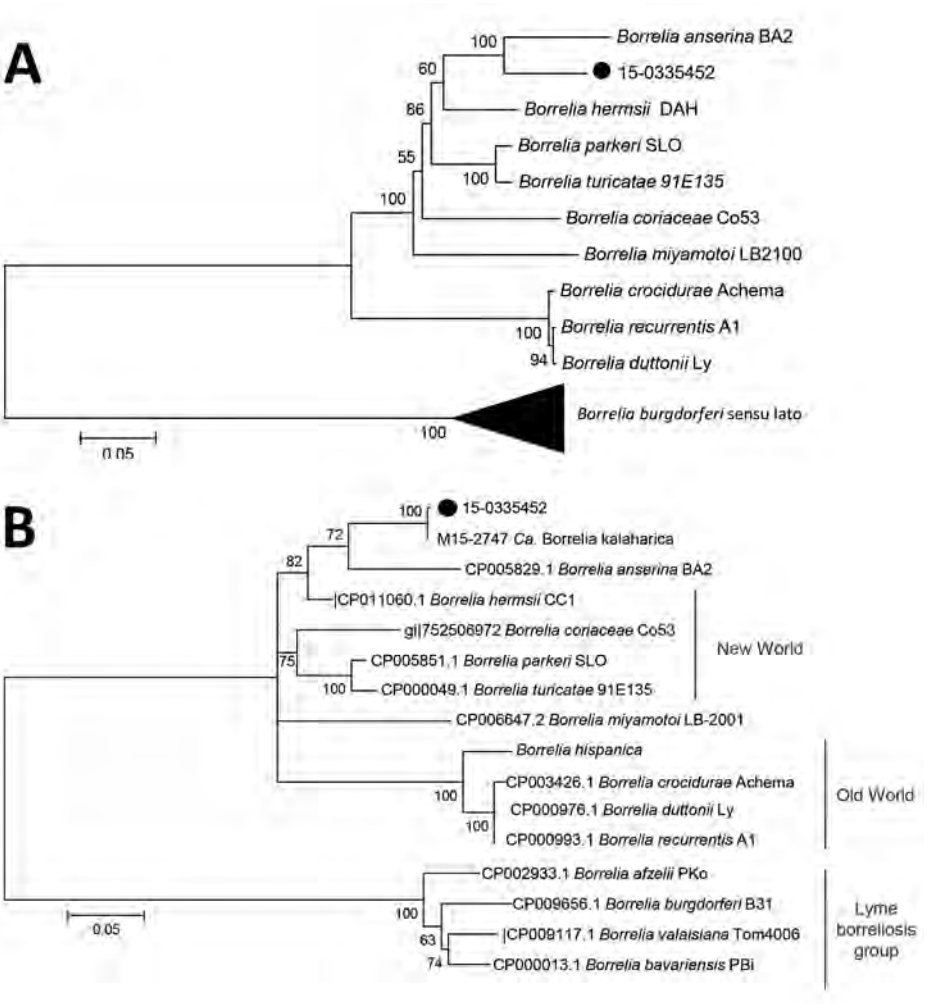

Figure 3. Molecular phylogenetic analysis by maximum-likelihood method of isolates from a patient in Germany with recurrent fever episodes after traveling to southern Africa, 2015. A) Phylogeny of *uvrA* sequence fragments. The tree with the highest log likelihood (–2566.8936) is shown. A discrete gamma distribution was used to model evolutionary rate differences among sites (4 categories [+G, parameter = 0.9541]). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 43.5691% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 19 nt sequences. There were a total of 570 positions in the final dataset. Bootstrap values >50 are shown. Black dot indicates the sample analyzed in this study. Black triangle represents the clade containing *B. burgdorferi* s.l. isolates, collapsed for simplicity. Scale bar indicates substitutions per site. B) Phylogeny of concatenated sequences of 7 MLST housekeeping loci (*clpX, nifS, pepX, pyrG, recG, rplB, uvrA*). The tree with the highest log likelihood (–31066.7852) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. A discrete gamma distribution was used to model evolutionary rate differences among sites (4 categories [+G, parameter = 0.7881]). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 36.6955% sites). The analysis involved 33 nt sequences. There were a total of 4,203 positions in the final dataset. The subtree containing the LB group of spirochetes was collapsed. Bootstrap values >50 are shown. Black dot indicates the sample analyzed in this study. Scale bar indicates substitutions per site.

Tables 4, 5) and phylogenetic inferences (Figure 3, panel B) further support the close genetic relationship of the specimen investigated here with *Ca*. B. kalaharica and both clusters next to *B. anserina*. For MLST analysis, only 7 genes could be included as *clpA* PCR did not yield a PCR product. The PCR for the *glpQ* locus also proved negative in spite of several amplification attempts suggesting that perhaps base differences in the primer regions prevented amplification. Despite our efforts, we were unable to cultivate the causative pathogen from blood.

Discussion

The case described here is the second report within a few months of TBRF in a tourist from Germany traveling to countries in southern Africa, such as South Africa, Namibia, and Botswana (*10*). In the previous case, a presumed soft tick bite in the Kalahari Desert was described, whereas our patient did not report any arthropod bite. However, contact with arthropods was likely as the patient was camping. Soft tick *Ornithodoros* species only need short blood meals and do not attach tightly to the host (*34*), making it conceivable that a feeding tick was not noticed. These cases underscore that, in returning travelers with RF, TBRF should be considered in the differential diagnosis, even if no tick bite is reported. Thick smears are the diagnostic procedure of choice and should be carefully evaluated for corkscrew-shaped spirochetes (*1*). However, the sensitivity of this method may change depending on febrile versus afebrile periods with different pathogen loads circulating in the blood. Thus, as we saw in this patient, thick smears may turn negative during infection and should therefore be repeated preferentially during febrile episodes.

Death as a result of TBRF is considered to be rare; however, higher mortality rates have been suspected as a result of Herxheimer reactions, even though there is a lack of data for TBRF in Africa (*1*,*35*). Clinicians need to be aware that the initiation of antimicrobial treatment might be associated with a severe Herxheimer reaction, necessitating aggressive supportive care.

Borrelia species can be identified and differentiated by means of DNA sequence analysis, although it may be hard to distinguish closely related *Borrelia* species, such as *B. duttonii*, *B. recurrentis,* and *B. crocidurae* (*36*,*37*). 16S rRNA sequences are available for many of the *Borrelia* species and strains that have been found in Africa and thus, although the locus may have low resolution, it can give a first indication of relationships. Other loci that have been used in previous reports were also used in the current study, including *flaB* and housekeeping loci (*37*). Because there is so little information about which RF-causing species do occur in southern Africa (*6*), a more thorough characterization of the DNA isolate would be beneficial to epidemiologists and other researchers in the field.

The traditional concept of strict division of geographic areas into Old World and New World *Borrelia* and division of species across Africa has been challenged by the description of new *Borrelia* species. This is the second report of a species that has not been described previously. Genetically, *Candidatus* B. kalaharica is most closely related to TBRF *Borrelia* described from the Mvumi region in Tanzania (*8*,*38*). In previous publications it was suggested that these *Borrelia* strains from Mvumi may belong to the new species (*8*,*38*). Unfortunately, the only available sequences were for 16S rRNA and *flaB,* but more sequence data will be needed to reveal the taxonomic position of these strains. Of interest, both the strains from Mvumi and *Candidatus* B. kalaharica show more genetic similarity to New World RF species than to the expected Old World species.

We report the second case of a human infection with the proposed new species *Candidatus* B. kalaharica. Our findings support the definition of *Candidatus* B. kalaharica as a new species that is genetically distant from other RF group species and more closely related to New World RF *Borreliae*. It appears to be an emerging pathogen for humans that should be considered in the differential diagnosis of febrile patients.

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About the Author

Dr. Stete is a resident in internal medicine and infectious diseases at the Division of Infectious Diseases, University Medical Center, Freiburg, Germany. Her primary research interests are in the field of parasitic diseases, travel medicine, and migration health.

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Address for correspondence: Katarina Stete, University of Freiburg Division of Infectious Diseases, Department of Medicine II, Medical Center, Faculty of Medicine, Hugstetterstr 55, 79106 Freiburg, Germany; email: katarina.stete@uniklinik-freiburg.de

Novel Orthopoxvirus and Lethal Disease in Cat, Italy

Gianvito Lanave, Giulia Dowgier, Nicola Decaro, Francesco Albanese, Elisa Brogi, Antonio Parisi, Michele Losurdo, Antonio Lavazza, Vito Martella, Canio Buonavoglia, Gabriella Elia

We report detection and full-genome characterization of a novel orthopoxvirus (OPXV) responsible for a fatal infection in a cat. The virus induced skin lesions histologically characterized by leukocyte infiltration and eosinophilic cytoplasmic inclusions. Different PCR approaches were unable to assign the virus to a defined OPXV species. Large amounts of typical brick-shaped virions, morphologically related to OPXV, were observed by electron microscopy. This OPXV strain (Italy_09/17) was isolated on cell cultures and embryonated eggs. Phylogenetic analysis of 9 concatenated genes showed that this virus was distantly related to cowpox virus, more closely related to to ectromelia virus, and belonged to the same cluster of an OPXV recently isolated from captive macaques in Italy. Extensive epidemiologic surveillance in cats and rodents will assess whether cats are incidental hosts and rodents are the main reservoir of the virus. The zoonotic potential of this novel virus also deserves further investigation.

Orthopoxviruses (OPXVs; family *Poxviridae*, subfamily *Chordopoxvirinae*, genus *Orthopoxvirus*) are complex, double-stranded DNA viruses with ongoing interest because of their potential use as bioterrorism agents and in gene therapy. Variola virus (VARV), the causative agent of smallpox, has been eradicated in nature; however, there is still the possibility of accidental or intentional release, and it is currently classified as a category A biologic agent (*1*). Another concern is the zoonotic potential of some OPXVs, such as monkeypox virus, camelpox virus, buffalopox virus, and cowpox virus (CPXV) (*2*,*3*).

CPXV, which has a wide host range and a distribution restricted to the Eurasian continent, causes localized dermatitis in humans, although severe disease might develop in immuneocompromised persons, occasionally with a fatal outcome. Natural hosts for CPXV are wild rodents (*4*), but the infection is acquired mainly through direct contact with cats, which are natural hosts, and rarely by exotic animals and wild species (*5*). Ectromelia virus (ECTV) is the causative agent of mousepox, a severe exanthematous disease of mice in laboratory colonies and has been reported worldwide in several outbreaks and causes high economic losses in biomedical research (*6*). ECTV has never been reported in humans, and little is known regarding its natural distribution and hosts (*7*).

Reports of OPXV infections in animals and humans have largely increased during recent decades, which has enhanced their zoonotic potential and led to the perception of an increasing risk for humans (*8*). For cats, there are several reports of poxvirus infections, but the causative agent has been characterized as CPXV (*9*–*14*) or has not been characterized (*15*–*18*).

We report a case of fatal infection with an OXPV in a household cat. This virus was more closely related to ECTV than to CPXV, putatively representing a novel OPXV species.

Materials and Methods

Case Study

A domestic, short-haired, male, 6-month-old cat was brought to a veterinarian because of multicentric, nodular, ulcerative dermatitis (Figure 1). The cat was regularly vaccinated for common feline diseases (feline panleukopenia, rhinhotracheitis, calicivirosis, and chlamydiosis) and showed negative test results for retroviral infections. An antiparasitic product had been applied monthly (Frontline Combo Spot On; Merial, Ingelheim, Germany). The cat was fed a balanced commercial diet and lived indoors, but it had access to outdoors and had a hunting behavior.

The cat had multiple nodular, plaque-like, ulcerative lesions on its body, particularly on the feet and face. Results of diagnostic testing, including a wood lamp examination, skin scrapings, trichogram, and fungal culture, were negative. Cytological examination showed a mixed inflammatory population of cells with a relevant amount of

Author affiliations: University of Bari, Valenzano, Italy (G. Lanave, G. Dowgier, N. Decaro, M. Losurdo, V. Martella, C. Buonavoglia, G. Elia); La Vallonea Veterinary Laboratory, Milan, Italy (F. Albanese); Centro Veterinario Montarioso, Siena, Italy (E. Brogi); Istituto Zooprofilattico Sperimentale di Puglia e Basilicata, Putignano, Italy (A. Parisi); Istituto Zooprofilattico Sperimentale di Lombardia ed Emilia Romagna, Brescia, Italy (A. Lavazza)

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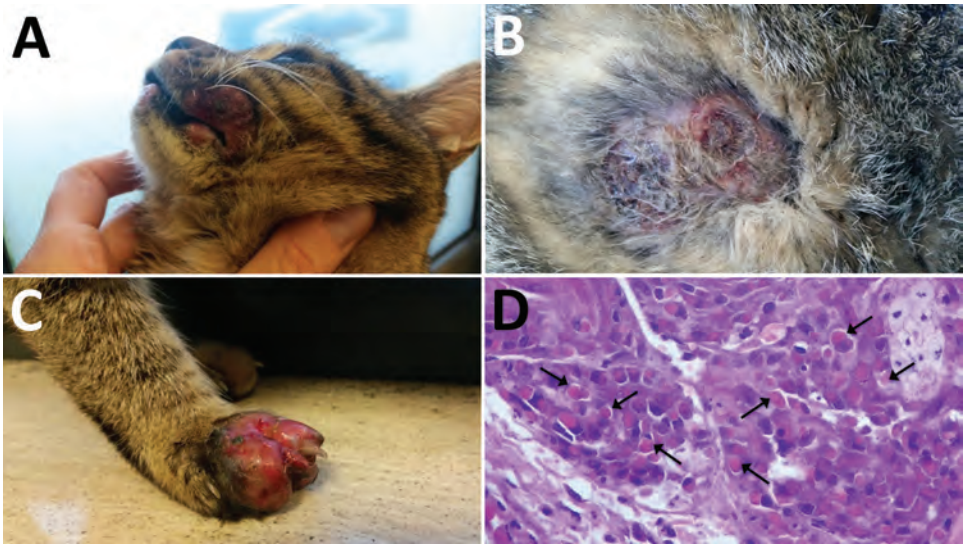

Figure 1. Cat with orthopoxvirus infection, Italy. Ulcerated nodules and plaques were observed on the lips (A), thorax (B), and forelimb (C). Skin punch biopsy specimen (D) showing leukocyte infiltration and cytoplasmic inclusion bodies (arrows) (hematoxylin and eosin stain, original magnification × 60).

eosinophils. A blood test showed only a mild leukocytosis with an increase in numbers of lymphocytes, neutrophils, and eosinophils. Because of rapid worsening of its clinical conditions, the cat was euthanized.

Histopathologic Analysis

We collected multiple skin biopsy specimens for histopathologic analysis by using an 8-mm biopsy punch and fixed these specimens in 10% buffered formalin. Samples were embedded in paraffin, sectioned, and stained with hematoxylin and eosin, according to standard protocols.

DNA Extraction and PCR Amplification

An OPXV infection was suspected on the basis of clinical presentation and histopathologic analysis. Therefore, we processed histologic preparations for molecular investigations to confirm the presumptive diagnosis. We purified total DNA from a thin section of ≈20 mg of formalin-fixed, paraffin-embedded tissue by using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions.

We tested the DNA extract by using 2 panchordopoxvirus PCRs specific for the variable GC content of the genera included in the subfamily *Chordopoxvirinae* and other unclassified chordopoxviruses (*19*). For the low GC content PCR, we used DNA from a laboratory vaccinia virus Western Reserve strain (VACV-WR) as a positive control. For the high GC content PCR, we used an Orf virus isolated during an outbreak of contagious ecthyma as a positive control. We initially conducted subsequent identification of OPXV by using a PCR specific for the gene coding A-type inclusion protein (*20*), followed by a second PCR specific for the hemagglutinin (HA) gene (*21*). In addition, we performed 2 species-specific PCRs, 1 for ECTV and 1 for CPXV, to further characterize the virus (*22*).

We conducted all PCR amplifications by using an LA PCR Kit (version r.2.1) (Takara Bio, Tokyo, Japan) in a 50-μL reaction containing 1 mmol/L of primers, LA PCR Buffer (Mg^{2+}), 8 μL of dNTP mixture (corresponding to 400 mmol/L of each dNTP), 2.5 units of TaKaRa LA Taq polymerase, and 1 μL of template DNA. The cycling protocol used for each assay was programmed as described (*19*). PCR products were subjected to electrophoresis on a 1.5% agarose gel containing a fluorescent nucleic acid marker (GelRed; Bio-Rad Laboratories, Hercules, CA, USA) at 80 V for 45 min and visualized under fluorescent light on the Gel Doc EZ Imaging System with Image Laboratory Software (Bio-Rad Laboratories). PCR products were directly sequenced by Eurofins Genomics GmbH (Ebersberg, Germany). We manually edited and analyzed sequences by using the Geneious platform version 10.1.3 (Biomatters Ltd., Auckland, New Zealand).

Virus Isolation

After diagnosis of OPXV infection, we collected additional biopsy specimens from skin lesions of the diseased cat intravitam and then used for subsequent virologic investigations. For virus isolation, we used African green monkey kidney fibroblast CV-1 cells and African green monkey kidney epithelial Vero cells. Cells were grown in Dulbecco's modified minimum essential medium (DMEM) supplemented with 10% fetal bovine serum. Tissues were homogenized in DMEM (10%, wt/vol) and centrifuged at 8,000 × *g* for 10 min. Supernatants were treated with antimicrobial drugs (penicillin 5,000 IU/mL, streptomycin 2,500 μg/mL, and amphotericin B 10 μg/mL) for 30 min, inoculated on partially confluent CV-1 and Vero cell cultures, and incubated at 37°C in a 5% CO_2 incubator. After an adsorption period of 45 min, DMEM was added. Cells were observed daily for cytopathic effects.

For hematoxylin and eosin staining and indirect immunofluorescence (IIF) assay, we grew cells on coverslips placed in 12-well plates. Cells were mock- or virus-infected and coverslips were harvested at 48 hours postinfection. For detection of inclusion bodies, we fixed cells in Bouin solution for 2 h and stained them with hematoxylin and eosin. For the IIF assay, cells were fixed with 80% acetone for 30 min. Coverslips were rinsed twice with phosphate-buffered saline and incubated 30 min in a humidified chamber at 37°C with a serum sample (diluted 1:50) collected from the ill cat. Coverslips were washed twice with phosphate-buffered saline and incubated with goat anti-cat IgG conjugated with fluorescein isothiocyanate (Sigma-Aldrich, Milan, Italy).

The homogenate of skin biopsy specimens was inoculated onto the chorioallantoic membrane of 12-day-old chick embryos. After 2 days of incubation at 37°C, membranes were collected from the eggs and pock morphology was observed.

Electron Microscopy

We performed negative staining and electron microscopic analysis of homogenates of skin punch biopsy specimens and supernatants of infected Vero cells that showed an evident cytopathic effect. Samples were frozen and thawed twice and centrifuged at 4,000 × *g* for 20 min and at 9,300 × *g* for 10 min to clarify the supernatant. The second supernatant (82 μL) was then ultracentrifuged in an Airfuge centrifuge (Beckman Coulter, Brea, CA, USA) for 15 min at 21 lbs/in^2 (82,000 × *g*). The Airfuge was fitted with an A 100 rotor that held six 175-μL test tubes containing specific adapters for 3-mm grids, which enables direct pelleting of virus particles on carbon-coated, formvar copper grids. These grids were stained with 2% sodium phosphotungstate, pH 6.8, for 1.5 min, and observed with a Tecnai G2 Biotwin Transmission Electron Microscope (Field Electron and Ion Company, Hillsboro, OR, USA) operating at 85 kV. We identified poxvirus particles, observed at magnifications of 11,000×–26,500×, on the basis of their typical morphologic characteristics.

Serologic Analysis

We tested the serum sample collected intravitam from the diseased cat for OPXV antibodies by virus neutralization and IIF assays. We used strains Italy_09/17 isolated from the same cat and VACV-WR in these tests.

For the virus neutralization test, we mixed 2-fold dilutions of heat-inactivated serum (starting at a dilution of 1:2) with 100 50% tissue culture infective doses of virus in 96-well microtiter plates. After incubation at room temperature for 60 min, 2 × 10^4 CV-1 cells were added to each well. Plates were read after 4 days of incubation at 37°C in a humidified atmosphere of 5% CO_2.

For the IIF assay, we fixed confluent monolayers of CV-1 cells grown on coverslips and infected with strain Italy_09/17 or VACV-WR with 80% acetone. We tested 2-fold dilutions of heat-inactivated serum (diluted 1:20 to 1:5,120) by using 1 coverslip/dilution. Goat anti-cat IgG conjugated with fluorescein isothiocyanate was used as a secondary antibody (Sigma-Aldrich).

Next-Generation Sequencing

For DNA extraction, we obtained virus stocks from semipurified virus particles. In brief, we infected CV-1 cells with strain Italy_09/17. At 48 hours postinfection, the cell medium was collected and nuclei and cell debris were discarded by centrifugation at 1,000 × *g* for 10 min at 4°C. We extracted virus DNA by using a QIAamp Cador Pathogen Mini Kit (QIAGEN) according to the manufacturer's instructions.

We quantified DNA by using the Fluorometric Qubit dsDNA High Sensitivity Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). We prepared a genomic DNA library by using the Nextera DNA Sample Prep Kit (Illumina, San Diego, CA, USA) according to the manufacturer's protocol and performed a size-selection step manually by using Ampure XP magnetic beads (Beckman Coulter). We performed quality control analysis of the sample library by using the QIAxcel Advanced System with QIAxcel ScreenGel Software 1.4.0 (QIAGEN). We normalized library samples as suggested by QIAGEN and performed sequencing by using a MiSeq instrument, version 2, and a MiSeq Reagent Kit (Illumina).

Genome Annotation and Comparison

We obtained 1,497,762 paired reads in next-generation sequencing (NGS) experiments (Illumina); these reads had an average length of 155.4 bp. We performed quality control of reads by using FastQC (*23*) and sequence trimming by using the plugin Trim Ends in Geneious software version 10.1.3 (https://www.geneious.com/). We filtered NGS sequences by using the genome of African green monkey (*Chlorocebus sabeus*), which yielded 217,236 unmapped reads. We used these unmapped reads for de novo assembling of the feline OPXV genome by using the Geneious Assembler.

We annotated the nearly full-length genome sequence of the Italy_09/17 isolate by using ECTV strain Naval as reference (GenBank accession no. KJ563295). We performed genome annotation by using FindORFs software in Geneious version 10.1.3 and a set of reference sequences, including ECTV Naval (accession no. KJ563295), ECTV Mos (accession no. AF012825), CPXV BR (accession no. AF482758), and VACV COP (accession no. 35027) for comparison. We further analyzed open reading frames that remained unassigned or with a lower similarity to the reference sequences by using MyOrfeome (http://myorfeome.sourceforge.net).

Phylogenetic Analysis

For characterization of the OPXV strain, we used the strategy proposed by Emerson et al. (*24*). We selected 9 coding sequences from the genome of the feline OPXV strain Italy_09/17: A7L, early transcription factor/VETF large subunit; A10L, major core protein; A24R, RNA polymerase 132; D1R, messenger RNA capping enzyme, large subunit; D5R, DNA-independent NTPase (DNA replication); E6R, hypothetical protein; E9L, DNA polymerase; H4L, RNA polymerase-associated protein; and J6R, RNA polymerase 147. Gene designations refer to the VACV COP genome. We aligned concatenated genome sequences of OPXVs representative of North American and Old World (African and Eurasian) viruses by using Geneious version 10.1.3 and the MAFFT algorithm (*25*). After searching the GenBank database, we retrieved complete HA gene sequences of 2 feline-derived human OPXV strains (accession nos. EF612709 and FJ445747) and of an OPXV isolated from captive macaques (accession no. KY100116) and aligned them with cognate OPXV sequences.

We performed phylogenetic analysis for concatenated DNA alignments with Bayesian inference by using 4 chains run for >1 million generations (*26*,*27*). We used ModelTest software (http://evomics.org/resources/software/molecular-evolution-software/modeltest/) to identify the most appropriate model of evolution for the entire dataset and for each gene individually. The identified program settings for all partitions, under the Akaike Information Criteria, included 6 character states (general time reversible model), a proportion of invariable sites, and a gamma distribution of rate variation across sites. We deposited nucleotide sequences of strain Italy_09/17 used for phylogeny in GenBank (accession nos. MF578930–9).

Detection of Other Pathogens

We subjected nucleic acids extracted from freshly collected skin biopsy specimens and serum of the affected cat to a TaqMan assay for detection of canine parvovirus 2/feline panleukopenia virus (*28*) and to a minor groove binder probe assay for rapid discrimination between true feline panleukopenia virus strains and antigenic variants of canine parvovirus 2 (*29*). We also used DNA extracts to detect proviral DNA of feline immunodeficiency virus (*30*) and feline leukemia virus (*31*) and DNA of feline hemoplasmas (*32*) and feline herpesvirus (*33*). We screened RNA extracts by real-time PCR or conventional reverse transcription PCR specific for carnivore coronaviruses (*34*) and caliciviruses (*33*,*35*).

Results

Histopathologic Analysis

Histopathologic analysis of multiple skin specimens showed mild hyperplasia of the epidermis and the follicular wall. Many roundish to oval brightly eosinophilic inclusion bodies were clearly evident in the cytoplasm of both epidermal and follicular keratinocytes, including in a few sebocytes. The morphology of cells suggested a possible OPXV infection (Figure 2, panel A). Nodular to diffuse dermatitis caused by mixed inflammatory cells was also present in dermis and hypodermis; those cells were mainly represented by eosinophils, histiocytes, and lymphocytes, together with few plasma cells and neutrophils.

Molecular Investigations

Molecular analysis of formalin-fixed, paraffin-embedded tissues showed positive results for the low GC panchordopoxvirus PCR and negative results for the high GC panchordopoxvirus PCR (*19*). This pattern of amplification was consistent with an OPXV infection. Amplification of the gene coding for the A-type inclusion protein generated an amplicon of ≈1,237 bp, and amplification of the HA gene (*20*,*21*) generated an amplicon of 846 bp, which are expected sizes for these genes in ECTV. All samples collected from the cat were negative for other pathogens by the molecular assays used.

Sequence analysis of the HA gene of strain Italy_09/17 showed high nucleotide identity (98%) with that of CPXV strain Germany (GenBank accession no. HQ420897) and to feline-derived human poxvirus IT1 (accession no. EF612709). In addition, strain Italy_09/17 was also highly related to most of the ECTV strains in GenBank; the highest (97%) nucleotide identity was with ECTV strain Naval (accession no. KJ563295). Strain Italy_09/17 showed positive results in the ECTV-specific PCR and negative results in the CPXV-specific PCR (*22*) (Table 1).

Virus Isolation

Virus isolation from freshly collected skin biopsy specimens was successful with Vero and CV-1 cells. We observed a cytopathic effect at 48 hours postinfection that showed rounding of cells, increased granularity, and detachment from the monolayer. In Vero cells, a cytopathic effect was less evident than in CV-1 cells. Cells stained with hematoxylin and eosin contained large eosinophilic cytoplasmic inclusion bodies that were compatible with infection by poxviruses, including CPXV (*36*) and ECTV (*37*) (Figure 2, panel B).

The IIF assay showed granular fluorescence areas that displayed the morphology of the inclusion bodies in cell cytoplasms (Figure 2, panel C). Both CV-1 and Vero cells showed positive IIF assay results, but there was no fluorescence staining in the negative control.

At 48 hours postinoculation on embryonated eggs, virus produced superficial pocks on the chorioallantoic membrane. Most of these pocks were small (diameter 1.0 mm), gray, and had central hemorrhages. Few (3%–5%)

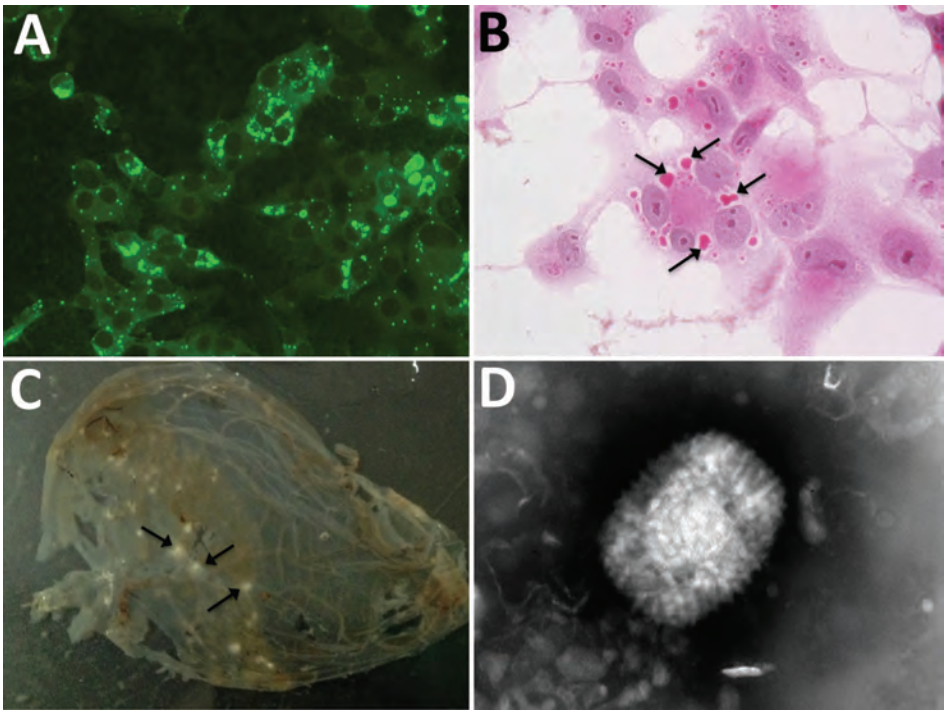

Figure 2. Analysis of an orthopoxvirus isolated from an infected cat, Italy. A) Cytoplasmic fluorescence in infected Vero cells using serum from the diseased cat (original magnification ×400). B) Cytoplasmic inclusion bodies (arrows) in infected Vero cells (hematoxylin and eosin stain, original magnification ×400). C) Pocks (arrows) in the inoculated chorioallantoic membrane of a 12-day-old chick embryo. D) Electron micrograph of orthopoxvirus-like particle from infected Vero cells. The virus preparation was negative-stained with sodium phosphotungstate (original magnification ×25,000).

pocks were larger (1.8 mm in diameter), white, and without hemorrhages.

Electron Microscopy

Many typical brick-shaped virions (≈320 × 240 nm) morphologically related to the genus *Orthopoxvirus* were observed by negative staining and electron microscopy. We observed these results for skin punch biopsy specimens and cell culture supernatants.

As in a previous study (*15*), few particles showed the characteristic ribbon structure of the M form of vaccinia virus (*38*) (Figure 2, panel D), which is usually prevalent in fresh preparations collected during acute-phase infections. Most virions were slightly larger, showed a uniform electron density, and had a thick capsule outlined by a ragged edge (i.e., the morphologic aspect known as the C form), which are less infective and prevalent during evolution of a chronic infection.

Serologic Analysis

The infected cat was negative by virus neutralization for strain Italy_09/17 and reference VACV isolates. However,

Table 1. PCR approach for identification of viruses of the subfamily *Chordopoxvirinae**

Specificity	Target gene	Reference	Result	Amplicon, bp	Sequence	First match by BLAST analysis†	Nucleotide identity, %
Panchordopoxvirus, low GC	Insulin metalloproteinase-like protein gene/IMV membrane protein gene	(*19*)	+	220	+	ECTV Naval KJ563295	100
Panchordopoxvirus, high GC	Insulin metalloproteinase-like protein gene/IMV membrane protein gene	(*19*)	ND	ND	ND	ND	ND
Eurasian/African OPXVs	A-type inclusion protein gene	(*20*)	+	1, 237‡	+	CPXV Germany 91–3 DQ437593	98
Eurasian/African OPXVs	Hemagglutinin gene	(*21*)	+	864§	+	Feline poxvirus ITA2 FJ445747	96
ECTV	Hemagglutinin gene	(*22*)	+	150	+	ECTV Naval KJ563295	99
CPXV	Hemagglutinin gene	(*22*)	ND	629–677	ND	ND	ND

*CPXV, cowpox virus: ECTV, ectromelia virus; IMV, intracellular mature virus; ND, not determined; OPXV, orthopoxvirus; +, sequence obtained.
†https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch. GenBank accession numbers are provided.
‡Expected size for CPXV: 1,601 or 1,673 bp; expected size for ECTV: 1,220 bp.
§Expected size for CPXV: 942 bp; expected size for ECTV: 846 bp.

the IIF assay detected antibody titers of 1:1,280 for virus Italy_09/17 and 1:640 for VACV-WR.

Identification of a Novel OPXV by NGS

We used 217,236 paired reads for de novo assembling and obtained 3 contigs (contig one, 195,015 bp; contig two, 21,014 bp; and contig three, 1,596 bp) and a quality score >99%. The mean coverage of the assembled contigs was 61×. The 9 open reading frames (A7L, A10L, A24R, D1R, D5R, E6R, E9L, H4L, and J6R) used for OPXV characterization were mapped in contig 1, and their sequences (total 27,228 nt) were concatenated and aligned with concatenated cognate sequences of selected OPXVs. In addition, because the HA gene of 2 feline-derived human virus isolates was available in the sequence databases, we performed an alignment based on the HA gene. We conducted phylogenetic analysis on the basis of the 9 concatenated sequences by using Bayesian inference. Posterior probabilities percentages were consistently high (≥90%) for all clades on phylograms, which supported inferred phylogenetic relationships.

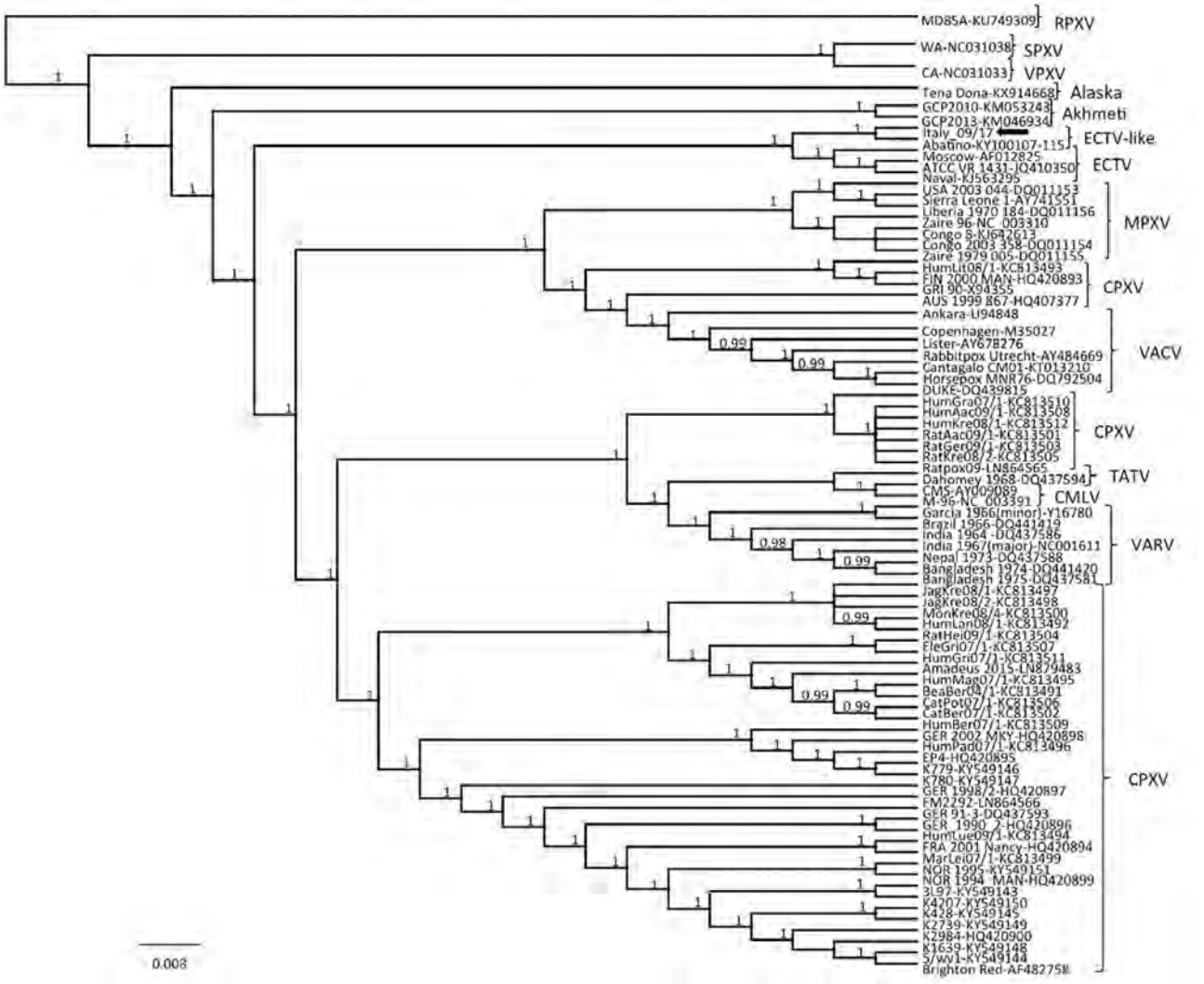

Figure 3. Phylogenetic relationship of extant orthopoxviruses with a feline poxvirus isolated from a cat, Italy. Phylogenetic tree shows 27,228 nt concatenated alignment of 9 coding gene (A7L, A10L, A24R, D1R, D5R, E6R, E9L, H4L, and J6R) sequences of orthopoxvirus. Gene designations refer to the VACV-COP genome (GenBank accession no. M35027). Posterior output of the tree was derived from Bayesian inference using 4 chains run for >1 million generations, a general time-reversible model, a proportion of invariable sites, a gamma distribution of rate variation across sites, and a subsampling frequency of 1,000. Posterior probability values >0.95 are indicated on the tree nodes. The black arrow indicates the feline poxvirus Italy_09/17 isolated in this study. Raccoonpox virus strain MD85A was used as an outgroup. Strain name, host and year of detection, location of origin, and GenBank accession numbers for orthopoxviruses used for phylogeny are shown in Table 2 (https://wwwnc.cdc.gov/EID/article/24/9/17-1283-T2.htm). Scale bar indicates nucleotide substitutions per site. CMLV, camelpox virus; CPXV, cowpox virus; ECTV, ectromelia virus; MPXV, monkeypox virus; RPXV, raccoonpox virus; SPXV, skunkpox virus; TATV, taterapox virus; VACV, vaccinia virus; VARV, variola virus; VPXV, volepox virus.

In the consensus phylogenetic tree (Figure 3), we found that strain Italy_09/17 was distantly related to other OPXVs, including all 10 CPXV lineages (*39*) and other recently identified, deep-branching OPXVs (*40,41*) and displayed a closer relatedness with ECTV prototypes, albeit forming a separate cluster. This cluster also included strain Abatino, which was recently isolated from a poxvirus outbreak in a captive colony of Tonkean macaques in Italy (*42*). Nucleotide identity of strain Italy_09/17 with strain Abatino was 99.66% and identity with reference ECTVs was 98.11%–98.13%. Higher nucleotide identities were found among ECTVs (99.97%–99.99%) and between VARV-Garcia1966 (variola minor) and VARV-India1967 (variola major) (99.68%). Thus, on the basis of current OPXV criteria of species demarcation, the cat and macaque isolates should be considered prototypes of a novel OPXV. Also, for the HA gene, strain Italy_09/17 appeared more closely related to strain Abatino (99.79% nt identity) than to feline-derived human OPXV strains (95.83%–95.99% nt identity) that were identified in Italy in 2009, for which a full-length genome and concatenated genes used for species demarcation using phylogeny are not available (*43*).

Discussion

OPXV infection in cats is frequently observed, and OPXV transmission from cats to humans has been demonstrated or at least suspected on several occasions (*8,11,14,43–47*). Cats are susceptible to CPXV infection, for which they represent only incidental hosts, as are humans, cattle, horses, and dogs. The virus is usually transmitted to cats by hunted rodents; cat-to-cat transmission is apparently rare (*47*). In contrast, ECTV has a host range restricted to laboratory mice, and cat or human infections have not been reported (*6*).

Additional OPXVs, such as raccoonpox virus and skunkpox virus, have been reported in wildlife and infect carnivores (*48*). Conversely, cats have been found to be susceptible to members of the genus *Parapoxvirus*, raccoonpox virus, and uncharacterized poxviruses (*49*). There are >400 reports of OPXV infection in domestic cats, but the total number of feline cases is considered to be much greater (*5*). Despite this large number of reports, genetic characterization of the detected poxvirus has been achieved in only a few instances. Thus, circulation in cats of other OPXVs cannot be ruled out.

We report detection of an OPXV strain that caused a fatal infection in a cat. The virus was not a classical CPXV, which is common in felids. Analysis of 9 concatenated genes showed that the poxvirus detected was only distantly related to all CPXV lineages currently known and formed a separate cluster with respect to ECTV, with which it was strictly related and grouped with an OPXV strain recently isolated from captive macaques in Italy (*42*). These 2 viruses had lower genetic identity with ECTV than that observed with reference ECTVs and between variola minor virus and variola major virus. Therefore, these ECTV-like poxviruses likely represent a novel OPXV species. However, the true animal reservoir of this novel OPXV needs to be assessed, and the idea that wild rodents can act as carriers for the new virus cannot be ruled out.

If one considers the close relatedness between strain Italy_09/17 and ECTV, which has been detected only in laboratory animals, it could be speculated that an ECTV-like virus circulating in wild rodents has resulted in ECTV strains adapted to laboratory mice. Alternatively, an ECTV strain might have escaped from laboratory mice and adapted to wild conditions. In addition, the zoonotic potential of the feline ECTV-like OPXV deserves an in-depth investigation. Feline poxvirus was also related to an unclassified OPXV, which was detected in a human in Italy almost 10 years ago and for which only partial HA gene has been identified (*43*). Consequently, this feline poxvirus could represent a threat to human health. Thus, veterinarians and cat breeders and owners should be aware of this additional risk associated with handling of cats with skin lesions.

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About the Author

Dr. Lanave is a postdoctoral fellow in the Department of Veterinary Medicine, University of Bari, Bari, Italy. His primary research interest is emerging viruses of animals.

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Address for correspondence: Nicola Decaro, Department of Veterinary Medicine, University of Bari, Strada Provinciale per Casamassima Km 3, 70010 Valenzano, Bari, Italy; email: nicola.decaro@uniba.it.

Emergence of Carbapenemase-Producing *Enterobacteriaceae*, South-Central Ontario, Canada[1]

Philipp P. Kohler,[2] Roberto G. Melano, Samir N. Patel, Shumona Shafinaz, Amna Faheem, Brenda L. Coleman, Karen Green, Irene Armstrong, Huda Almohri, Sergio Borgia, Emily Borgundvaag, Jennie Johnstone, Kevin Katz, Freda Lam, Matthew P. Muller, Jeff Powis, Susan M. Poutanen, David Richardson, Anu Rebbapragada, Alicia Sarabia, Andrew Simor, Allison McGeer, for the Toronto Invasive Bacterial Diseases Network (TIBDN)[3]

We analyzed population-based surveillance data from the Toronto Invasive Bacterial Diseases Network to describe carbapenemase-producing *Enterobacteriaceae* (CPE) infections during 2007–2015 in south-central Ontario, Canada. We reviewed patients' medical records and travel histories, analyzed microbiologic and clinical characteristics of CPE infections, and calculated incidence. Among 291 cases identified, New Delhi metallo-β-lactamase was the predominant carbapenemase (51%). The proportion of CPE-positive patients with prior admission to a hospital in Canada who had not received healthcare abroad or traveled to high-risk areas was 13% for patients with oxacillinase-48, 24% for patients with New Delhi metallo-β-lactamase, 55% for patients with *Klebsiella pneumoniae* carbapenemase, and 67% for patients with Verona integron-encoded metallo-β-lactamase. Incidence of CPE infection increased, reaching 0.33 cases/100,000 population in 2015. For a substantial proportion of patients, no healthcare abroad or high-risk travel could be established, suggesting CPE acquisition in Canada. Policy and practice changes are needed to mitigate nosocomial CPE transmission in hospitals in Canada.

Author affiliations: Sinai Health System, Toronto, Ontario, Canada (P.P. Kohler, S. Shafinaz, A. Faheem, B.L. Coleman, K. Green, E. Borgundvaag, S.M. Poutanen, A. McGeer); Public Health Ontario Laboratories, Toronto (R.G. Melano, S.N. Patel); University of Toronto, Toronto (R.G. Melano, S.N. Patel, B.L. Coleman, I. Armstrong, J. Johnstone, K. Katz, M.P. Muller, J. Powis, S.M. Poutanen, A. Sarabia, A. Simor, A. McGeer); Toronto Public Health, Toronto (I. Armstrong); LifeLabs, Toronto (H. Almohri); McMaster University, Hamilton, Ontario, Canada (S. Borgia); William Osler Health System, Brampton, Ontario, Canada (S. Borgia, D. Richardson); St. Joseph's Health Centre, Toronto (J. Johnstone); Public Health Ontario, Toronto (J. Johnstone, K. Katz, F. Lam); North York General Hospital, Toronto (K. Katz); St. Michael's Hospital, Toronto (M.P. Muller); Toronto East Health Network, Toronto (J. Powis); University Health Network, Toronto (S.M. Poutanen); Dynacare, Brampton (A. Rebbapragada); Trillium Health Partners, Toronto and Mississauga, Ontario, Canada (A. Sarabia); Sunnybrook Health Sciences Centre, Toronto (A. Simor)

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The global emergence of carbapenemase-producing *Enterobacteriaceae* (CPE) poses a threat to the achievements of modern medicine. The Centers for Disease Control and Prevention and the World Health Organization have recently classified CPE as one of the most urgent antimicrobial-resistance threats (*1*,*2*). CPE rarely arise de novo; rather, colonization and infection occur as a result of transmission of organisms, plasmids, or transposons from person to person, with such transmission occurring predominantly in healthcare institutions. An understanding of the epidemiology of the emergence of CPE and the changing burden over time is critical to the implementation of control programs and the management of individual patients.

In Canada, CPE were first reported in 2008 and have until recently been limited to individual cases and small outbreaks (*3*–*8*). Laboratory surveillance suggests substantial geographic variability, with *Klebsiella pneumoniae* carbapenemase (KPC) predominating in Quebec, whereas New Delhi metallo-β-lactamase (NDM) is most frequent in British Columbia (*9*,*10*). Nationally, time trends for CPE are discrepant; data from Canada's Nosocomial Infection Surveillance Program suggest stable CPE numbers in recent years, but data from voluntary laboratory reporting indicate a clear increase (*11*–*13*). To avoid the limitations of these surveillance systems and to better assess changes in disease burden and epidemiology in Ontario, we analyzed data from population-based surveillance for CPE.

[1]Preliminary results from this study were presented at IDWeek, October 26–30, 2016, New Orleans, Louisiana, USA.

[2]Current affiliation: Cantonal Hospital, St. Gallen, Switzerland.

[3]Additional members are listed at the end of this article.

Methods

Setting

Metropolitan Toronto (Toronto) and the Regional Municipality of Peel (Peel) are adjacent municipalities in south-central Ontario, Canada; the 2016 populations were 2.7 million for Toronto and 1.4 million for Peel (*14*,*15*). The Toronto Invasive Bacterial Diseases Network (TIBDN) is a collaborative network of microbiology laboratories, infection-control practitioners, and public health departments that performs population-based surveillance for infectious diseases in Toronto and Peel. TIBDN laboratories provide service to all hospitals and >87% of long-term care homes and physician offices serving area residents. Among TIBDN hospitals, 13% (3/23) perform admission screening for CPE colonization for all previously hospitalized patients, and an additional 65% (15/23) screen only if patients have been hospitalized outside of Canada (A. Jamal, Sinai Health System, unpub. data, 2018).

Data Sources

In Ontario, cases and clusters of CPE were first reported in 2008 (*16*). In 2011, guidelines for laboratory identification of CPE were published, and voluntary reporting to Public Health Ontario was initiated. In July 2014, TIBDN started active, population-based surveillance for laboratory-confirmed episodes of colonization or infection attributable to CPE. To identify CPE-colonized or -infected patients before July 2014, TIBDN laboratories and infection prevention and control programs accessed data from voluntary surveillance, searched microbiology laboratory databases for meropenem-nonsusceptible *Enterobacteriaceae*, reviewed hospital infection control department line lists and databases, and analyzed data from annual antimicrobial resistance reports from the Ontario Institute for Quality Management in Healthcare (IQMH). In addition, all isolates submitted for confirmatory testing to the Public Health Ontario Laboratory (PHOL), Canada's National Microbiology Laboratory (NML), and the Canadian Nosocomial Infection Surveillance Program were identified. During active surveillance, each newly identified case in TIBDN laboratories was reported to the central study office, with annual audits of participating and reference laboratories conducted to ensure complete case identification (*17*). Patient information was reviewed for each isolate to ensure that patients were counted only once.

Laboratory Identification of CPE

All 18 TIBDN laboratories are accredited by IQMH and follow IQMH recommendations for CPE identification, which include screening of all clinical isolates with an ertapenem MIC ≥1 mg/L or a meropenem disc diffusion diameter ≤25 mm. Before 2010, laboratories (including PHOL) used the modified Hodge test for screening; during 2010–2015, laboratories either sent all such isolates to PHOL for confirmation (n = 7) or screened with the modified Hodge test (n = 1), the ROSCO KPC + MBL confirm ID KIT (Rosco Diagnostica, Taastrup, Denmark) (n = 9), or by direct in-house PCR (n = 1) (*18*). All isolates with a positive screen in all years were tested by PCR for the presence of bla_{KPC}, bla_{OXA-48}–like, bla_{VIM}, bla_{NDM}, bla_{IMP}, and bla_{SME} genes at either PHOL (16 laboratories) (*19*) or NML (2 laboratories) (*20*).

For laboratory specimens yielding CPE, we recorded date of collection, body site, bacterial species, carbapenemase gene (or genes), reason for collection (i.e., screening versus clinical), and results of susceptibility testing. We used the first isolate from each patient to describe the distribution of bacterial species and carbapenemases. We reviewed charts associated with all isolates to identify CPE infections.

Data Collection and Definitions

We collected data by performing chart review for all patients. We approached patients first identified on or after January 1, 2013, to obtain consent, and we collected additional data by conducting interviews with patients or with next of kin if the patient was deceased or otherwise not able to provide information. We used a standard case report form to extract data from hospital or office charts from the admission or outpatient visit during which CPE was first identified and for any TIBDN hospital admissions in the prior year. We recorded demographic information, postal code of residence, co-occuring conditions (including Charlson index score) (*21*), antimicrobial drug use, proton-pump and immunosuppressive therapies, surgeries, intensive care unit admissions, and medical interventions.

We collected dates, hospital names, country, and reason for consultation for healthcare contacts within and outside of Canada in the year before the culture that identified each patient as being CPE colonized or infected. We obtained travel history within 1 year before CPE detection from patient interviews conducted by study staff or infection control practitioners. We defined high-risk travel as travel to the Indian subcontinent (India, Sri Lanka, Bangladesh, Pakistan, and Afghanistan) (*22*,*23*).

For bacteremia, a positive blood culture result sufficed for the diagnosis of infection. For all other culture sites, we defined infection as the presence of a positive clinical culture, a chart-documented physician diagnosis, and the initiation of targeted antimicrobial therapy. We calculated the 30-day mortality rate starting from the date the relevant clinical culture was obtained.

Statistical Analysis

We used SAS University Edition (SAS Institute, Cary, NC) for statistical analyses. We reported categorical variables as frequencies and proportions and continuous variables as median with interquartile range. We used

χ^2 or Fisher exact test, as appropriate, for comparison of dichotomous variables. For continuous variables, we used the Mann-Whitney U or Kruskal-Wallis test. We used the Benjamini and Hochberg procedure, with a false discovery rate of <0.05, to correct for multiple comparisons (*24*). We calculated incidence of CPE infection and bacteremia by using the first CPE infection or bacteremia from each resident of Toronto and Peel, on the basis of population estimates from Statistics Canada (*25*). We performed Poisson regression to assess time trends for all CPE infections, bloodstream infections, and sterile sites or urine isolates (*26*). We considered p values <0.05 statistically significant.

Results

Incidence and Outcome of CPE Infections

We identified 291 residents of Toronto or Peel who were colonized or infected with CPE during October 2007–December 2015. Charts were not available for 21 patients, and 12 patients declined consent. Among the remaining 258 patients, median age was 70 years (range 3 months–95 years), and 65% were male. Overall, 149 (58%) patients had ≥1 clinical isolate, and 92 (36%) had an infection caused by CPE. Urinary tract infections (n = 75 [82%]) were most common, followed by pneumonia and primary bacteremia (n = 13 [14%] each) (Table 1). Thirty-day mortality was 16% (15/92) for all infected patients and 31% (9/29) for patients with primary (5/13) or secondary (4/16) bacteremia.

The incidence of all CPE infections increased from 0 before 2007 to 0.33 cases/100,000 population in 2015 (p<0.0001); incidence of CPE bloodstream infections (primary and secondary) increased from zero before 2007 to 0.19 cases/100,000 population in 2015 (p = 0.045) (Figure 1). For patients with ≥1 sterile site (i.e., blood, pleural or peritoneal space, or bone) or urine isolate, the incidence in 2015 was 0.52 cases/100,000 population.

Patient Factors Associated with CPE Acquisition

In the year before CPE identification, 67% of patients had received antimicrobial drugs, 35% had undergone ≥1 surgical procedure, and 30% had had an intensive care unit admission. Overall, 71% (183/258) of CPE infections were categorized as hospital acquired (*27*). Risk profiles differed somewhat between patients with different carbapenemases (Table 2).

Travel history was available for 238 patients (92% of patients for whom clinical data were available); information was collected through patient interviews by study staff for 93 patients (39%) and from infection prevention and control staff for 145 patients (61%). A total of 142 patients (60%) had received healthcare abroad (n = 111) or reported travel to high-risk countries without a healthcare encounter (n = 31). Among these

Table 1. Isolate source and infection type among patients colonized or infected with carbapenemase-producing *Enterobacteriaceae*, Metropolitan Toronto and the Regional Municipality of Peel, south-central Ontario, Canada, 2007–2015*

	No. (%)				
Characteristic	All patients, n = 258	*Escherichia coli*, n = 86	*Klebsiella pneumoniae*, n = 122	*Enterobacter* spp., n = 30	Other,† n = 20
Isolate source‡					
Only screening	115 (45)	58 (67)	47 (39)	6 (20)	4 (20)
≥1 clinical	149 (58)	30 (35)	79 (65)	24 (80)	16 (80)
Positive specimen types at first identification§					
Rectal or colostomy	138 (54)	61 (71)	64 (53)	9 (30)	4 (20)
Urine	89 (35)	19 (22)	52 (43)	11 (37)	7 (35)
Blood	21 (8)	4 (5)	9 (7)	3 (10)	5 (25)
Wound	15 (6)	2 (2)	10 (8)	1 (3.3)	2 (10)
Sputum or broncoalveolar lavage	12 (5)	1 (1)	6 (5)	3 (10)	2 (10)
Other	17 (7)	4 (5)	5 (4)	6 (20)	2 (10)
Infection‡					
Any	92 (36)	21¶ (24)	46 (38)	13 (43)	12 (60)
Urinary tract	75 (29)	19 (22)	46 (38)	6 (20)	4 (20)
Pneumonia	13 (5)	4 (5)	3 (3)	4 (13)	2 (10)
Other#	13 (5)	3 (4)	5 (4)	3 (10)	2 (15)
Primary bacteremia**	13 (5)	2 (2)	4 (3)	2 (7)	1 (5)

*****Among first patient isolates; sums of specimen types exceed the number of patients because >1 specimen type may have yielded carbapenemase-producing *Enterobacteriaceae*.
†*Citrobacter* spp. (n = 7), *Morganella morganii* (n = 4), *Serratia marcescens* (n = 4), *Klebsiella oxytoca* (n = 3), *Providencia rettgeri* (n = 1), *Proteus mirabilis* (n = 1).
‡Including all follow-up isolates available and all infections during the patients' hospitalization.
§Including isolates from all specimens obtained within 2 days of the first positive specimen.
¶One patient originally colonized with a carbapenemase-producing *E. coli* subsequently experienced an infection with a carbapenemase-producing *Enterobacter cloacae*.
#Includes 7 skin or soft tissue infections, 5 bone or joint infections, and 1 abdominal infection.
**Patients with secondary bacteremia were classified according to their primary source of infection (urinary tract [n = 12] and pneumonia [n = 6]). Two bacteremic patients had both urinary tract infection and pneumonia diagnosed.

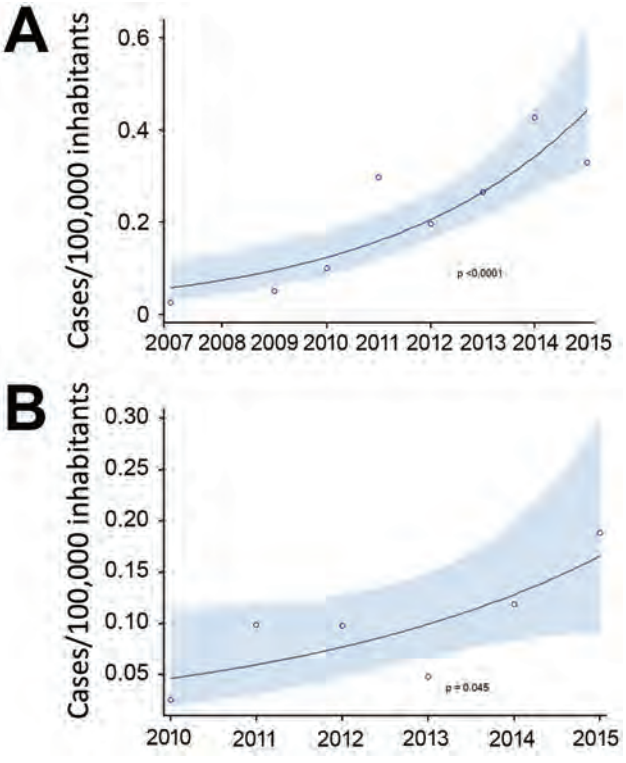

Figure 1. Incidence of all carbapenemase-producing enterobacterial infections per 100,000 inhabitants, 2007–2015 (A), and bloodstream infections per 100,000 inhabitants, 2010–2015 (B), calculated by using a Poisson regression model, Metropolitan Toronto and the Regional Municipality of Peel, south-central Ontario, Canada, 2007–2015. Shading indicates 95% CI.

patients, 95/97 (97%) with NDM-producing isolates and 14/19 (74%) with oxacillinase 48 (OXA-48)–producing isolates reported travel to the Indian subcontinent with or without a healthcare encounter. In contrast, 15 (68%) of 22 patients with KPC-producing isolates had received healthcare in the United States or southern Europe, and 2 of 3 patients with Verona integron-encoded metallo-β-lactamase (VIM)–producing isolates had been admitted to hospitals in Croatia (n = 1) and Portugal (n = 1).

The proportion of CPE-positive patients with prior admission to a hospital in Canada who had not received healthcare abroad or traveled to high-risk areas was 13% for patients with OXA-48, 24% for patients with NDM, 55% for patients with KPC, and 67% for patients with VIM (p = 0.001). Of the 17 patients without healthcare encounters in Ontario or elsewhere (i.e., patients with presumptive community-acquired CPE), 9 (8 with NDM and 1 with OXA-48) reported high-risk travel in the year before CPE identification. Of an additional 8 patients (4 with OXA-48 and 1 each with NDM, KPC, *Serratia marcescens* enzyme, and VIM), 4 had detailed interviews conducted by study staff and reported neither healthcare exposure nor high-risk travel (Figure 2).

Microbiology

Overall, NDM was the most common carbapenemase (148/291 isolates [51%]), followed by KPC (72/291 isolates [25%]). NDM was most commonly found in *Escherichia coli* (69/148 isolates [47%]) and *K. pneumoniae* (60/148 isolates [41%]), whereas KPC was found predominantly in *K. pneumoniae* (44/72 isolates [61%]). The type of carbapenemases varied considerably over time and between Toronto and Peel (Figure 3).

Fourteen percent (12/86) of tested isolates were susceptible to nitrofurantoin, 14% (18/131) to ciprofloxacin, 25% (36/142) to trimethoprim/sulfamethoxazole, 30% (43/142) to gentamicin, 52% (15/29) to tigecycline, and 88% (15/17) to colistin. Isolates containing NDM genes were less susceptible to all antimicrobial drugs than isolates with other carbapenemase genes (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/24/9/18-0164-Techapp1.pdf).

Discussion

Since the first detection of CPE in Ontario in 2007, the incidence of CPE infections has been increasing steadily. Most patients with CPE had a recent history of healthcare abroad or travel to high-risk countries; NDM and OXA-48 producers were associated with travel in the Indian subcontinent and KPC producers with healthcare encounters in the United States and Mediterranean countries (*22*,*23*). However, a notable proportion of CPE patients had received healthcare in Canada but had no history of healthcare or travel abroad, suggesting that CPE transmission is occurring in Canada. The small number of patients without a history of healthcare abroad or high-risk travel might represent community acquisition in Canada but might also have resulted from travel or healthcare encounters that occurred >1 year before CPE detection.

Measuring population-based incidence is key to understanding the burden of disease and prioritizing public health interventions; however, population-based surveillance for CPE is complex and has rarely been performed. A non–population-based US study using 2012–2013 data estimated that the population incidence of CPE from urine or sterile sites combined was 1.4 cases/100,000 population (*26*). In our study, the incidence of urine or sterile site CPE isolates was 0.5 cases/100,000 population for 2015, which is ≈40% of the overall US CPE incidence and higher than the incidence in Oregon or New Mexico. Comparing this incidence in Canada with incidence elsewhere in the world is difficult because of the lack of published data; nevertheless, our data emphasize the steady increase and the geographic variability in CPE occurrence.

In immediately adjacent urban areas in south-central Ontario, substantial differences exist in the incidence and epidemiology of CPE infection. The higher incidence of NDM producers in Peel is probably associated with the

Table 2. Characteristics of patients with carbapenemase-producing *Enterobacteriaceae* infections, by type of carbapenemase, Metropolitan Toronto and the Regional Municipality of Peel, south-central Ontario, Canada, 2007–2015*

Patient characteristics and risk profile	All patients, n = 258†	NDM, n = 145	KPC, n = 64	OXA-48, n = 32	VIM, n = 12	p value‡
Sex						
M	168 (65)	94 (65)	37 (58)	25 (78)	8 (67)	0.32
F	90 (35)	51 (35)	27 (42)	7 (22)	4 (33)	
Age, y, median (IQR‡)	70 (57–79)	70 (59–79)	70 (50–79)	70 (52–77)	77 (65–88)	0.37
Charlson index score >2§	88 (34)	47 (32)	25 (39)	7 (22)	7 (58)	0.15
Inpatient at time of diagnosis	233 (90)	129 (89)	58 (91)	29 (91)	12 (100)	0.85
Days from admission to diagnosis, median (IQR)¶	2.5 (0–21)	0 (0–11)	14 (0–41)	0 (0–11)	19 (5–67)	**0.03**
CPE acquisition according to SHEA definitions#						
Hospital acquired, hospital onset	113 (44)	55 (38)	35 (55)	12 (38)	8 (67)	0.10
Hospital acquired, community onset	70 (27)	41 (28)	21 (33)	4 (13)	3 (25)	0.24
Undetermined	58 (23)	40 (28)	7 (11)	11 (34)	0	**0.024**
Community acquired	17 (7)	9 (6)	1 (2)	5 (16)	1 (8)	0.12
Residing in long-term care facility	9 (4)	2 (2)	4 (7)	0	3 (25)	**0.018**
Healthcare abroad or high-risk travel**	142/238 (60)	98/135 (73)	22/59 (37)	19/27 (70)	3/12 (25)	**0.0012**
Exposures and medical interventions††						
Intensive care stay	78 (30)	39 (27)	26 (41)	6 (19)	5 (42)	0.13
Mechanical ventilation	52 (20)	24 (17)	20 (31)	3 (9)	3 (25)	0.11
Previous surgery	91 (35)	29 (20)	41 (64)	13 (41)	4 (33)	**0.0012**
Central venous catheter	86 (33)	41 (28)	32 (50)	9 (28)	2 (17)	**0.03**
Antibiotic exposure, any	173 (67)	92 (64)	51 (80)	16 (50)	10 (83)	**0.03**
3rd- and 4th-generation cephalosporins	74 (29)	40 (28)	18 (28)	7 (22)	7 (58)	0.16
Carbapenems	33 (13)	14 (10)	12 (19)	3 (9)	3 (25)	0.17
Quinolones	81 (31)	41 (28)	26 (41)	6 (19)	7 (58)	0.05

*****Values are no. (%) except as indicated. All characteristics and risk profile descriptors apply to the 1-year period preceding CPE detection. CPE, carbapenemase-producing *Enterobacteriaceae*; IQR, interquartile range; KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; OXA-48, oxacillinase 48; SHEA, Society for Healthcare Epidemiology of America; VIM, Verona integron-encoded metallo-β-lactamase.
†Three patients with *Serratia marcescens* enzyme and 2 with non–metallo-carbapenemase are not listed separately.
‡p values corrected for multiple testing with the Hochberg and Benjamini procedure. Bold type indicates statistical significance (p<0.05).
§No significant differences observed for any comorbid conditions.
¶Only patients included where first isolate is a clinical sample (n = 126).
#Defined as hospital acquired if hospital admission occurred within 90 days before CPE detection.
**High-risk countries and the Indian subcontinent. Denominators indicate no. patients with travel information available.
††Not listed because of nonsignificance: bronchoscopy, cystoscopy, dialysis, Foley catheter, urostomy, colostomy, tracheostomy, blood transfusion, proton-pump inhibitors, steroids, chemotherapy, immunosuppression, previously identified antibiotic-resistant pathogens (e.g., methicillin-resistant *Staphylococcus aureus* and extended-spectrum β-lactamase).

fact that ≈28% of the local population is of South Asian descent compared with ≈12% in Toronto (*14*,*15*). Our finding that 51% of NDM carriers had healthcare encounters and an additional 21% reported travel to the Indian subcontinent supports the hypothesis that NDM is often introduced from these highly endemic countries. In contrast, patients with KPC and VIM producers more often do not have a history of high-risk travel or healthcare abroad, suggesting that CPE was acquired in hospitals in Canada. The facts that 1) KPC and VIM most commonly occurred in species associated with hospital-acquired infections (*K. pneumoniae* and *Enterobacter* spp.) whereas *E. coli*, the main cause of community-acquired enterobacterial infections, almost exclusively harbored NDM and OXA-48; 2) clinical isolates producing KPC or VIM were detected later in the course of hospitalization; and 3) most patients with KPC producers had had previous surgery or a central venous catheter, are consistent with other studies and with these isolates having been acquired during hospital admission (*28*,*29*). Similarly, in a Germany study, a higher proportion of patients with OXA-48 had traveled before CPE detection compared with patients with VIM, suggesting nosocomial acquisition of VIM producers (*30*). In a multicenter study conducted in 34 hospitals in Spain, VIM producers were also more likely to be hospital acquired than OXA-48 producers (*31*).

The fact that most CPE in our study population appear to be acquired in healthcare settings strongly suggests that intensification of control programs in this population is needed if we wish to protect patients from the impacts of CPE (*32*,*33*). Although the cost of control programs is a concern, the relatively low incidence of CPE in our population should be an incentive to implement such programs; control programs have been shown to be cost-effective in low-prevalence areas (*34*,*35*), and success in transmission control programs is more likely when they are implemented while prevalence of colonization is low (*33*). Our data are consistent with a recent assessment of CPE transmission in England; although we might perceive that large problems in India pose the greatest risk, the much larger number of our patients exposed to a smaller problem in Ontario likely poses the greater risk to our patient population (*36*).

Relative to isolates from other countries, CPE isolates in Toronto and Peel are more susceptible to commonly used antimicrobial drugs (*37*). Nonetheless, most isolates are resistant to all commonly used orally available

	Healthcare abroad, n = 111	High-risk travel, n = 31	Low-risk travel, n = 16
NDM	Indian subcontinent (n = 69), Vietnam (n = 1), Thailand (n = 1), Egypt (n = 1)	Indian subcontinent (n = 26)	China (n = 1), Philippines (n = 2), United States (n = 1), France (n = 1), Jamaica (n = 1), Vietnam (n = 1), Israel (n = 1)
KPC	United States (n = 6), Italy (n = 3), Greece (n = 3), Portugal (n = 2), Indian subcontinent (n = 2), Barbados (n = 2), Brazil (n = 1), Vietnam (n = 1), France (n = 1)	Indian subcontinent (n = 1)	US (n = 3), France (n = 1)
OXA-48	Indian subcontinent (n = 10), Egypt (n = 2), Saudi Arabia (n = 2), United States (n = 1)	Indian subcontinent (n = 4)	Spain (n = 1), Turkey (n = 1)
VIM	Croatia (n = 1), Portugal (n = 1), United States (n = 1)	None	Austria–Germany (n = 1)
Other	None	None	Dominican Republic (n = 1)

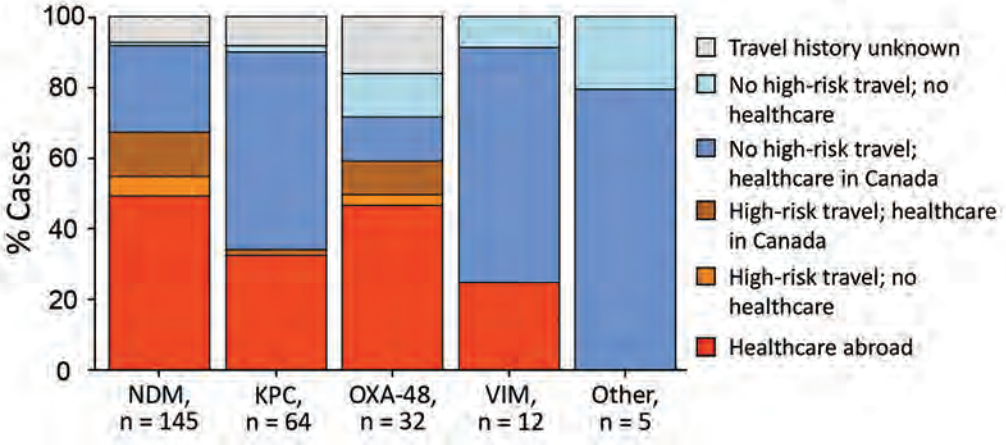

Figure 2. Healthcare visits abroad and travel history in patients with carbapenemase-producing *Enterobacteriaceae* infection in the 1 year before detection, stratified by type of carbapenemase, Metropolitan Toronto and the Regional Municipality of Peel, south-central Ontario, Canada, 2007–2015. Patients who traveled to any location other than the Indian subcontinent were classified as low-risk travel and indicated as no high-risk travel in the graph. n values indicate number of patients. KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; OXA-48, oxacillinase 48; VIM, Verona integron-encoded metallo-β-lactamase.

antimicrobial drugs, and choices for parenteral therapy are limited. These concerns emphasize the need for the continued development of new antimicrobial drugs active against these resistant organisms.

Our study has several limitations. Although laboratory testing in Ontario is standardized, the modified Hodge test, the only screening test available before 2011, might have missed a small number of CPE during this period. However, PCR screening of all meropenem-nonsusceptible *Enterobacteriaceae* isolates from 4 TIBDN teaching hospitals during 2009–2011 at NML identified only a single additional CPE. Further, the increase in CPE infection incidence during 2010–2015 remains statistically significant. Because most TIBDN hospitals screen only patients who have accessed healthcare outside of Canada, our data on colonization will be biased toward the identification of CPE in these populations. This bias will underestimate the number of patients with colonization acquired in Canada. Similarly, our surveillance system detects only laboratory-confirmed infections, and infections for which cultures are not obtained will have been missed. This misclassification error might be lower for CPE than other organisms because resistance by CPE means that they might fail empiric therapy. We used a definition of high-risk countries for travel and healthcare currently used in Ontario hospitals (A. Jamal, Sinai Health System, unpub. data, 2018), but surveillance data are not available for many countries to validate this definition. In addition, we asked only about travel in the preceding year, and some infection control departments might only have asked about high-risk travel. We do not have molecular typing data for all isolates, which limits our ability to detect transmission within Canada. Similarly, we do not have data regarding the investigation of transmission or environmental reservoirs at individual hospitals. Although we have corrected for multiple comparisons, particular caution should be used in interpreting the statistical significance of comparisons with p values close to 0.05. We did not identify endoscopy as a risk factor for acquisition of CPE; however, our power to do so might have been limited, and exposure to outpatient endoscopy might not have been captured in patients with data from chart review only.

In conclusion, the incidence of CPE infection is increasing in south-central Ontario. Our data suggest that, even early on in the emergence of CPE, a substantial

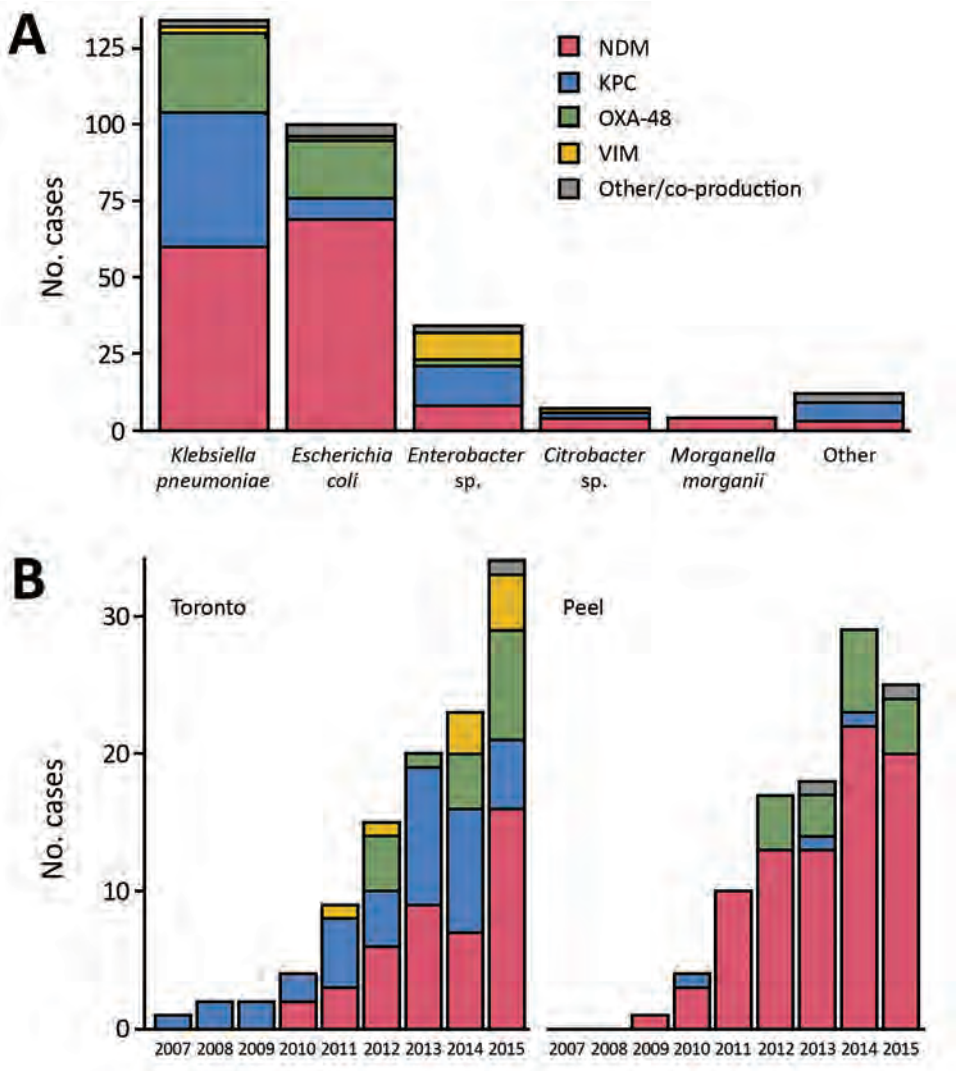

Figure 3. Distribution of carbapenemases in 291 first isolates of carbapenemase-producing *Enterobacteriaceae*, by enterobacterial species (A) and region (B), Metropolitan Toronto and the Regional Municipality of Peel, south-central Ontario, Canada, 2007–2015. Other enterobacterial species were *Serratia marcescens* (n = 4), *Klebsiella oxytoca* (n = 3), *Providencia rettgeri* (n = 1), and *Proteus mirabilis* (n =1). Other carbapenemases or co-productions were NDM–OXA-48 (n = 2) and *S. marcescens* enzyme (n = 1). KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; OXA-48, oxacillinase 48; VIM, Verona integron-encoded metallo-β-lactamase.

proportion of CPE infections are autochthonous cases, including most of those with KPC- and VIM-producing isolates. Policy and practice changes are needed to better protect patients from CPE exposure and acquisition in southern Ontario.

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About the Author

Dr. Kohler is an infectious disease specialist currently working at the Cantonal Hospital in St. Gallen, Switzerland. His research interests include epidemiologic aspects of antibiotic resistance with a focus on gram-negative pathogens and nosocomial outbreaks.

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Address for correspondence: Philipp P. Kohler, Cantonal Hospital St. Gallen, Clinic for Infectious Diseases and Hospital Epidemiology, Rorschacher Strasse 95, 9007 St. Gallen, Switzerland; email: philipp.kohler@kssg.ch

From Culturomics to Clinical Microbiology and Forward

Grégory Dubourg, Sophie Baron, Frédéric Cadoret, Carine Couderc, Pierre-Edouard Fournier, Jean-Christophe Lagier, Didier Raoult

Culturomics has permitted discovery of hundreds of new bacterial species isolated from the human microbiome. Profiles generated by using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry have been added to the mass spectrometer database used in clinical microbiology laboratories. We retrospectively collected raw data from MALDI-TOF mass spectrometry used routinely in our laboratory in Marseille, France, during January 2012–March 2018 and analyzed 16S rDNA sequencing results from misidentified strains. During the study period, 744 species were identified from clinical specimens, of which 21 were species first isolated from culturomics. This collection involved 105 clinical specimens, accounting for 98 patients. In 64 cases, isolation of the bacteria was considered clinically relevant. MALDI-TOF mass spectrometry was able to identify the species in 95.2% of the 105 specimens. While contributing to the extension of the bacterial repertoire associated with humans, culturomics studies also enlarge the spectrum of prokaryotes involved in infectious diseases.

The diagnosis of bacterial diseases in clinical microbiology has relied on phenotypic identification, based on the bacterial repertoire known to be associated with humans. This mode of identification, which is, in fact, recognition of previously described microorganisms, does not allow for the identification of new bacteria. Recently, the systematic use of universal 16S rDNA gene sequencing of cultivated bacteria that presented an atypical phenotypical profile paved the way for identifying rare, fastidious, and new microorganisms (*1*,*2*). However, this method implies redefining specific phenotypical characteristics, which sometimes cannot be done because of the limited number of available biochemical tests. More recently, the revolution provided by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry identification permits comparison of a protein spectrum obtained from a colony with a database, which can be permanently incremented with newly identified bacteria (*3*,*4*). The use of a cutoff identification score, with values in the range of 1.7–2, enables correct identification of the isolate. However, when MALDI-TOF mass spectrometry recognizes bacteria never previously associated with humans, it is reasonable to carry out confirmation by sequencing the 16S rDNA gene. The main advantage of MALDI-TOF mass spectrometry compared with sequencing methods is that it is extremely fast and cost-effective (*3*,*4*). Indeed, the cost involves mainly the cost of the machine; the individual cost per test is insignificant. Thus, the ease in testing bacterial colonies led us to establish the repertoire of commensal bacteria of the human microbiota in the laboratory at IHU Méditerranée Infection in Marseille, France, by using a high-throughput culture and MALDI-TOF mass spectrometry identification. Sequencing of the 16S rDNA gene enables identification of atypical bacteria with definition of new bacterial species, whose genomes are then sequenced. This approach, called culturomics (*5*,*6*), has made possible the addition of 672 bacteria to the known repertoire of the bacteria already isolated from the human mucosa. Other teams, in parallel, have used similar approaches (*7*,*8*).

The usefulness of culturomics in increasing knowledge of the repertoire of cultivable bacteria from human mucous membranes appears clear for microbiota studies. However, the benefit of this process in clinical microbiology is prone to controversy. We speculated that commensal bacteria found in humans may be involved in opportunistic infections. In our experience, the creation of new spectra enabled us to increment our MALDI-TOF mass spectrometry database used for clinical microbiology, thus enabling recognition of bacterial species first isolated as a part of culturomics studies and improving the accuracy of diagnosis of infectious diseases involving bacteria.

Author affiliations: Aix Marseille University, Institut de Recherche pour le Développement (IRD), Assistance Publique des Hôpitaux de Marseille (APHM), Microbes, Evolution, Phylogeny and Infections (MEPHI), Institut Hospitalo-Universitaire (IHU) Méditerranée Infection, Marseille, France (G. Dubourg, S. Baron, J.-C. Lagier, D. Raoult); APHM, IHU Méditerranée Infection, Marseille (F. Cadoret, C. Couderc); Aix Marseille University, IRD, APHM, Vecteurs–Infections Tropicales et Méditerranéennes, IHU Méditerranée Infection, Marseille (P.-E. Fournier)

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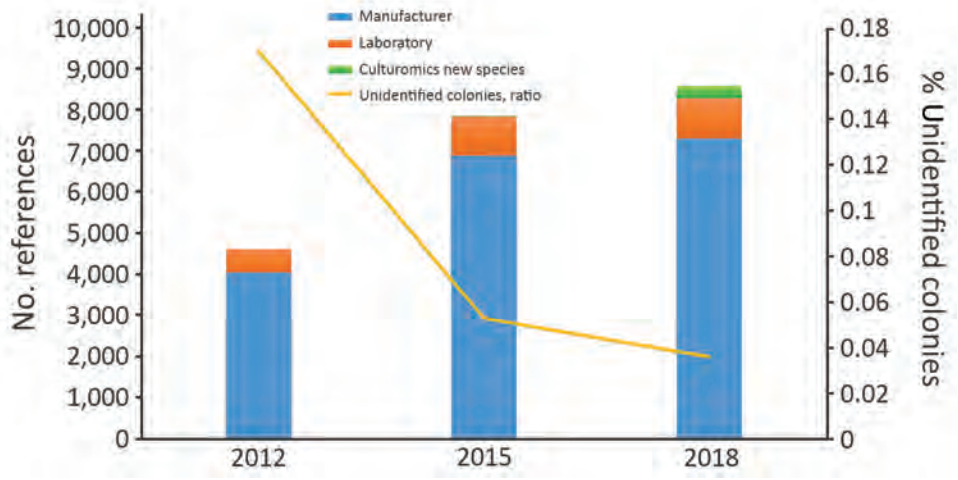

Figure. Annual ratio of unidentified bacteria and evolution of the number of spectral references available in the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry database in a clinical laboratory in Marseille, France.

Materials and Methods

Settings

All data included in this study were obtained from the routine microbiology laboratory at IHU Méditerranée Infection, which receives a mean annual number of 350,400 samples from the 4 Marseille university hospitals (Timone, Conception, North, and Sainte-Marguerite hospitals), which contain a total of 3,700 beds. Retrospective data were collected for January 2012–March 2018.

Routine Bacteriological Practices

We analyzed samples according to standard microbiological procedures, as previously described, depending on the specimen (*9*–*12*). This process included systematic inoculation onto Columbia agar with 5% sheep blood (BioMérieux, Craponne, France), chocolate agar (BioMérieux) (excluding urine and fecal samples), and specific media such as colistin-nalidixic agar or MacConkey agar (both BioMérieux) for specimens potentially contaminated by resident flora. Blood cultures were incubated into a Bactec device (Becton Dickinson, Le Pont de Claix, France) and analyzed as previously described (*9*).

Specific Cultures

We plated fecal specimens taken following a regional outbreak of *Clostridioides* (formerly *Clostridium*) *difficile* 027 during May 2013–March 2018 (*13*), in which toxin detection was positive using GeneXpert *C. difficile* PCR (Cepheid, Paris, France) after ethanol treatment (*14*), to obtain *C. difficile* isolates. We also investigated possible multidrug-resistant bacteria carriage by plating on chromID MRSA agar for methicillin-resistant *Staphylococcus aureus*, chromID CARBA SMART medium (BioMérieux) for carbapenemase-producing *Enterobacteriaceae* (CPE), and Drigalski/MacConkey agar (BioMérieux) for third-generation, cephalosporin-resistant, gram-negative bacteria.

Identification of Colonies

We performed bacterial identification on colonies using MALDI-TOF mass spectrometry, as previously described (*3*,*4*). We considered identification to be correct when the identification score was ≥1.9 and when the same single species was recognized. When identification did not meet these criteria, we performed proteic extraction using formic acid and acetonitrile (*15*). If identification was still incorrect following the proteic extraction protocol, we performed 16S rDNA sequencing systematically, as previously described (*16*), in 3 situations: when the identification score was <1.9 despite proteic extraction, when multiple different species were recognized with a correct identification score, and when a bacterium was isolated for the first time in the clinical microbiology laboratory.

Culturomics Studies

In brief, culturomics consists of the multiplication of culture conditions applied to human specimens to increase the repertoire of the human microbiome. The pioneering study used 212 conditions (*5*); this number was reduced to 70 in 2012 (*17*,*18*) and then to 18 in 2014 (*6*). In addition, several specific conditions were designed for archaea, microcolonies, proteobacteria, and microaerophilic and halophilic bacteria. Most specimens used were fecal samples. However, respiratory, vaginal, and urine samples have been analyzed recently in the context of culturomics studies. Identification has also been performed using MALDI-TOF mass spectrometry. Colonies were considered correctly identified when 2 colonies exhibited an identification score ≥1.9. If identification scores were not correct after 3 attempts, sequencing of the 16S rDNA gene was performed (*16*). If there was <98.7% similarity with the closest neighbor, the bacterial isolate was considered to be a new species (*19*).

Updating the MALDI-TOF Mass Spectrometry Database

The database used for routine bacterial identification is updated through 3 sources: updates from the MALDI-TOF mass spectrometry manufacturer, updates from culturomics studies, and routine laboratory results. Updates from culturomics studies and routine laboratory results are based on 16S rDNA sequencing results.

Analysis of Data from MALDI-TOF Mass Spectrometry Used in the Clinical Microbiology Laboratory

We retrospectively collected raw data from MALDI-TOF mass spectrometry used in the microbiology laboratory involving identifications performed during January 2012–March 2018, which are saved monthly. Data were deduced from the samples. These data do not consider the clinical relevance of the identified microorganism, the final result, or multiple attempts to identify the colony using MALDI-TOF mass spectrometry.

Results

Bacterial Identification in Clinical Microbiology Laboratory

During January 2012–March 2018, the clinical microbiology laboratory performed 351,937 nondereplicated bacterial identifications using MALDI-TOF mass spectrometry. Of these, 28,391 (8.1%) were unidentified or misidentified. When we looked at the yearly ratio of unidentified bacteria, we noticed that it fell from 17.7% in 2012 to 3.6% in 2018 (Figure). Overall, we identified 744 unique bacterial species correctly using MALDI-TOF mass spectrometry.

Contribution to MALDI-TOF Mass Spectrometry Database Updates

During the study period, we added 4,539 references to our database. Updates from the manufacturer comprised 3,255 references, whereas 983 references came from routine

Table 1. Main features of the bacteria discovered as part of culturomics studies identified in a clinical microbiology laboratory*

Species	Culturomics study	CSUR no.	Strain	GenBank accession no.	Date of spectrum implementation	No. cases	References
Actinomyces bouchesdurhonensis	Gut microbiota (storied samples)	P2825	Marseille-P2825T	LT576385	2017 Apr	3	Unpub. data
Actinomyces ihuae	Gut microbiota (HIV)	P2006	SD1	LN866997	2015 Jul	17	(*6*,*20*)
Actinomyces marseillensis	Respiratory microbiota	P2818	Marseille-P2818T	LT576400	Not added	1	(*21*)
Alistipes jeddahensis	Gut microbiota	P1209	AL1	LK021116	2015 Oct	4	(*6*,*22*)
Anaerosalibacter massiliensis	Gut microbiota (Polynesia)	P762	ND1	HG315673	2013 Apr	1	(*6*,*23*)
Bacteroides timonensis	Gut microbiota (anorexia nervosa)	P194	AP1	JX041639	2016 Apr	2	(*6*,*24*)
Butyricimonas phocaeensis	Gut microbiota (obese)	P2478	AT9	LN881597	2015 Nov	1	(*6*,*25*)
Clostridium culturomicsense	Gut microbiota (Saudian obese)	P1184	CL6	LK021117	2014 Sep	1	(*6*,*26*)
Clostridium jeddahtimonense	Gut microbiota (obese)	P1230	CL2	LK021118	2014 Aug	7	(*6*)
Clostridium massilioamazoniense	Gut microbiota (Polynesia)	P1360	ND2	HG315672	2013 May	1	(*6*)
Clostridium saudii	Gut microbiota (Saudian obese)	P697	JCC	HG726039	2014 Aug	11	(*6*,*27*)
Corynebacterium ihuae	Gut microbiota (antimicrobials)	P892	GD6	JX424768	2013 May	3	(*6*,*28*)
Corynebacterium lascolaense	Urinary microbiota	P2174	MC3	LN881612	2013 Sep	6	(*6*)
Corynebacterium phoceense	Urinary microbiota	P1905	MC1	LN849777	2015 May	12	(*6*,*29*)
Gabonia massiliensis	Gut microbiota	P1910	GM3	LN849789	2017 Apr	1	(*6*,*30*)
Nosocomicoccus massiliensis	Gut microbiota (HIV)	P246	NP2	JX424771	2012 Feb	1	(*6*,*31*)
Peptoniphilus grossensis	Gut microbiota (obese)	P184	ph5	JN837491	2015 Nov	18	(*6*,*32*)
Polynesia massiliensis	Gut microbiota (Polynesia)	P1280	MS3	HF952920	2013 Mar	1	(*6*)
Prevotella ihuae	Gut microbiota (fresh feces)	P3385	Marseille-P3385T	LT631517	Not added	1	(*33*)
Pseudomonas massiliensis	Gut microbiota (Polynesia)	P1334	CB1	LK985396	2015 Apr	5	(*6*,*34*)
Varibaculum timonense	Gut microbiota (fresh feces)	P3369	Marseille-P3369T	LT797538	Not added	1	(*33*)

*Accession numbers indicate nucleotide sequences. CSUR, Collection de Souches de l'Unité des Rickettsies (an international strain collection).

Table 2. Identification of bacterial pathogens by MALDI-TOF mass spectrometry, Marseille, France*

Species	MALDI-TOF identification (score)	Specimen	Duplicates per patient?†
Actinomyces bouchesdurhonensis	*Actinomyces bouchesdurhonensis* (1.85)	Pharynx swab	No
A. bouchesdurhonensis	*A. bouchesdurhonensis* (1.9)	Abscess	No
Actinomyces ihuae	*Actinomyces ihuae* (1.97)	Abscess	No
A. ihuae	*A. ihuae* (1.9)	Abscess	No
A. ihuae	*A. ihuae* (2.5)	Abscess	No
A. ihuae	*A. ihuae* (1.92)	Abscess	No
A. ihuae	*A. ihuae* (2.5)	Abscess	No
A. ihuae	*A. ihuae* (1.73)	Abscess	No
A. ihuae	*A. ihuae* (2.23)	Abscess	No
A. ihuae	*A. ihuae* (2.2)	Abscess	No
A. ihuae	*A. ihuae* (2.1)	Abscess	No
A. ihuae	*A. ihuae* (2.1)	Bone	No
A. ihuae	*A. ihuae* (2.52)	Puncture fluid	No
A. ihuae‡	*A. ihuae* (2.47)	Puncture fluid	No
A. ihuae‡	*Actinomyces* spp. (1.65)	Biopsy	No
A. ihuae‡	*A. ihuae* (2.32)	Abscess	No
A. ihuae‡	*A. ihuae* (2.33)	Abscess	No
A. ihuae‡	*A. ihuae* (1.95)	Puncture fluid	No
A. ihuae‡	*A. ihuae* (2.07)	Abscess	No
Actinomyces marseillensis	*Actinomyces marseillensis* (NA)	Blood culture	No
Alistipes jeddahensis	*Alistipes jeddahensis* (1.97)	Abscess	No
Bacteroides timonensis	*Bacteroides timonensis* (1.95)	Blood culture	Yes
B. timonensis	*B. timonensis* (1.88)	Blood culture	Yes
B. timonensis	*B. timonensis* (1.96)	Blood culture	No
Corynebacterium ihuae‡	*Corynebacterium ihuae* (2)	Blood culture	No
C. ihuae	*C. ihuae* (2.2)	Wound	No
C. ihuae	*C. ihuae* (1.8)	Blood culture	No
Corynebacterium lascolaense	*Corynebacterium lascolaense* (2.2)	Urine	No
C. lascolaense	*C. lascolaense* (2.3)	Pacemaker	No
C. lascolaense	*C. lascolaense* (2.1)	Urine	Yes
C. lascolaense	*C. lascolaense* (2.14)	Urine	Yes
C. lascolaense‡	*C. lascolaensis* (2.2)	Urine	No
Corynebacterium phoceense	*Corynebacterium phoceense* (1.91)	Urine	No
C. phoceense	*Corynebacterium* spp. (2.3)	Unknown	No
C. phoceense	*C. phoceense* (2.6)	Blood culture	No
C. phoceense‡	No reliable identification	Blood culture	No
Nosocomicoccus massiliensis‡	*Nosocomicoccus massiliensis* (2.3)	Blood culture	No
Peptinophilus grossensis	*Peptinophilus grossensis* (2.1)	Abscess	No
P. grossensis	*P. grossensis* (2.18)	Biopsy	No
P. grossensis	*P. grossensis* (1.9)	Abscess	No
P. grossensis	*P. grossensis* (2.3)	Biopsy	No
P. grossensis	*P. grossensis* (1.9)	Biopsy	Yes
P. grossensis	*P. grossensis* (2.2)	Biopsy	Yes
P. grossensis	*P. grossensis* (2.18)	Biopsy	No
P. grossensis	*P. grossensis* (2)	Material	No
P. grossensis	*P. grossensis* (1.78)	Abscess	No
P. grossensis	*P. grossensis* (2.1)	Abscess	No
P. grossensis	*P. grossensis* (2.15)	Abscess	No
P. grossensis	*P. grossensis* (1.9)	Puncture fluid	No
P. grossensis	*P. grossensis* (2.1)	Puncture fluid	Yes
P. grossensis	*P. grossensis* (2.3)	Puncture fluid	Yes
P. grossensis	*P. grossensis* (2.2)	Puncture fluid	No
P. grossensis	*P. grossensis* (2.1)	Abscess	Yes
P. grossensis	*P. grossensis* (2.1)	Abscess	Yes
P. grossensis	*P. grossensis* (1.9)	Puncture fluid	No
P. grossensis	*P. grossensis* (2.3)	Biopsy	No
P. grossensis	*P. grossensis* (2.31)	Biopsy	No
P. grossensis	*P. grossensis* (1.86)	Abscess	No
Polynesia massiliensis	*Polynesia massiliensis* (2.21)	Peritoneal fluid	No
Prevotella ihuae	No reliable identification	Abscess	No
Pseudomonas massiliensis	*Pseudomonas massiliensis* (2.5)	Blood culture	No
Pseudomonas massiliensis	*Pseudomonas massiliensis* (2)	Blood culture	No
Pseudomonas massiliensis‡	*Pseudomonas massiliensis* (1.9)	Blood culture	No
Varibaculum timonense	No reliable identification	Abscess	No

*MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; NA, not available.
†Replicated isolates in different specimens from the same patient.
‡Strains for which 16S rDNA sequencing was performed.

Table 3. Identification of bacteria discovered as a part of culturomics studies in the clinical microbiology laboratory as commensals, Marseille, France*

Species	MALDI-TOF mass spectrometry identification (score)	Specimen	Duplicates per patient?†	Additional information
Actinomyces bouchesdurhonensis	*A. bouchesdurhonensis* (2)	Larynx biopsy	No	Polymicrobial
Alistipes jeddahensis	*Alistipes jeddahensis* (2.38)	Liquid feces	No	Seeking *Salmonella* spp.
A. jeddahensis	*A. jeddahensis* (2.45)	Liquid feces	No	Seeking *Salmonella* spp.
A. jeddahensis	*A. jeddahensis* (2.5)	Liquid feces	No	Seeking *Salmonella* spp.
Anaerosalibacter massiliensis	*Anaerosalibacter massiliensis* (1.78)	Rectal swab	No	Seeking MDR bacteria
Butyricimonas phocaeensis	*Butyricimonas phoaceensis* (2.36)	Liquid feces	No	Seeking toxigenic CD
Clostridium culturomicsense	*Clostridium culturomicsense* (2)	Liquid feces	No	Seeking toxigenic CD
Clostridium jeddahtimonense	*Clostridium jeddahtimonense* (2.1)	Liquid feces	No	Seeking toxigenic CD
C. jeddahtimonense	*C. jeddahtimonense* (2.3)	Liquid feces	No	Seeking toxigenic CD
C. jeddahtimonense	*C. jeddahtimonense* (2.4)	Liquid feces	No	Seeking toxigenic CD
C. jeddahtimonense	*C. jeddahtimonense* (2.1)	Liquid feces	No	Seeking toxigenic CD
C. jeddahtimonense	*C. jeddahtimonense* (2.2)	Liquid feces	Yes	Seeking toxigenic CD
C. jeddahtimonense	*C. jeddahtimonense* (1.72)	Liquid feces	Yes	Seeking toxigenic CD
C. jeddahtimonense	*C. jeddahtimonense* (2.3)	Liquid feces	No	Seeking toxigenic CD
C. jeddahtimonense	*C. jeddahtimonense* (2.1)	Liquid feces	No	Seeking toxigenic CD
Clostridium massilioamazoniense	*Clostridium massilioamazoniense* (1.7)	Liquid feces	No	Seeking toxigenic CD
Clostridium saudii	*Clostridium saudii* (2.5)	Liquid feces	No	Seeking toxigenic CD
C. saudii	*C. saudii* (1.74)	Liquid feces	No	Seeking toxigenic CD
C. saudii	*C. saudii* (2.5)	Liquid feces	No	Seeking toxigenic CD
C. saudii	*C. saudii* (1.94)	Liquid feces	No	Seeking toxigenic CD
C. saudii	*C. saudii* (2.18)	Liquid feces	No	Seeking toxigenic CD
C. saudii	*C. saudii* (1.77)	Liquid feces	No	Seeking toxigenic CD
C. saudii	*C. saudii* (1.93)	Liquid feces	No	Seeking toxigenic CD
C. saudii	*C. saudii* (2.1)	Liquid feces	No	Seeking toxigenic CD
C. saudii	*C. saudii* (1.9)	Liquid feces	No	Seeking toxigenic CD
C. saudii	*C. saudii* (2.47)	Liquid feces	No	Seeking toxigenic CD
C. saudii	*C. saudii* (1.83)	Liquid feces	No	Seeking toxigenic CD
Corynebacterium lascolaense	*Corynebacterium lascolaense* (1.85)	Intrauterine device	No	Not considered
C. lascolaense	*C. lascolaense* (2.1)	Urine	No	Growth not significant
Corynebacterium phoceense	*Corynebacterium phoceense* (2.1)	Vagina	No	Not considered
C. phoceense	*C. phoceense* (1.9)	Vagina	No	Not considered
C. phoceense	*C. phoceense* (2.1)	Vagina	No	Not considered
C. phoceense	*C. phoceense* (2)	Vagina	No	Not considered
C. phoceense	*C. phoceense* (2.2)	Vagina	No	Not considered
C. phoceense	*C. phoceense* (2)	Vagina	No	Polymicrobial
C. phoceense	*C. phoceense* (2.2)	Vagina	No	Not considered
C. phoceense	*C. phoceense* (1.97)	Urine	No	Polymicrobial
Gabonia massiliensis	*Gabonia massiliensis* (2.3)	Liquid feces	No	Seeking MDR bacteria
Pseudomonas massiliensis	*Pseudomonas massiliensis* (2)	Skin swab	Yes	Seeking *S. aureus* carriage
P. massiliensis	*P. massiliensis* (2.5)	Skin swab	Yes	Seeking *S. aureus* carriage

*MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight.
†Replicated isolates in different specimens from the same patient.

laboratory results. In addition, 306 (23.4%) updates were from new bacterial species discovered as a part of culturomics studies. Overall, references from the manufacturer represented 87% of the total database, routine laboratory results represented 8%, and new culturomics species represented 5% (Figure).

Routine Identification of Species Isolated as Part of Culturomics Studies

Among the 351,937 bacterial identifications performed routinely during the study period, we identified species first isolated from culturomics studies in 105 clinical specimens, accounting for 98 patients. This collection represents a total of 21 species, accounting for 2.8% (21/744) of the overall microbiology laboratory bacterial diversity (Table 1).

Among the 105 colonies identified as new species isolated as a part of culturomics studies, identification was correct for 100 (95.2%) using MALDI-TOF mass spectrometry. Thus, 16S rDNA gene sequencing was required for 5 strains to achieve final identification. MALDI-TOF mass spectrometry was not able to provide a reliable identification for *Varibaculum timonense*, *Prevotella ihuae*, *Actinomyces ihuae*, and 2 *Corynebacterium phoceense* isolates. We confirmed identification of 9 supplementary strains, representing 5 species (*Corynebacterium lascolaense*, *Actinomyces ihuae*, *Corynebacterium ihuae*, *Nosocomiicoccus massiliensis*, and *Pseudomonas massiliensis*),

Table 4. Characteristics of 17 persons with *A. ihuae* infection, Marseille, France, April 2015–March 2018*

Patient no.	Patient age, y/sex	Sampling site	Incubation time, h	Culture result	MALDI-TOF mass spectrometry score	16S rRNA result
1	24/F	Periareolar right breast	48	Polymicrobial	2.54	NA
2	26/F	Umbilical collection	48	Pure	2.5	NA
3	37/M	Periareolar left breast	72	Polymicrobial	1.97	NA
4	33/F	Breast	72	Polymicrobial	2.1	NA
5	77/F	Bone	72	Polymicrobial	2.1	NA
6	22/M	Testicular collection	96	Pure	1.95	*A. ihuae* 99.70%
7	56/M	Back	48	Pure	2.32	*A. ihuae* 99.70%
8	55/F	Labia majora	72	Polymicrobial	2.07	*A. ihuae* 99.70%
9	30/F	Labia majora	72	Pure	2.47	*A. ihuae* 99.70%
10	26/F	Labia majora	72	Polymicrobial	2.33	*A. ihuae* 99.60%
11	44/M	Leg ulcer	48	Polymicrobial	NA	*A. ihuae* 99.50%
12	66/M	Cervical collection	72	Polymicrobial	2.23	NA
13	49/M	Superinfected sebaceous cyst	48	Polymicrobial	1.9	NA
14	18/F	Sacrococcygeal cyst	96	Pure	2.45	NA
15	26/F	Labia majora	72	Polymicrobial	2.2	NA
16	45/F	Breast abcess	72	Polymicrobial	1.73	NA
17	44/M	Axillar abcess	96	Polymicrobial	1.92	NA

* MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; NA, not available.

using 16S rDNA gene sequencing (Tables 2, 3). Overall, we sequenced 14 isolates, accounting for 8 species, for the 16S rDNA gene.

Species Potentially Relevant as Human Pathogens

Among the 105 isolates included in this work, 64 were isolated as potential pathogens, accounting for 14 different species. Most were anaerobes that were cultured from abscesses or punctures, often involved in cases of polymicrobial infections. *Peptoniphilus grossensis* (18 cases) and *Actinomyces ihuae* (17 cases) were the most commonly isolated bacteria (Table 1). These species were initially cultured from the human gut. Special attention was given to *A. ihuae* infections (Table 4), which were strongly associated with breast abscess or genital area infections. Also, 10 bacteremia-involved species were isolated as a part of culturomics studies. *Bacteroides timonensis* was thus isolated in 3 blood cultures from 2 patients, whereas *Pseudomonas massiliensis* was found in 3 bacteremia episodes. *Corynebacterium phoceense* and *Corynebacterium ihuae* were recovered from 2 bloodstream infection episodes, whereas *Actinomyces marseillensis* and *Nosocomicoccus massiliensis* were each isolated from 1 blood sample (from 2 different patients). *B. timonensis*, *P. massiliensis*, *N. massiliensis*, and *C. ihuae* were first cultured from the human gut, whereas *A. marseillensis* was first isolated from respiratory microbiota and *C. phoceense* was first isolated from urinary microbiota. Overall, species cultured as part of culturomics studies were found to be potential pathogens in 59 different patients (Table 2). The significance of the presence of *P. massiliensis* in a lens from a patient with keratitis was ultimately not interpreted.

Species Isolated as Human Commensal Members

In this work, 40 isolates corresponding to 12 species discovered as a part of culturomics studies were isolated as belonging to the human flora. Of these, 22 were recovered when evaluating for toxigenic *C. difficile*, following a positive result with the GeneXpert *C. difficile* test. *C. saudii* was isolated in this context 11 times, followed by *C. jeddahtimonense* (8 times), *C. culturomicsense*, *Butyricimonas phocaeensis*, and *Anaerosalibacter massiliensis* (1 time each) (Table 3). These 5 species were first cultured from fecal specimens (Table 1).

In addition, *Corynebacterium lascolaense* was identified in 1 urine specimen, but in an insufficient quantity to be considered clinically relevant. Similarly, *C. phoceense* was recovered from 1 urine sample and from 7 vaginal swabs but was never reported to a physician in this context. These species were first cultured from urinary microbiota. Finally, *Pseudomonas massiliensis*, which was cultured from the human gut, was also recovered twice from skin swabs collected from the same physician after an epidemiologic investigation. Overall, species cultured for the first time as a part of culturomics studies were found as commensals in 38 different patients.

Discussion

This work constitutes the proof of concept that exploration of the repertoire of commensal bacteria enables identification of microorganisms involved in clinical microbiology. Indeed, the strategy of combining high-throughput culture techniques, MALDI-TOF mass spectrometry identification, and 16S rDNA gene sequencing of misidentified isolates enabled us to add 306 spectral references for 292 different new bacterial species to our laboratory's database. Thus, with culturomics, 21 new species were identified 105 times, in 98 patients. The results are robust; identification scores were all ≥1.9 with exclusion of multiple identifications. In addition, identification of 9 strains using 16S rDNA sequencing, accounting for 5 species, confirmed

the initial recognition by MALDI-TOF mass spectrometry (Table 2). These results strengthen our belief that identifying commensal microbes provides a valuable contribution to clinical microbiology, as revealed by the decrease in the number of unidentified colonies by MALDI-TOF mass spectrometry over time (Figure).

As exemplified for *A. ihuae* infections (Table 4), these microorganisms, which were isolated mainly from the human gut, can probably be found frequently in polymicrobial cultures. Thus, the microbiologist may be tempted to abandon the final identification of a microorganism found in such a situation, concluding that the infection is polymicrobial.

The extension of the bacterial repertoire associated with humans will considerably increase the number of bacteria associated with human diseases. In this study alone, over a 5-year period, 2.8% (21/744) of the overall identified bacteria would not have been identified without incrementing the MALDI-TOF mass spectrometry database with spectra obtained from culturomics studies.

On the whole, pathogenic microbes are also often found as commensals, as is currently well known for *C. difficile*, *S. aureus*, and *S. pneumoniae* (*35*–*37*). In our study, for example, *Corynebacterium phoceense*, *Pseudomonas massiliensis*, and *C. lascolaense* were found as both commensals and pathogens. This finding highlights the need for establishment of a repertoire of human microbes (*38*), which was recently estimated at 2,776 species, of which more than 10% were recovered by culturomics studies. Such a repertoire of prokaryotes associated with humans not only benefits microbiota studies, through notation of unknown sequences with new species genome sequencing, but also enables studying the role of these species in human infections (*39*). We estimate that, among the cases included here, the presence of species cultured as part of culturomics studies was potentially clinically relevant for 60 of them (61.2%). The online availability of the MALDI-TOF mass spectrometry spectra obtained from these species discovered by culturomics (http://www.mediterranee-infection.com/article.php?laref = 256&titre = urms-database) ensures their further identification by other laboratories.

Culturomics was initially designed to exhaustively identify commensals inhabiting human surfaces and thus can potentially lead in the future to personal medical interventions as a part of microbiome studies. However, the thinnest barrier between commensalism and pathogenicity, which should lead researchers to rethink Koch's postulate (*40*), has rendered culturomics studies useful in the field of clinical microbiology despite a potential skepticism. We show herein that, while contributing to the extension of the bacterial repertoire associated with humans, culturomics studies also enlarge the spectrum of prokaryotes involved in infectious diseases.

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About the Author

Dr. Dubourg is an assistant professor of bacteriology at IHU Méditerranée Infection, Marseille, France. His primary research interest is human microbiome studies.

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Address for correspondence: Didier Raoult, IHU Méditerranée Infection, 19-21 Boulevard Jean Moulin, 13385 Marseille CEDEX 5, France; email: didier.raoult@gmail.com

Association of Batai Virus Infection and Encephalitis in Harbor Seals, Germany, 2016

Wendy K. Jo,[1] Vanessa M. Pfankuche,[1] Annika Lehmbecker, Byron Martina, Ana Rubio-Garcia, Stefanie Becker, Jochen Kruppa, Klaus Jung, Daniela Klotz, Julia Metzger, Martin Ludlow, Wolfgang Baumgärtner, Erhard van der Vries,[2] Albert Osterhaus

We isolated Batai virus from the brain of a euthanized, 26-year-old, captive harbor seal with meningoencephalomyelitis in Germany. We provide evidence that this orthobunyavirus can naturally infect the central nervous system of a mammal. The full-genome sequence showed differences from a previously reported virus isolate from a mosquito in Germany.

Batai virus (BATV) is a member of the Bunyamwera serogroup of orthobunyaviruses of the family *Peribunyaviridae*. Orthobunyaviruses are single-stranded, negative-sense RNA viruses with a tripartite genome composed of small, medium, and large segments, which encode nucleocapsid, glycoproteins, and polymerase, respectively (*1*). These segments can be interchanged between viruses of the same genus, resulting in stable reassortant bunyaviruses. For example, the Ngari virus genome consists of segments from BATV and Bunyamwera virus. Ngari virus is associated with outbreaks of hemorrhagic fever in humans and shows a clinical spectrum different from that of both parent viruses (*2*).

BATV has been documented to cause mild illness in ruminants and humans (*3*,*4*). Other hosts include domestic pigs and wild birds (*3*). BATV is transmitted mainly by *Anopheles* and *Culex* spp. mosquitoes and is widely distributed throughout Europe, Asia, and Africa (*3*).

In Germany, BATV was first detected in *Anopheles maculipennis* mosquitoes in 2009 (*5*). Enzootic transmission cycles involving domestic and wild mammals was reported in a serologic study in which 3 (0.55%) of 548 cattle had BATV-neutralizing serum antibodies (*6*). We report natural BATV infection of 2 captive harbor seals (*Phoca vitulina*) in Germany, in which meningoencephalomyelitis developed in 1 of them.

The Study

In September 2016, a 26-year-old male harbor seal (*Phoca vitulina*) in a zoo in northern Germany showed peracute deterioration of its general condition. Because of progression and severity of illness, the seal was euthanized and the carcass sent to the Department of Pathology, University of Veterinary Medicine Hannover (Hannover, Germany), for necroscopic analysis. Macroscopic examination showed signs of distress but no gross lesions were detected. Histologic analysis showed a mild to moderate, multifocal, perivascularly accentuated, lymphohistiocytic meningoencephalomyelitis, which affected the cerebrum, cerebellum (Figure 1, panel A), brain stem, medulla oblongata, and cervical spinal cord. Histologic analysis indicated a virus etiology.

Routine immunohistochemical tests of the seal brain for morbilliviruses, Borna disease virus, and tick-borne encephalitis virus (*7*,*8*) and immunofluorescence analysis for rabies virus were performed by the Department of Consumer and Food Safety of Lower Saxony (Hannover, Germany). All tests showed negative results.

We attempted virus isolation from homogenized brain in Vero cells. Cytopathic changes were observed within 3 days and continued to emerge in subsequent passages. To identify the pathogen, we investigated supernatant from the initial Vero cell isolation by using deep sequencing and a modified sequence-independent, single-primer amplification protocol as described (*9*,*10*). Analysis of raw reads with Bowtie 2 version 2.2.9 (https://sourceforge.net/projects/bowtie-bio/files/bowtie2/2.2.9/) for DNA mapping and Pauda version 1.0.1 (https://bioconda.github.io/recipes/pauda/README.html) for amino acid mapping identified BATV.

We created a reference assembly for all 3 genome segments (GenBank accession nos. S, MH299972; M, MH299973; and L, MH299974) by using CLC Genomics

Author affiliations: University of Veterinary Medicine Hannover, Hannover, Germany (W.K. Jo, V.M. Pfankuche, A. Lehmbecker, S. Becker, J. Kruppa, K. Jung, D. Klotz, J. Metzger, M. Ludlow, W. Baumgärtner, E. van der Vries, A. Osterhaus); Center for Systems Neuroscience, Hannover (W.K. Jo, V.M. Pfankuche, W. Baumgärtner, A. Osterhaus); Artemis One Health, Delft, the Netherlands (B. Martina, A. Osterhaus); Seal Centre, Pieterburen, the Netherlands (A. Rubio-Garcia)

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[1]These authors contributed equally to this article.

[2]Current affiliation: Utrecht University, Utrecht, the Netherlands.

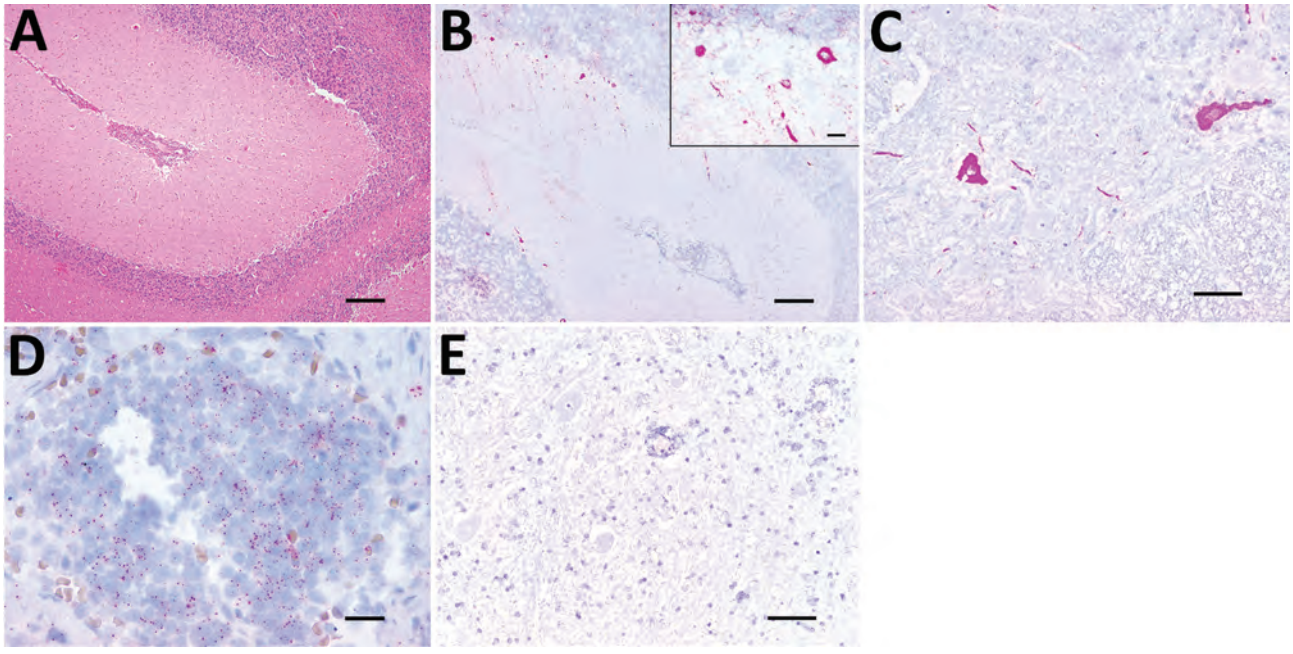

Figure 1. Histologic analysis and fluorescent in situ hybridization (FISH) of a Batai virus BATV)–infected harbor seal, Germany, 2016. A) Cerebellum showing mild to moderate, perivascularly accentuated, lymphohistiocytic inflammation (hematoxylin and eosin stain; scale bar indicates 200 µm). B) Purkinje cells and neurons of granular cell layer showing intracytoplasmic BATV–specific pink, positive result detected by FISH (fast red stain; scale bar indicates 200 µm). Inset: Higher magnification view of analysis using the QuantiGene ViewRNA ISH Tissue 1-Plex Assay Kit and the QuantiGene ViewRNA Chromogenic Signal Amplification Kit (Affymetrix-Panomics, Santa Clara, CA, USA) (fast red stain; scale bar indicates 20 µm). C) Scattered neurons of spinal cord showing a strong, pink, intracytoplasmic BATV-specific result detected by FISH (fast red stain; scale bar indicates 100 µm). D) Cortical and medullary lymphocytes of pulmonary lymph node showing a mild, pink, intracytoplasmic BATV-specific result detected by FISH (fast red stain; scale bar indicates 20 µm). E). Negative control (incubation without probe) of spinal cord showing no BATV-specific result (fast red stain; scale bar indicates 100 µm).

Workbench version 9.0 (QIAGEN, Hilden, Germany). The isolated virus was closely related to previously identified BATV strains from Europe (Figure 2) but had the highest sequence homology with strains from Russia (nucleotide pairwise identity S, 99%; M, 98.6%; and L, 98.5%).

We tested seal tissues (Table) for BATV by using real-time PCR and fluorescent in situ hybridization (FISH) as described (*5*,*12*). We used BATV-specific probe 5′-FAM-AACAGTCCAGTTC-CAGACGATGGTC-BHQ-1-3′ and primers Fwd-5′-GCTGGAAGGTTACTGTATTTAATAC-3′ and Rv-5′-CAAGGAATCCACTGAGTCTGTG-3′ specific for the S segment (*5*). A BATV-specific probe for nucleotides 28–899 of the S segment was designed for FISH experiments (QuantiGene ViewRNA Kits; Affymetrix-Panomics, Santa Clara, CA, USA), which were performed according to the manufacturer's protocol with minor modifications (*12*).

The highest virus load (by real-time PCR) was found in the central nervous system, and lesion-associated Purkinje cells and neurons of the granular cell layer of the cerebellum showed positive FISH results (Figure 1, panel B). A positive cytoplasmic result was also obtained for single spinal cord neurons (Figure 1, panel C). More limited BATV infection was found in peripheral organs, and the lowest cycle threshold was for the intestine. We also found BATV in single cells of the tunica mucosa of the small intestine and in cortical and medullary lymphocytes of the pulmonary lymph node by FISH (Figure 1, panel D). Other organs showed negative results in both assays.

We also performed histopathologic analysis of archived formalin-fixed paraffin-embedded (FFPE) organ samples of a seal that had shared the enclosure with the BATV-infected seal and had died 2 months before the euthanized seal showed the first clinical signs. Glomerular and tubular epithelial kidney cells (online Technical Appendix Figure, https://wwwnc.cdc.gov/EID/article/24/9/17-1829-Techapp1.pdf), cells of the tunica mucosa of the small intestine, and cortical and medullary lymphocytes of the pulmonary lymph node showed positive results for BATV by FISH. These FFPE samples did not show positive results by real-time PCR, probably because of low sensitivity of the assay for FFPE samples (*13*).

Retrospective analysis of FFPE brain samples of seals (n = 7) that had histopathologic changes suggestive of an unknown virus etiology and were isolated from harbor

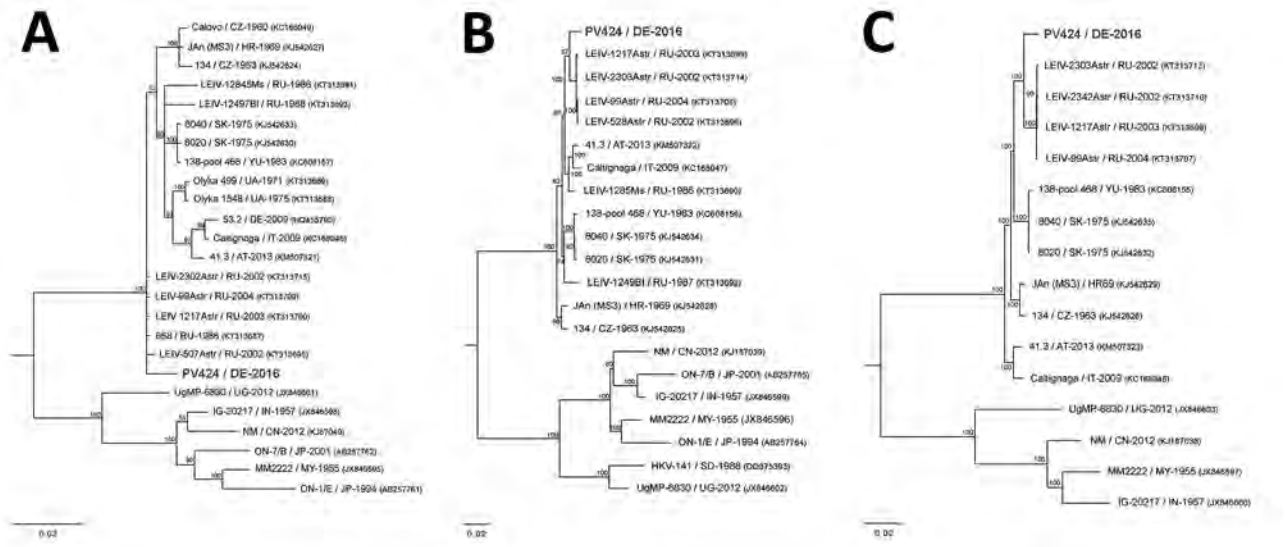

Figure 2. Bayesian phylogeny trees based on full-genome coding region sequences of small, medium, and large RNA segments of Batai virus and comparison viruses. A) Small RNA segments (69–770 bp). Bunyamwera virus (GenBank accession no. D00353) was used as the outgroup. B) Medium RNA segments (42–4,346 bp). Bunyamwera virus (GenBank accession no. M11852) was used as the outgroup. C) Large RNA segments (49–6,762 bp). Bunyamwera virus (GenBank accession no. X14383) was used as the outgroup. Bold indicates virus isolated in this study. Analysis was performed for 1 million generations and sampled every 100 steps. The first 25% of samples were discarded as burn-in according to MrBayes (*11*). Hasegawa-Kishino-Yano nucleotide substitution model was selected as best-fit model according to Bayesian information criteria. Numbers at the nodes indicate posterior probabilities percentage. GenBank accession numbers are provided for comparison isolates; accession nos. of the isolated Batai virus strain PV424/DE-2016 are small, MH299972; medium, MH299973; large, MH299974. Scale bars indicate nucleotide substitutions per site.

seals in coastal waters of Germany in the past decade all had negative results for BATV by FISH. Therefore, we screened 100 serum samples from harbor seals and 100 serum samples from gray seals (*Halichoerus grypus*) collected in 2016 and 2017 after admission to a seal rehabilitation center in the Netherlands that covers seal populations partially overlapping those of coastal waters of Germany. We neutralized isolated seal BATV (100 50% tissue culture infective doses) with diluted serum samples before application to reporter cells and examined for cytopathic effects after 3 days. However, no BATV antibodies (titer >1:20) were detected.

Table. Analysis of a Batai virus–infected harbor seal with meningoencephalomyelitis, Germany, 2016*

Sample material	Histopathologic finding	Real-time PCR (cycle threshold)†	FISH
Brain	Cerebrum, cerebellum, brain stem, medulla oblongata, and cervical spinal cord: mild to moderate, multifocal, and lymphohistiocytic meningoencephalomyelitis, perivascularly accentuated; parietal lobe: multiple glial nodules; thoracic spinal cord: mild to moderate and multifocal meningitis, perivascularly accentuated, lymphohistiocytic with few eosinophilic granulocytes; cauda equina: mild to moderate, multifocal, and lymphohistiocytic perineuritis	+ (15)	+
Lung	Mild and multifocal anthracosis; acute, diffuse, and severe hyperemia; acute, diffuse, and moderate edema	– (>35)	–
Spleen	Moderate to severe and diffuse hyperemia	– (35)	–
Kidney	Mild, interstitial, and lymphohistiocytic nephritis with single, intratubular concrements	– (>35)	–
Pulmonary lymph node	Mild follicular hyperplasia	NI	+
Mesenteric lymph node	Mild to moderate follicular hyperplasia and hemosiderosis	NI	–
Liver	Mild, multifocal, lymphohistiocytichepatitis, mild to moderate hepatocellular storage of iron	– (>35)	–
Small intestine	Mild, diffuse, lymphoplasmacytic, and partially eosinophilic enteritis	+ (28)	+
Large intestine	NSML	NI	–
Nose	NSML	NI	–
Heart	NSML	NI	–
Stomach	NSML	NI	–

*FISH, fluorescent in situ hybridization; NI, not investigated; NSML, no major microscopic lesions; –, negative; +, positive.
†Negative result >35; positive result ≤35.

Conclusions

We isolated and characterized BATV from the brain of a captive harbor seal in Germany. This seal had lymphohistiocytic meningoencephalomyelitis and evidence of virus replication in Purkinje cells, neurons, enterocytes, and lymphocytes in peripheral tissues. Evidence of BATV infection by FISH was also obtained for a second harbor seal that had died 2 months before in the same enclosure. No additional evidence was found for seals as natural hosts for BATV infection by investigating brains from seals with encephalitis in coastal waters of Germany and by conducting a serosurvey among free-living harbor and gray seals. Results obtained from the 2 BATV-infected animals indicated BATV circulation in the area during the mosquito season and that captive seals were possible dead-end hosts. Because seals in their natural environment are most likely less exposed to mosquitoes than seals in captivity, the observed seal BATV infections might be unnatural captivity-associated events. Phylogenetic analysis indicated that BATV isolated from the seal brain differed from BATV isolated from a mosquito in Germany and is more closely related to strains identified in Russia.

This study provides evidence of BATV associated with central nervous system disease in a naturally infected mammal. Other orthobunyaviruses have also been shown to cross the blood–brain barrier and show neurotropic properties (*14*). For example, a virus from the same serogroup, Bunyamwera virus, was recently associated with neurologic disease and abortion in horses (*15*). Moreover, possible human BATV infection in disease-endemic regions requires further investigation because BATV infection of mammals, including humans, has been reported in Europe (*3*) and Sudan (*4*).

Furthermore, BATV is the donor of the M segment of Ngari virus, which causes hemorrhagic fever in humans (*2*). The 2 BATV-infected seals could have been exceptionally sensitive to BATV infection because of predisposing factors, such as advanced age, concurrent conditions, genetic predisposition, or immunologic deficiencies. This possibility raises the question whether immunocompromised humans or other mammals might be at increased risk for development of neurologic BATV infection. Collectively, our data indicate the need for increased surveillance of BATV infection in mosquitoes, mammals, and birds in Europe.

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About the Author

Ms. Jo is a doctoral candidate at the University of Veterinary Medicine Hannover Research Center for Emerging Infections and Zoonoses, Hannover, Germany. Her research interests are virus discovery, emerging and reemerging infectious diseases, cross-species transmission of viruses, virus evolution, and host adaptation.

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Address for correspondence: Albert Osterhaus, Research Center for Emerging Infections and Zoonoses, University of Veterinary Medicine, Bünteweg 17, Hannover D-30559, Germany; email: albert.osterhaus@tiho-hannover.de

Use of Favipiravir to Treat Lassa Virus Infection in Macaques

Kyle Rosenke, Heinz Feldmann, Jonna B. Westover, Patrick William Hanley, Cynthia Martellaro, Friederike Feldmann, Greg Saturday, Jamie Lovaglio, Dana P. Scott, Yousuke Furuta, Takashi Komeno, Brian B. Gowen, David Safronetz

Lassa virus, the cause of Lassa fever in humans, is endemic to West Africa. Treatment of Lassa fever is primarily supportive, although ribavirin has shown limited efficacy if administered early during infection. We tested favipiravir in Lassa virus–viremic macaques and found that 300 mg/kg daily for 2 weeks successfully treated infection.

Lassa virus (LASV; family *Arenaviridae*, genus *Mammarenavirus*) is the etiologic agent of the severe hemorrhagic disease Lassa fever. Annually, ≈300,000 persons become infected with LASV, 20% of which experience life-threatening clinical manifestations including edema, hemorrhage, and multiorgan failure, resulting in an estimated 5,000 deaths (*1*). Most human infections are acquired from the natural rodent reservoir, the multimammate rat (*Mastomys natalensis*). Human-to-human transmission, mostly nosocomial, occurs (*1*). LASV has a relatively well-defined region of endemicity exclusive to West Africa. Incidence of LASV infections is highest in Nigeria, Sierra Leone, Liberia, and Guinea, although sporadic cases and moderate outbreaks of Lassa fever have been documented in many other West Africa nations (online Technical Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/24/9/18-0233-Techapp1.pdf) (*2*). Over several decades, importation of Lassa fever into Europe, Asia, and the Americas has increased (*2*).

Treatment of Lassa fever is largely supportive, although ribavirin is used off label, despite side effects and limited efficacy data (*3*). Recently, the antiviral favipiravir (T-705; 6-fluoro-3-hydroxy-2-pyrazinecarboxamide) has gained attention as a broad-spectrum antiviral drug against RNA viruses (*4*) including LASV; initial studies using small animal models have been conducted (*5*,*6*). We assessed the antiviral efficacy of favipiravir in LASV-infected cynomolgus macaques; this animal model reliably recapitulates several hallmarks of Lassa fever infection in humans (*7*).

Author affiliations: National Institutes of Health, Hamilton, Montana, USA (K. Rosenke, H. Feldmann, P.W. Hanley, C. Martellaro, F. Feldmann, G. Saturday, J. Lovaglio, D.P. Scott); University of Manitoba, Winnipeg, Manitoba, Canada (H. Feldmann, D. Safronetz); Utah State University, Logan, Utah, USA (J.B. Westover, B.B. Gowen); Toyama Chemical Co., Ltd., Toyama, Japan (Y. Furuta, T. Komeno); Public Health Agency of Canada, Winnipeg (D. Safronetz)

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The Study

We randomly divided 8 female cynomolgus macaques (*Macaca fascicularis*) into 2 groups of 4 each (treatment and control) and injected each animal intramuscularly with a lethal dose of LASV, strain Josiah (1×10^4 50% tissue culture infective dose [$TCID_{50}$]) (Table). Treatment began at 4 days postinfection (dpi), 1 day after the onset of viremia (*7*). The initial treatment (300 mg/kg favipiravir) was administered intravenously; subsequent treatments (300 mg/kg favipiravir every 24 h for 13 d) were administered subcutaneously. The dosage was based on the successful treatment of Lassa fever in guinea pigs (*5*). To avoid the confounding effects of ribavirin, we did not give it in combination. LASV-infected control animals received an equivalent volume of vehicle by the same route and schedule. Animals were assessed twice daily; physical examinations, including hematologic, blood chemistry, and virologic assessments, were conducted regularly.

Reduced activity and appetite, probably resulting from being anesthetized daily, was noted early in animals in both groups (Figure 1, panel A). At 6 dpi (2 d after treatment began), clinical scores for 3 of 4 animals in the treatment group plateaued and remained consistent for the remainder of the study. Although the score for the remaining animal was higher, all favipiravir-treated animals survived LASV challenge ($p<0.01$; Figure 1, panel B). In contrast, clinical scores for the control group increased dramatically after 8 dpi; all control animals displayed anorexia, hunched posture, piloerection, and lethargy (Figure 1, panel A). The control animals reached the humane endpoint and were euthanized on 10, 11, and 12 dpi (Figure 1, panel B).

Although we detected viral RNA by quantitative PCR as early as 3 dpi (online Technical Appendix Figure 2, panel A), at no time was infectious LASV isolated from blood (Figure 1, panel C) or postmortem tissue samples (data not shown) from animals in the favipiravir group. However, in the

Table. Study design for treatment of Lassa virus infection in cynomolgus macaques*

Study, no. animals	Treatment	Frequency†	Dose, mg/kg	Loading dose, mg/kg	Total daily dose, mg/kg	Survived/total, no.
First						
4	Placebo	Every 24 h	Volume equivalent	Volume equivalent	Volume equivalent	0/4
4	Favipiravir	Every 24 h	300	300	300	4/4
Second						
4	Placebo	Every 8 h	Volume equivalent.	Volume equivalent	Volume equivalent	0/4
4	Favipiravir	Every 8 h	50	300	150	0/4

*All animals were challenged with a previously determined lethal dose of 10^4 50% tissue culture infective dose of Lassa virus (strain Josiah) via intramuscular injection. At day 4 after infection, a time coinciding with the earliest onset of detectable viremia, treatment with placebo or drug was initiated by intravenous injection of the loading dose followed by daily (days 5–17) subcutaneous dosing. Animals were examined daily for clinical signs of disease, and samples were taken for hematologic, blood chemistry, and virologic analyses at 12 times throughout the study, beginning on the day of virus challenge and ending on day 56 after infection.
†For 14 d.

control animals, increased liver enzyme levels were detected at 6 dpi, coinciding with infectious LASV (Figure 1, panels D, E). One animal in the favipiravir group demonstrated moderately increased levels of alanine aminotransferase and aspartate aminotransferase at 6–12 dpi, which resolved after treatment cessation. In the control animals, albumin and

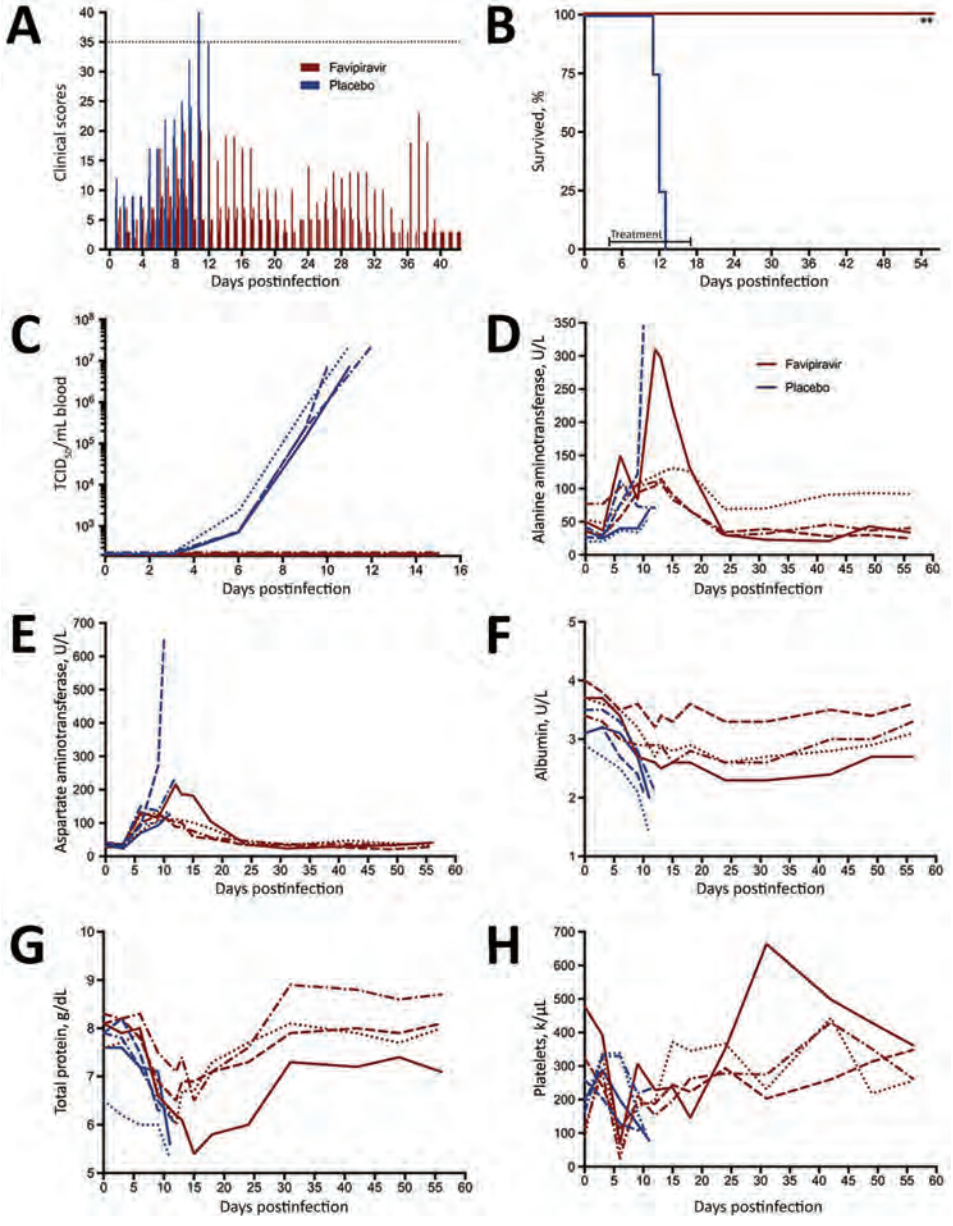

Figure 1. Effect of daily favipiravir treatments on morbidity and mortality rates, viral loads, and selected blood chemistry and hematology values during the course of the efficacy study in cynomolgus macaques challenged with 10^4 $TCID_{50}$ of Lassa virus. Groups of 4 animals each were given 300 mg/kg/d of favipiravir or placebo for 14 consecutive days, beginning on day 4 postinfection. A) Daily clinical scores (dotted line indicates euthanasia score of 35). B) Survival curve (*p<0.01 compared with placebo-treated animals by the Mantel-Cox log-rank test). C) Viremia as assessed by $TCID_{50}$ assay. D) Alanine aminotransferase levels. E) Aspartate aminotransferase levels. F) Albumin levels. G) Total protein levels. H) Platelet levels. $TCID_{50}$, 50% tissue culture infective dose.

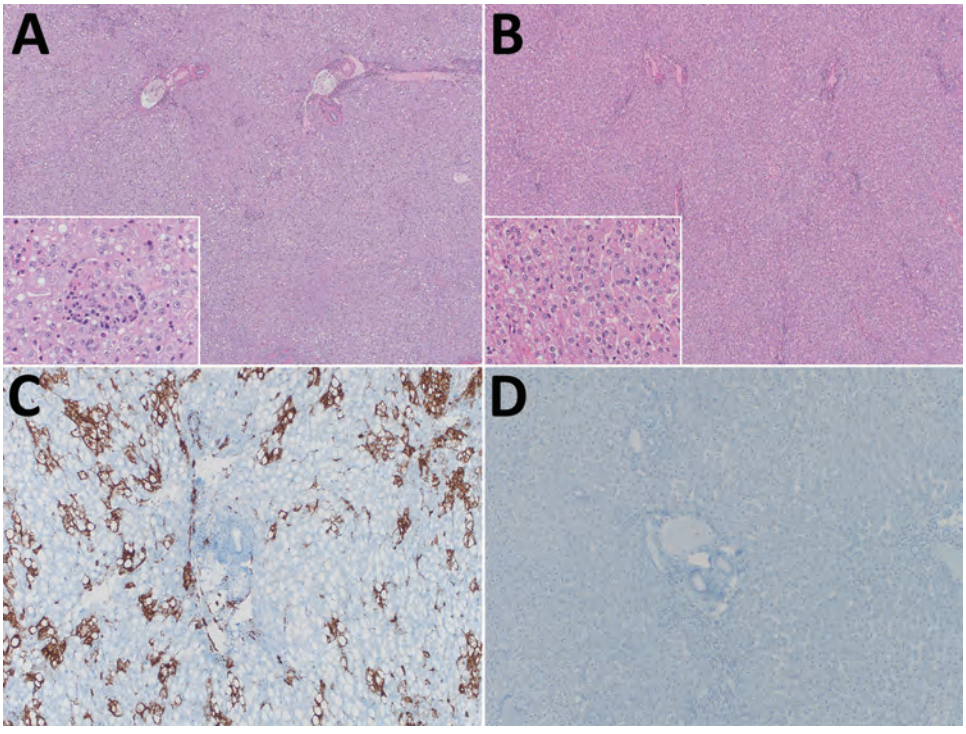

Figure 2. Histologic and in situ hybridization analyses of livers from cynomolgus macaques infected with Lassa virus (LASV) (1×10^4 50% tissue culture infective dose [$TCID_{50}$]) and treated 1 time daily with favipiravir (treated) or vehicle only (control). A–B) Histologic analyses. A) Control, showing multifocal neutrophilic infiltrates with hepatocyte necrosis and degeneration (original magnification ×40). Inset shows hepatocyte necrosis (original magnification ×400). B) Treated, showing essentially healthy hepatic tissue (original magnification ×40). Inset shows healthy hepatic tissue (original magnification ×400). C–D) In situ hybridization analyses. C) Control, showing multifocal and coalescing viral RNA detected within hepatocytes (original magnification ×100). D) Treated, showing no viral RNA detected (original magnification ×100).

total protein levels decreased dramatically throughout LASV infection; in the favipiravir-treated animals, these levels displayed a more moderate decrease before returning toward reference levels (Figure 1, panels F, G).

Hematologic profiles were less uniform between the 2 groups, although we observed several abnormalities previously associated with LASV infection (online Technical Appendix Figure 3). After an initial increase, platelet counts in the control animals consistently decreased until euthanasia. In 2 of the favipiravir-treated animals, a similar increase was noted initially; however, a marked decrease occurred at 6 dpi (Figure 1, panel H). Platelet levels rebounded by 9 dpi and continued to increase. In addition, all 4 favipiravir-treated animals had lipemia (>301 mg/dL) during the treatment phase of the study (4–18 dpi).

Histopathologic examination of livers from control animals demonstrated hepatitis with neutrophil invasion and substantial steatosis, consistent with Lassa fever in this model (Figure 2, panel A) (*7*). In contrast, livers from favipiravir-treated animals showed no abnormalities (Figure 2, panel B). In situ hybridization detected LASV RNA within hepatocytes of control animals but not treated animals (Figure 2, panels C, D).

Conclusions

Over the past 3 years, total numbers of Lassa fever cases and case-fatality rates among humans have increased (*8*). The current Lassa fever outbreak in Nigeria and Benin continues to show large numbers of Lassa fever cases and case-fatality rates >30% (*8*), again highlighting the lack of available treatments. Furthermore, the recent person-to-person transmission of LASV in Germany (*9*) serves as a reminder that Lassa fever is of global health concern.

Lassa fever patients are largely managed by supportive care in combination with ribavirin (*3*). Experimental efficacy of ribavirin was initially tested in a Lassa fever rhesus macaque model in which intramuscular injections 3 times daily for 14 days improved survival rates (*10*). In clinical trials in Sierra Leone, both oral and intravenous ribavirin increased survival rates of Lassa fever patients (*11*). Oral dosing of ribavirin has since remained the standard of treatment for Lassa fever, despite unproven efficacy from clinical studies (*3*,*12*). Moreover, side effects of ribavirin therapy have resulted in noncompliance (*12*).

The successful daily administration of favipiravir to macaques at an elevated dosing regimen of 300 mg/kg/d may partially explain the transient thrombocytopenia, elevated liver enzyme levels, and lipemia found in this study. Experimentally, favipiravir is a well-known broad-spectrum antiviral drug with in vitro inhibitory activity against a multitude of RNA viruses (*4*). In Japan, favipiravir is approved for influenza treatment; in the United States, phase 3 clinical trials for the same indication have been completed (*4*). During the West Africa Ebola outbreak, favipiravir was administered to humans on an emergency basis under a different dosing regimen, but effectiveness was limited (*13*). We therefore designed a second study that more closely followed the dosing in the Ebola trial (50 mg/kg every 8 h) (*13*). However, this

multiple dose per day format failed to protect cynomolgus macaques from Lassa fever and did not alter disease progression (Table; online Technical Appendix Figure 1, panel B, and Figure 4, panels A–D). Of note, the high-dose favipiravir therapy in macaques successfully abated the pathophysiologic parameters associated with LASV infection, resulting in survival. These results suggest that low doses of favipiravir have limited therapeutic effect, whereas higher doses are therapeutic and will improve clinical outcomes.

Favipiravir was recently administered in combination with ribavirin to successfully treat Lassa fever in 2 human patients. Although it is not possible to determine how effective favipiravir was in controlling these 2 cases, administration reduced viremia in both patients (*9*). Combination therapy with favipiravir and ribavirin in immunocompromised mice with LASV infection showed efficacy with suboptimal doses of each drug (*6*). The synergistic effect of the 2 compounds is also supported by several other studies in rodents (*14*). Recently, human monoclonal antibody therapy protected cynomolgus macaques from LASV infection (*15*). Combination therapy with favipiravir may be a future therapeutic strategy (*15*). On the basis of our findings, improved favipiravir tolerability in humans (*4*), availability of an oral formulation, and its advanced preclinical status (according to the US Food and Drug Administration), we recommend that favipiravir enter clinical trials as a treatment for Lassa fever.

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Y.F. and T.K. are employees of Toyama Chemical Co., the manufacturers of favipiravir. All other authors declare no conflict of interest.

About the Author

Dr. Rosenke is a microbiologist in the Laboratory of Virology, NIAID, NIH. His research interests are the epidemiology, ecology, pathogenesis, and treatment of emerging viral diseases.

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Address for correspondence: Heinz Feldmann, National Institutes of Health, Laboratory of Virology, Rocky Mountain Laboratories, 903 S 4th St, Hamilton, MT 59840, USA; email: feldmannh@niaid.nih.gov; David Safronetz, National Microbiology Laboratory, PHAC, Viral Zoonoses, 1015 Arlington St, Winnipeg, MB R3E 3R2, Canada; email: david.safronetz@phac-aspc.gc.ca

Aortic Endograft Infection with *Mycobacterium chimaera* and *Granulicatella adiacens*, Switzerland, 2014

Andreas Plate, Thomas A. Kohl, Peter M. Keller, Sabine Majer, Rosamaria Fulchini, Carol Strahm, Cristoforo Medugno, Zoran Rancic, Lars Husmann, Hugo Sax, Stefan Niemann, Barbara Hasse

We describe an aortic endograft infection caused by *Mycobacterium chimaera* and *Granulicatella adiacens*, successfully treated with prolonged antimicrobial drug therapy after complete explantation of the infected endoprosthesis and extra-anatomical reconstruction. Whole-genome sequencing analysis did not indicate a close relationship to bacterial strains known to cause infections after cardiac surgery.

Aortic endograft infection (AGI) is a serious complication of aortic repair, and treatment involves prolonged antimicrobial drug therapy and complete or partial graft explantation with subsequent in situ or extra-anatomic arterial reconstruction. AGI attributable to nontuberculous mycobacteria (NTM) is a rare condition, and sporadic cases have been described (*1*). *Mycobacterium chimaera* is a slow-growing NTM and a member of the *M. avium* complex. Recent publications show the emergence of disseminated *M. chimaera* infections occurring after open heart surgery (*2*). A field investigation identified contaminated heater–cooler units (HCUs) as the source of infection (*3*,*4*). In addition to valve reconstructions, these cases also involved thoracic aortic grafts. We describe an abdominal AGI caused by *M. chimaera* and *Granulicatella adiacens*. Our aim was to find the source of the *M. chimaera* infection by using whole-genome sequencing (WGS) to compare the patient's isolate to strains implicated in infections known to occur after cardiac surgery.

Author affiliations: University Hospital Zurich, Zurich, Switzerland (A. Plate, Z. Rancic, L. Husmann, H. Sax, B. Hasse); Research Center Borstel–Leibniz-Center for Medicine and Biosciences, Borstel, Germany (T.A. Kohl, S. Niemann); German Center for Infection Research, Borstel (T.A. Kohl, S. Niemann); National Center for Mycobacteria, University of Zurich, Zurich (P.M. Keller); Institute of Medical Microbiology, University of Zurich, Zurich (P.M. Keller); Cantonal Hospital Münsterlingen, Münsterlingen, Switzerland (S. Majer); Cantonal Hospital St. Gallen, St. Gallen, Switzerland (R. Fulchini, C. Strahm); Cantonal Hospital Frauenfeld, Frauenfeld, Switzerland (C. Medugno)

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The Study

In March 2014, a formerly healthy 60-year-old man underwent an elective endovascular aortic repair because of an infrarenal aortic aneurysm. In May 2015, the patient sought medical care for low back pain radiating into the left leg. Laboratory examinations showed elevated C-reactive protein (57 mg/L [reference range <5 mg/L]), leukocytosis (14.8 g/L [reference range <9 g/L]), and acute kidney injury (estimated glomerular filtration rate 44 mL/min [reference range >80 mL/min]). A ^{18}Fluorodeoxyglucose positron emission tomography–computed tomography (PET–CT) examination indicated an AGI and showed an abscess formation in the iliopsoas muscle in close contact with the left common iliac artery; the intraoperative situs was highly suspicious for AGI, including erosion of the left common iliac artery and a visible endograft. The patient was transferred to the University Hospital Zurich (Zurich, Switzerland) for repeat surgery. The surgical procedure entailed complete endoprosthesis removal, closure of the aortic stump below the renal arteries with polypropylene sutures, and omentum coverage. All tissues were debrided, and treatment included vacuum-assisted open-abdomen treatment. Perfusion of the lower limbs' arteries was maintained with an axillo-bifemoral reconstruction using a polytetrafluoroethylene graft (Figure 1).

Deep wound cultures obtained during surgical revisions revealed *M. chimaera* (in 3/3 cultures) and *Granulicatella adiacens* (in 4/18 cultures). Histopathologic test results were compatible with mycobacterial infection (online Technical Appendix Tables 1, 2, https://wwwnc.cdc.gov/EID/article/24/9/18-0247-Techapp1.pdf). Results of blood cultures and mycobacteriologic blood and sputum cultures remained negative. The patient received a combination therapy containing clarithromycin, rifabutin, ethambutol, and amikacin in the early postoperative phase. After 6 weeks, amikacin was replaced by moxifloxacin. For coverage of *G. adiacens*, amoxicillin was added to the regimen. We treated the patient for a total of 12 months after the extra-anatomic reconstruction. Several PET–CT scans showed a complete metabolic response.

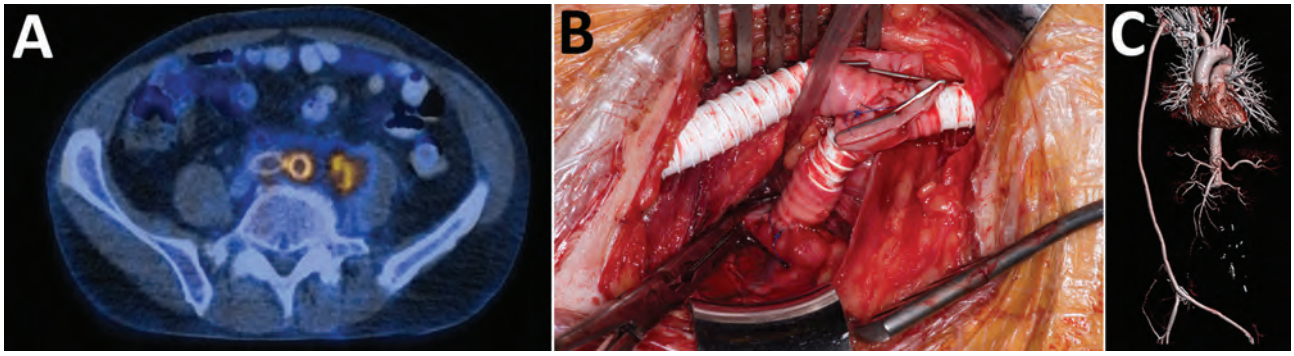

Figure 1. Preoperative, intraoperative, and postoperative images in the case of a patient who received an abdominal aortic endograft and was later diagnosed with *Mycobacterium chimaera* and *Granulicatella adiacens* infection, Switzerland, 2014. A) ^{18}Fluorodeoxyglucose positron emission tomography–computed tomography scan at diagnosis indicating a strong, metabolically active (maximum standard uptake value 9.7) aortic endograft infection and an adjacent abscess formation in the iliopsoas in close contact with the left common iliac artery. B) Intraoperative extra-anatomic position of a polytetrafluoroethylene graft through noninfected subcutaneous operative field. C) Satisfactory postoperative result of the axillo-bifemoral bypass on volume-rendered reconstructions of a contrast-enhanced computed tomography.

The diagnostic workup in May 2015 revealed an incidental 5-mm small pulmonary nodulus in the right upper lobe, which was observed to be metabolically active in PET–CT. After recovery from the abdominal intervention, the patient underwent wedge resection, and a localized squamous-cell carcinoma of the lung was confirmed. In April 2016, a relapse of his neoplasia occurred. Despite intensified chemotherapy, the patient died in August 2017 because of progressive pulmonary cancer; no autopsy was performed.

We cultured mycobacteriologic samples in BD MGIT tubes (BD, Franklin Lakes, NJ, USA) on Middlebrook 7H11 agar plates (BD) according to previously published methods (*3*). Air and water mycobacterial cultures were performed as suggested by the European Centre for Disease Prevention and Control (*5*).

We analyzed WGS data from the patient's isolate and strains from published studies (*2*,*6*–*10*) by using a reference mapping approach with the *M. chimaera* DSM-44623 genome (GenBank accession no. NZ_CP015278.1), aided by Burrows-Wheeler Aligner (http://bio-bwa.sourceforge.net), SAMtools (http://samtools.sourceforge.net/cns0.shtml), and GATK (https://software.broadinstitute.org/gatk) software. We combined variant positions to construct a phylogenetic tree with DnaSP 5.0 (http://www.ub.edu/dnasp/index_v5.html), FastTree (http://www.microbesonline.org/fasttree), FigTree (http://tree.bio.ed.ac.uk/software/figtree), and EvolView (http://www.evolgenius.info/evolview) software (online Technical Appendix).

The HCU-related outbreak of disseminated *M. chimaera* infections led us to investigate the hybrid operating

Table. Microbiologic test results of air and water samples from the operating room where an abdominal aortic endograft was performed on a patient later diagnosed with *Mycobacterium chimaera* and *Granulicatella adiacens* infection, Switzerland, 2014

Sample no.	Type	Place of sampling	Result
1	Water	NaCl heater machine	Negative
2	Water	Respirator 1, suction water tank ID 3393	Negative
3	Water	Respirator 1, breathing hose	Negative
4	Water	Respirator 2, suction water tank	Negative
5	Water	Respirator 2, breathing hose	Negative
6	Water	Operating pre-theater, wash basin, siphon	*M. intracellulare**
7	Water	Operating pre-theater, wash basin, cold water	Negative
8	Water	Operating pre-theater, wash basin, hot water	Negative
9	Water	Operating pre-theater, sink, siphon	Negative
10	Water	Operating pre-theater, sink, cistern	Negative
11	Water	Operating pre-theater, sink, cold water	*M. paragordonae*
12	Water	Scrub room 2, right side, wash basins 1–3, siphon water	Negative
13	Water	Scrub room 2, right side, wash basins 1–3, after flushing	Negative
14	Water	Scrub room 2, left side, wash basins 4–6, siphon water	Negative
15	Water	Scrub room 2, left side, wash basins 4–6, after flushing	Negative
16	Water	Operating pre-theater, sink, warm water	Negative
17	Air	Air sample 1	Negative
18	Air	Air sample 2	Negative
19	Air	Air sample 3	Negative
20	Air	Air sample 4	Negative
21	Air	Air sample 5	Negative

*Misidentification of *M. chimaera* excluded by partial 16S rDNA sequencing.

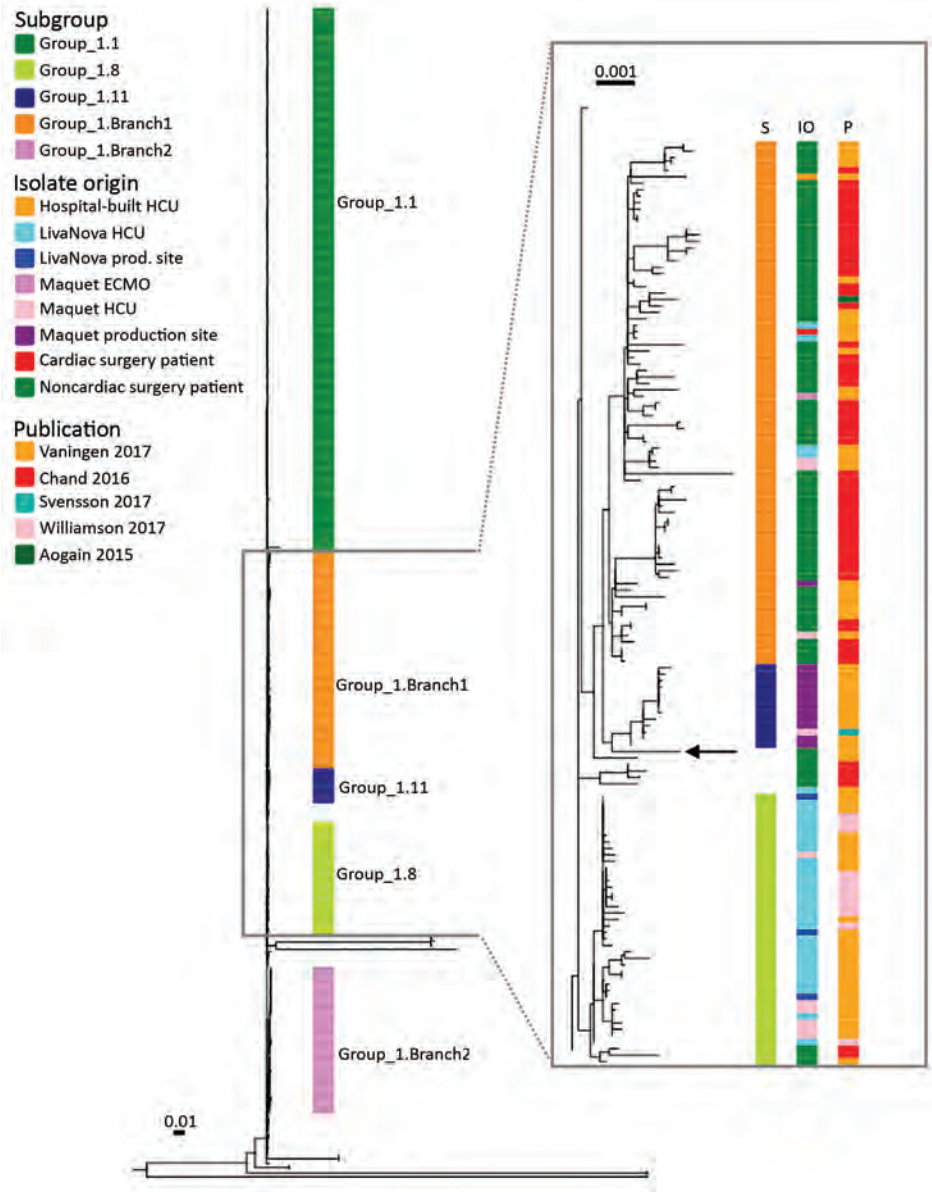

Figure 2. Phylogeny of isolate from case-patient who received an abdominal aortic endograft and was later diagnosed with *Mycobacterium chimaera* and *Granulicatella adiacens* infection, Switzerland, 2014, and comparison isolates. Maximum-likelihood tree was built from 14,192 single-nucleotide polymorphism positions of 437 group 1 *Mycobacterium chimaera* isolates mapped to the DSM-44623 *M. chimaera* genome (GenBank accession no. NZ_CP015278.1). DSM-44623 is shown as a rectangular phylogram with the inferred subgroups indicated. Inset box shows subgroups 1.8, 1.11, and 1.Branch1, annotated with isolate origin and the source publication. Black arrow indicates position of the patient isolate. Group 1.11 consisted mainly of samples collected at the Maquet production site in Rastatt, Germany (n = 12); 1 isolate came from an in-use Maquet HCU. Branch 1 contained primarily strains from patients with pulmonary *M. chimaera* infections (n = 70) and strains from LivaNova HCUs (n = 4), Maquet HCUs (n = 3), Maquet ECMOs (n = 11), a hospital-built HCU (n = 1), Maquet production site (n = 1), and a patient infected after cardiac surgery (n = 1). ECMO, extracorporeal membrane oxygenation; HCU, heater–cooler unit. Scale bars indicate numbers of substitutions per site.

room where the patient had undergone his initial surgery (online Technical Appendix Figure). The referring hospital did not use HCUs or extracorporeal membrane oxygenation devices. In summer 2015, we obtained water and air samples from the operating room (Table); results were negative for *M. chimaera*.

According to a signature single-nucleotide polymorphism–based classification, the patient isolate was similar to the group 1 strains of *M. chimaera* (*2*). We therefore included all group 1 strains with sufficient WGS data from published studies together with the patient isolate in a combined analysis of a total of 437 strains (Figure 2). The patient isolate did not cluster with subgroup 1.1, which represented all but 1 of the reported cases of disseminated *M. chimaera* infections associated with contaminated HCUs. Instead, the patient strain clustered with strains from subgroup 1.11 and branch 1 of group 1 (*2*); however, the patient strain had no close relationship to any other strain included in the comparison.

The endoprosthetic graft (Excluder RMT261214/PXC121200) of our patient was produced by Gore Medical (Newark, DE, USA). The Swiss Agency for Therapeutic Products submitted a medical device report for the implicated graft to the manufacturer.

Conclusions

We report an endovascular AGI caused by *M. chimaera* and *G. adiacens*, which was successfully treated with extra-anatomic bypass and prolonged antimicrobial therapy. Because of the histopathology results showing focal

granulomatous necrotizing inflammation and detection of sparse acid-fast rods in Ziehl Neelsen stain, we outweighed the importance of *M. chimaera* compared with *G. adiacens*.

Patients at risk for NTM infections are elderly patients with preexisting pulmonary conditions or immunocompromised patients. At AGI diagnosis, the localized pulmonic cancer in this patient was in an early stage, and the patient was not known to be immunocompromised. Blood cultures and repeated sputum specimens were negative for mycobacteria, and PET–CT did not reveal any distant foci. Therefore, we considered a hematogenous spread of a localized and naturally acquired infection to be unlikely. Water and air samples from the operating room were negative for *M. chimaera*; thus, local contamination in the operating room was unlikely. When we compared the patient's isolate with other available *M. chimaera* strains with available WGS data (*2*,*6*–*10*), we observed no association with the cardiac surgery cluster or any other closely related strain in the collection. Because the cardiac surgery cluster originated from *M. chimaera*–contaminated water in medical devices, a contamination of the medical prosthesis at the production site was considered, especially because the poorly soluble polytetrafluoroethylene polymerization is conducted as an emulsion in purified water. However, according to the graft manufacturer, its grafts are produced in a controlled environment, and ethylene oxide gas (EOG) is used for sterilization as recommended by the International Organization for Standardization (standard no. 11135-2007). EOG is widely used because of its good bactericidal activity on many bacterial species and even bacillus spores (*11*). However, studies showing the effect of EOG on mycobacteria are lacking, and cases of NTM infections caused by inadequate implant sterilization have been reported (*12*). As the logical next step in the investigation, testing environmental water samples from the production site or from fresh implants for NTM contamination was proposed. However, because of a paperwork assessment, the company decided not to pursue the case further.

Because our investigation involved a single case of an abdominal AGI caused by *M. chimaera* and *G. adiacens*, it is too early to draw any conclusions. If further infections emerge, investigations into the adequacy of EOG sterilization for arterial implants should be conducted. In this case, the combination of prolonged antimicrobial therapy, graft explantation, and extra-anatomic reconstruction resulted in sustained healing.

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About the Author

Dr. Plate is an internal medicine specialist working as an infectious disease fellow in the Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, Switzerland. His primary research interests are epidemiology and foreign body–associated infections.

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Address for correspondence: Andreas Plate, University Hospital Zurich, Division of Infectious Diseases and Hospital Epidemiology, Raemistrasse 100 CH-8091, Zurich, Switzerland; email: andreas.plate@usz.ch

etymologia

Granulicatella [gran'yoo-lik-ə-tel"ə]

Ronnie Henry

In 1961, Frenkel and Hirsch described strains of streptococci isolated from cases of bacterial endocarditis that grew only in the presence of other bacteria, around which they formed satellite colonies, or in media enriched with sulfhydryl compounds, such as cysteine. These nutritionally variant streptococci were eventually assigned the species *Streptococcus defectivus* (Latin for "deficient") and *S. adjacens* (because it grows adjacent to other bacteria).

On the basis of later research, these were placed in a new genus *Abiotrophia* (Greek *a*, "un-," + *bios*, "life," + *trophe*, "nutrition") as *A. adiacens* and *A. defectiva*. In 1998 and 1999, 2 additional species of *Abiotrophia* were described, *A. elegans* (Latin, "fastidious," referring to fastidious growth requirements) and *A. balaenopterae* (isolated from a minke whale [*Balaenoptera acutorostrata*]). In 2000, these new species, along with *A. adiacens*, were reclassified in the new genus *Granulicatella* (Latin *granulum*, "small grain," + *catella*, "small chain").

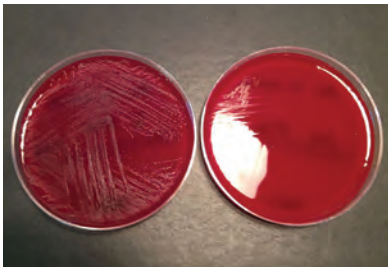

Figure. Blood agar plates with (left) and without (right) pyridoxal supplement from a study of neonatal *Granulicatella elegans* bacteremia, London, UK. Image from Neonatal *Granulicatella elegans* Bacteremia, London, UK; Emerging Infectious Diseases Vol. 19, no. 7, July 2013.

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Address for correspondence: Ronnie Henry, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop E03, Atlanta, GA 30329-4027, USA; email: boq3@cdc.gov

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Estimating Frequency of Probable Autochthonous Cases of Dengue, Japan

Akiyoshi Senda,[1] Anavaj Sakuntabhai, Shinako Inaida, Yoann Teissier, Fumihiko Matsuda, Richard E. Paul[1]

Imported dengue into naive areas is a recognized but unquantified threat. Differentiating imported and autochthonous cases remains problematic. A threshold approach applied to Japan identified several aberrant incidences of dengue. Despite these alerts, no epidemics occurred other than 1 in Yoyogi Park in Tokyo, which was probably an unusual event.

Dengue is a major international public health concern, and the number of dengue outbreaks has escalated over the past decade (*1*). International travel will ensure importation of dengue virus (DENV) from dengue-endemic regions into nonendemic countries (*2*). The potential threat of DENV invasion into naive areas is illustrated by autochthonous dengue cases in France and the United States (*3*,*4*) and unprecedented epidemics in the Madeira Islands of Portugal and Tokyo, Japan (*5*,*6*). Most human DENV infections are asymptomatic (*7*), but the virus can still be transmitted to mosquitoes (*8*), so repeated "silent" DENV invasion will probably become increasingly frequent.

In 2012, the World Health Organization released a global strategy for dengue prevention and control, with the objective of reducing dengue-attributable deaths by 50% and dengue-attributable illness by 25% by 2020 (*9*). These reductions are to be achieved, at least in part, by implementing improved outbreak prediction and detection through coordinated epidemiologic and entomologic surveillance. This approach is also important for areas where dengue is nonenedemic, that have no defined surveillance strategy.

Within this context, we examine the case of Japan, which had an unprecedented autochthonous DENV type 1 epidemic in Yoyogi Park in Tokyo in 2014 and is experiencing an ever-increasing number of dengue cases. Analyzing dengue case surveillance data from a 6-year period, we assess whether other incidents occurred when the number of dengue cases exceeded the expected number because of importation and whether these incidents represented potential foci of epidemics. We discuss whether the Tokyo epidemic was a rare event, the probability of a repeat epidemic, and the value of establishing a dengue alert threshold.

The Study

Since 1999, dengue has been 1 of the notifiable diseases under national surveillance across Japan. The case definition of dengue fever includes the presence of suspicious clinical symptoms and laboratory confirmation. The definition of an imported case is DENV infection in a patient who had traveled to a dengue-infected area within 2 weeks before symptom onset; all other cases are defined as autochthonous. All diagnosed dengue cases are registered in the surveillance system database (*10*).

We selected as study sites the 2 largest metropolitan areas, Greater Tokyo (including the prefectures of Tokyo, Saitama, Kanagawa, and Chiba) and Greater Osaka (including the prefectures of Osaka, Hyogo, and Kyoto), which encompass the largest number of dengue cases during the study period and can be considered as work commuting zones. Incidence rate was the number of cases divided by the population according to the 2015 national census. To calculate the threshold, we extracted data from annual reports for 2005 through 2014 (http://survey.tokyo-eiken.go.jp/epidinfo/csvinfo.do). We used Tukey's box plot method to establish the median background weekly incidence of dengue in each study area based on the previous 6 years' data. We defined the weekly threshold as the rounded-up value of the third quartile + 1.5 times the interquartile range of the number of cases from the same week. We applied the 6 previous years' weekly thresholds of cases in each prefecture to the weekly reported cases for 2011–2016. We defined an outlier as a week when the number of cases was ≥1 above or equal to the threshold. We defined the threshold for an autochthonous epidemic alert as ≥2 consecutive weeks in which outliers were detected.

Author affiliations: Kyoto University Faculty of Medicine, Kyoto, Japan (A. Senda); Kyoto University Graduate School of Medicine, Kyoto (S. Inaida, F. Matsuda); Pasteur Kyoto International Joint Research Unit for Integrative Vaccinomics, Kyoto (A. Sakuntabhai, F. Matsuda, R.E. Paul); Institut Pasteur, Paris, France (A. Sakuntabhai, R.E. Paul); Centre National de la Recherche Scientifique, Paris (A. Sakuntabhai, R.E. Paul); Institut Louis Malardé, Papeete, French Polynesia (Y. Teissier)

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[1]These authors contributed equally to this article.

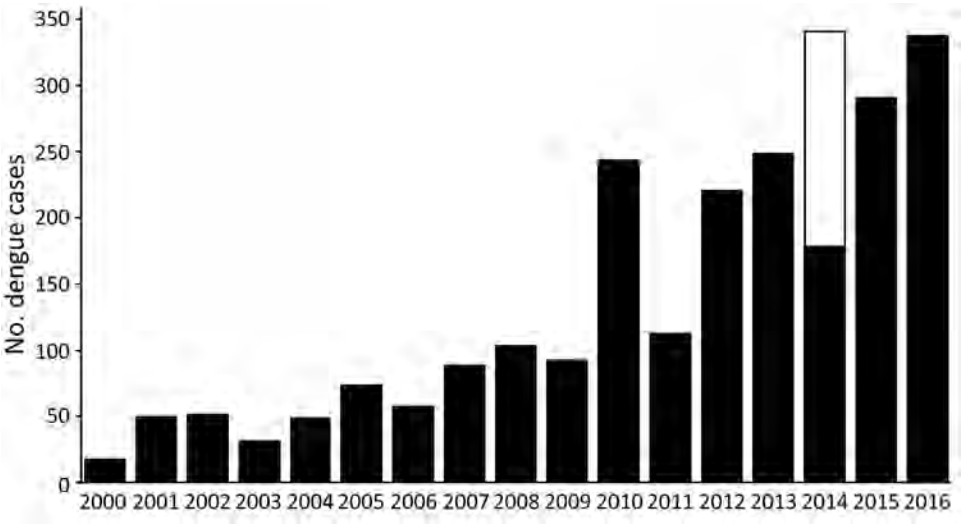

Figure 1. Annual reported dengue cases reported in dengue surveillance system, Greater Tokyo and Greater Osaka areas, Japan, 2011–2016. Black indicates imported cases; white indicates autochthonous cases detected during Tokyo epidemic.

The number of imported cases in Japan has been rising steadily over the past decade (Figure 1), concomitant with the increase in visitors, especially from South Korea, China, Taiwan, and Thailand (online Technical Appendix Figures 1, 2, https://wwwnc.cdc.gov/EID/article/24/9/17-0408-Techapp1.pdf). Until 2015, the number of outbound travelers from Japan exceeded that of inbound foreign travelers; one third of travelers from Japan went to dengue-endemic countries.

In the Greater Tokyo and Greater Osaka areas, the threshold value of the incidence rate varied by year and place (Table). Outlying dengue case weeks were detected in all 7 prefectures of the 2 aggregated greater areas during the 6-year study period (Table; Figure 2). We noted several occasions when outliers were reported for 2 consecutive weeks (7 times in Greater Tokyo and 4 times in Greater Osaka). In Greater Tokyo, conditions warranting an alert occurred in 2012 (weeks 10–11 and 36–37), 2013 (weeks 19–20), 2015 (weeks 2–3), and 2016 (weeks 1–2, 12–14, and 17–19); in Greater Osaka, conditions warranting an alert occurred in 2012 (weeks 34–36), 2013 (weeks 41–42), 2014 (weeks 12–15), and 2016 (weeks 12–13).

At the prefecture level, an alert condition was detected August 25–September 7, 2014, in Kanagawa Prefecture; this timing coincided with the Tokyo autochthonous epidemic. In addition, alert conditions were identified in Tokyo in 2013 (weeks 32–33) and 2016 (weeks 13–14), in Chiba in 2016 (weeks 33–34), and in Osaka in 2016 (weeks 12–13). The Tokyo and Osaka 2016 alert conditions occurred during the cold season and were probably caused by an increase in imported cases from Indonesia (*11*). By contrast, the alert condition in Chiba was followed by 2 additional cases reported in week 39 and 2 in week 42; both occurrences are outliers but are not in consecutive weeks and thus do not warrant an alert. The alert condition in 2013 in Tokyo was the first such occurrence observed in our data and coincided with a visit by a traveler from Germany who was allegedly infected with dengue in Japan (*12*) and had visited Tokyo.

Conclusions

We have addressed the increasing probability of dengue invasion into Japan in light of the 2014 Yoyogi Park epidemic. Although the increase in dengue cases in Japan is concomitant with the increase in human travel between Japan and dengue-endemic areas, several reports exist of travelers contracting dengue while visiting Japan, which suggests that DENV is circulating in the form of subclinical infections; by extrapolation, allegedly imported cases might be autochthonous (*12*).

Table. Thresholds and conditions warranting an autochthonous dengue case alert, Greater Tokyo and Greater Osaka areas, Japan, 2011–2016

Area and prefecture	Population	Total no. cases	Incidence rate, cases/10^6 person-years	Threshold range, maximum (mean)	No. outliers	No. occurrences of alert conditions
Greater Tokyo area	36,126,355	609	2.8	17 (4.5)	52	7
Tokyo	13,513,734	358	4.4	11 (3.1)	32	2
Kanagawa	9,127,323	111	2.0	5 (0.77)	16	1
Saitama	7,261,271	45	1.0	3 (0.12)	4	0
Chiba	6,224,027	95	2.5	7 (0.89)	14	1
Greater Osaka area	16,986,037	230	2.3	9 (2.0)	25	4
Osaka	8,838,908	140	2.6	5 (1.4)	12	1
Hyogo	5,536,989	52	1.6	4 (0.29)	6	0
Kyoto	2,610,140	38	2.4	3 (0.24)	1	0

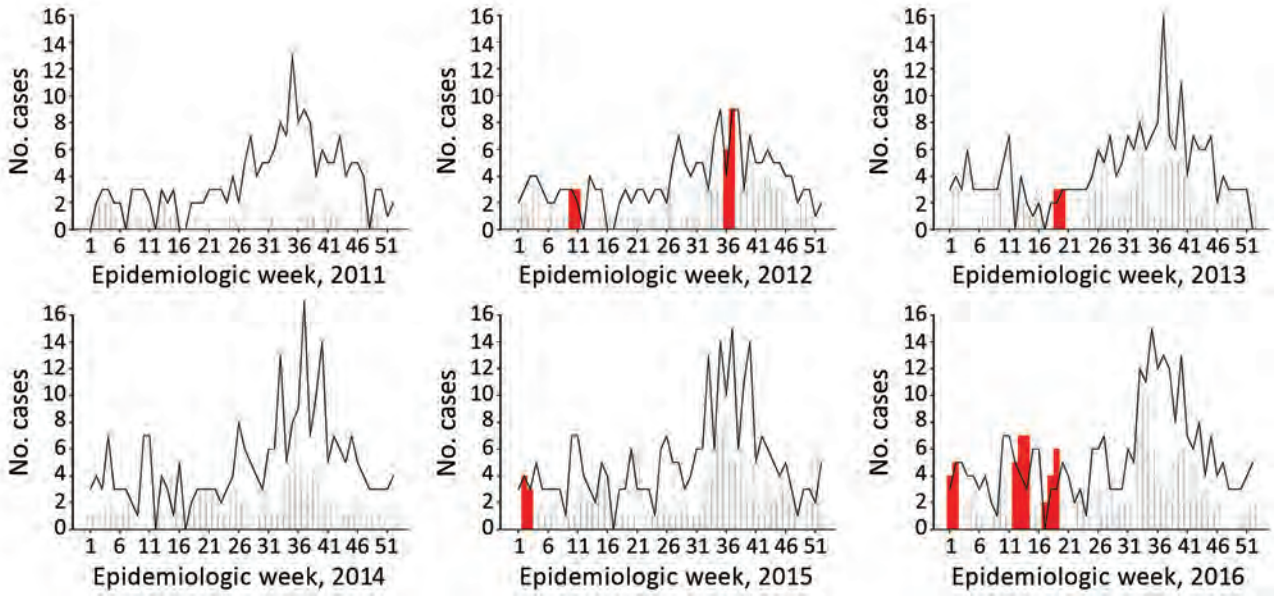

Figure 2. Detection of conditions warranting an autochthonous dengue case alert (red bars) compared with number of reported dengue cases per week (histogram) and estimated background threshold (black line), by year, Greater Tokyo area, Japan, 2011–2016.

Differentiating imported and autochthonous cases based on recent travel history might be misleading. A case-patient in Hyogo Prefecture, ≈400 km from Tokyo, had stayed in Malaysia during the 12 days before symptom onset but had recollection of mosquito bites 6 days before onset, and the virus strain 100% matched the Yoyogi Park strain (*13*). Unusual above-threshold incidences of dengue might provide an additional criterion for differentiation. Although no official epidemic coincided with the occurrence of dengue in the traveler from Germany (*12*), our alert threshold pinpointed this period as being aberrant. Unusual above-threshold dengue incidences were noted during several periods, but no subsequent epidemic progression was noted. A substantial stochastic dieout of circulating DENV is occurring, despite permissive temperatures that would enable efficient transmission of DENV by the predominant mosquito vector, *Aedes albopictus*, which occurs at high densities in urban areas of Japan (*14*). However, most infections probably will go unnoticed, and the actual spread of DENV is greater than estimated from surveillance. DENV seroprevalence results for 207 persons who frequented Yoyogi Park indicated that 10 persons without recollection of symptoms were seropositive (*15*).

In conclusion, although increased human movement and permissive temperatures pose a threat for DENV invasion, evidence suggests that the Yoyogi Park epidemic was an exception and that a considerable viral biomass after repeated introduction might be required for successful viral implantation. The added utility of using a threshold approach to detect aberrant incidence rates for public health activities remains to be developed but could provide a basis for performing seroprevalence studies around cases detected during weeks with aberrantly high incidence to establish the extent of the problem.

About the Author

Dr. Senda is a medical school graduate of Kyoto University, Kyoto, Japan. His primary research interest is the epidemiology of vectorborne infectious diseases.

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Address for correspondence: Richard E. Paul, Institut Pasteur, 25 Rue du Dr. Roux, Paris 75015, France; email: richard.paul@pasteur.fr

Correlation of Severity of Human Tick-Borne Encephalitis Virus Disease and Pathogenicity in Mice

Chaitanya Kurhade,[1] Sarah Schreier,[1] Yi-Ping Lee,[1,3] Loreen Zegenhagen,[4] Marika Hjertqvist, Gerhard Dobler, Andrea Kröger,[2] Anna K. Överby[2]

We compared 2 tick-borne encephalitis virus strains isolated from 2 different foci that cause different symptoms in tick-borne encephalitis patients, from neurologic to mild gastrointestinal symptoms. We compared neuroinvasiveness, neurovirulence, and proinflammatory cytokine response in mice and found unique differences that contribute to our understanding of pathogenesis.

Tick-borne encephalitis (TBE) is an emerging arthropod-borne viral (arboviral) disease in Europe and Asia characterized by severe central nervous system (CNS) disease in humans. New areas of endemicity and increased TBE incidence have been reported (*1*). In Sweden, TBE cases have increased dramatically; during 2017, a record year, 391 cases were reported, compared with 238 cases during 2016. TBE virus (TBEV) is transmitted by tick bites and ingestion of contaminated milk (*2*,*3*). Infection (TBEV, European subtype) usually follows a biphasic course in which, during the primary phase, patients show symptoms of fatigue, headache, myalgia, and fever, followed by a second phase of neurologic involvement with signs of meningitis, encephalitis, and paralysis and high fever. Neurologic sequelae develop in 20%–60% of cases (*4*–*7*). The ability of the virus to cause CNS disease (neurovirulence) depends on its ability to enter the brain (neuroinvasiveness).

Recently, a focus of TBE in southeastern Germany was identified with 5 patients (2005–2011), who showed only mild gastrointestinal and constitutional symptoms, without neurologic symptoms. One strain, MucAr HB171/11, was isolated from 6 questing adult *Ixodes ricinus* ticks from this natural focus (49°17′N, 12°12′E) (*8*). To investigate this low pathogenic strain and the absence of neurologic symptoms, we compared its pathogenesis with another European strain, Torö-2003. Torö-2003 was rescued from a cDNA infectious clone (*9*) generated from RNA extracted from a pool of *I. ricinus* ticks (9 adults, 106 nymphs) collected in September 2003 on the island of Torö (58°49′N, 17°50′E) in the Stockholm archipelago of Sweden (*10*). In the focus on Torö, 32 TBE patients (1986–2016) were reported. Data on 4 of these TBE case-patients show relatively mild neurologic disease with a few days of hospitalization for 2 of them. In the mouse model, Torö-2003 shows similar pathogenicity as the highly virulent Hypr strain (*9*). Knowledge of differential clinical courses and severity of disease caused by strains of TBEV can be an important criterion for diagnosing and treating the disease.

The Study

All animal experiments were performed in compliance with the German animal welfare law (TierSchG BGBl. S. 1105; 25.05.1998). Mice were housed and handled in accordance with good animal practice as defined by the Federation for Laboratory Animal Science Associations. All animal experiments were approved by the responsible state office (Lower Saxony State Office of Consumer Protection and Food Safety) under permit no. AZ 33.9-42502-04-11/0528. Experiments were performed in the Biosafety Level 3 facility at the Helmholtz Center for Infection Research (Braunschweig, Germany). Mice used for primary cell isolation were maintained under specific pathogen-free conditions, and studies were conducted according to the guidelines set out by the Regional Animal Ethical Committee (Umeå, Sweden; approval no. A77-14).

To assess the pathogenicity of the Torö-2003 and HB171/11 strains, we challenged C57BL/6 mice with 10^4 focus-forming units of Torö-2003 and HB171/11 (second passage in Vero cells) subcutaneously. Mice were highly susceptible to Torö-2003 infection and 100% succumbed to the infection; median survival time was 13 days. Mice showed paralysis, lethargy, hunchback posture, fur ruffling, and weight loss 2 days before death. In contrast, only 60% of the HB171/11-infected mice died (median survival time

Author affiliations: Umeå University, Umeå, Sweden (C. Kurhade, Y.-P. Lee, A.K. Överby); Helmholtz Centre for Infection Research, Braunschweig, Germany (S. Schreier, L. Zegenhagen, A. Kröger); Otto-von-Guericke-University Magdeburg, Magdeburg, Germany (S. Schreier, A. Kröger); Public Health Agency of Sweden, Solna, Sweden (M. Hjertqvist); Bundeswehr Institute of Microbiology, Munich, Germany (G. Dobler); DZIF Partner Site Munich, Munich (G. Dobler)

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[1]These first authors contributed equally to this article.

[2]These senior authors contributed equally to this article.

[3]Current affiliation: National Cheng Kung University, Tainan, Taiwan.

[4]Current affiliation: Charles River Laboratories, Freiburg, Germany.

18.5 days) (Figure 1, panel A). Analysis of viral RNA in peripheral organs after infection showed viral replication of Torö-2003 6 days postinfection (dpi) in the spleen (Figure 1, panel B). In inguinal lymph nodes (Figure 1, panel C) and lung (data not shown), we detected Torö-2003 RNA only at 10 dpi. In contrast, viral RNA from HB171/11 could hardly be detected in peripheral organs. Because gastrointestinal and constitutional symptoms reported for HB171/11 in humans (*8*) are hardly detectable in mice, we analyzed viral RNA in colon, appendix, and small intestine. No viral RNA was detected in these tissues (data not shown).

To investigate whether infection with the different virus strains changes immune response, we looked for proinflammatory gene induction in lymphoid tissue. Tumor necrosis factor–α, interleukin-6, and CXC motif ligand–10 were highly up-regulated in spleen and lymph nodes on infection with HB171/11, compared with Torö-2003 4 dpi. At later time points, we detected similar expression levels in

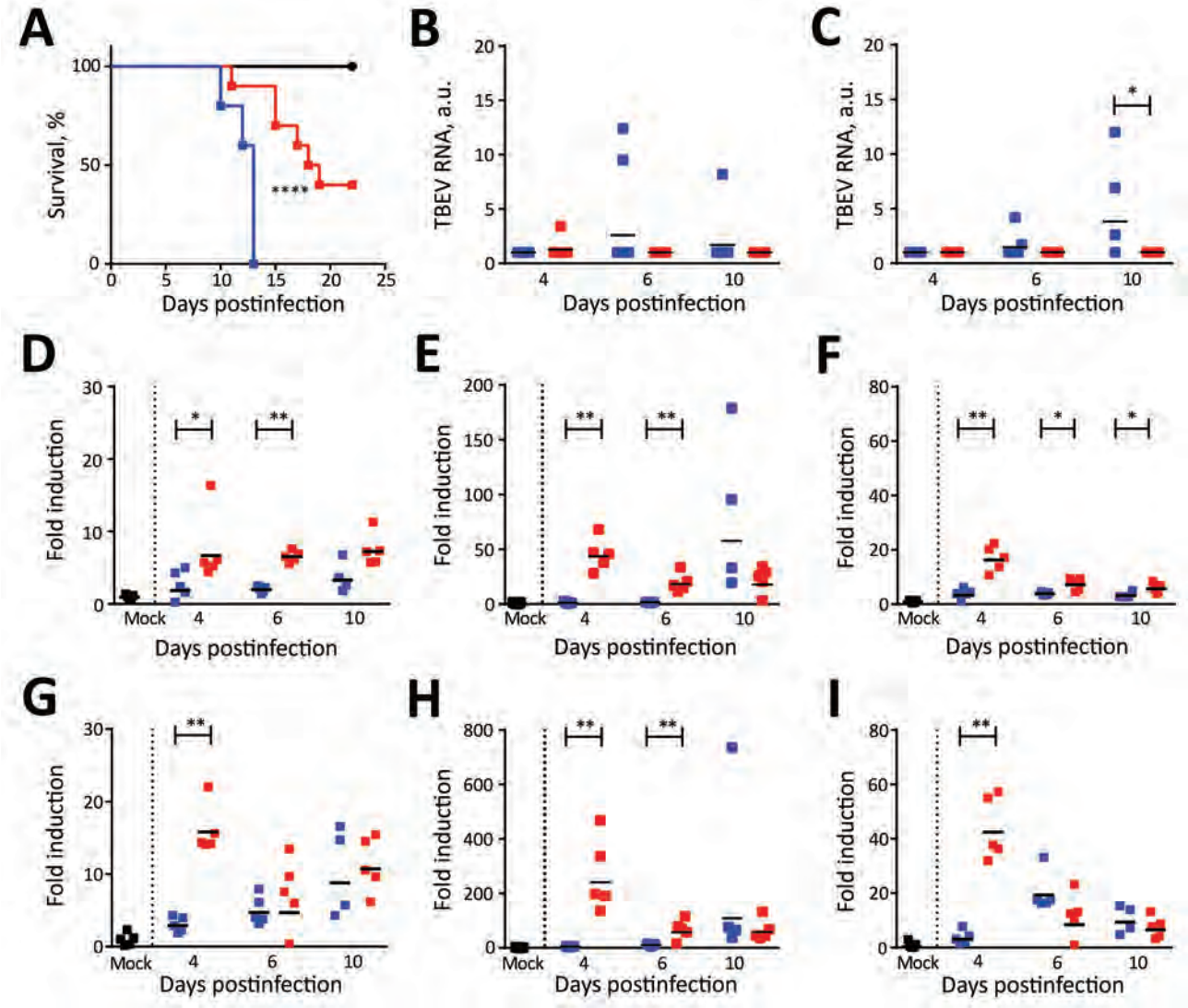

Figure 1. Survival analysis and TBEV burden in peripheral organs of Torö-2003–infected and HB171/11-infected C57BL/6 mice. A) Survival analysis of ten 6–10-week-old female C57BL/6 mice after subcutaneous inoculation with phosphate-buffered saline (mock, black) or with 10^4 focus forming units (FFU) of Torö-2003 (blue) or HB171/11 (red) in 100 μL phosphate-buffered saline. Survival differences were tested for statistical significance by log-rank test. B, C) Viral burdens in spleen (B) and lymph node (C) after subcutaneous infection of Torö-2003 or HB171/11 (10^4 FFU, n = 5) were measured by quantitative PCR and normalized to intracellular glyceraldehyde 3-phosphate dehydrogenase (GAPDH) levels as previously described (*14*). Each data point represents an individual mouse. D–I) Cytokine response in spleen (D–F) and lymph node (G–I). Expression levels of GAPDH, TNF-α (D,G), IL-6 (E,H), and CXCL-10 (F,I) were determined by validated QuantiTect primer assays (QIAGEN, Hilden, Germany) and quantitative PCR from organs prepared in B and C. Signals of indicated mRNA were normalized to the GAPDH mRNA signal. Bars indicate mean values. Asterisks indicate statistical significance calculated by Mann-Whitney test (*p<0.05; **p<0.01). Horizontal black bars indicate mean values. a.u., arbitrary units; CXCL, CXC motif ligand; IL, interleukin; TBEV, tick-borne encephalitis virus; TNF, tumor necrosis factor.

Torö-2003–infected and HB171/11-infected mice (Figure 1, panels D–I). Macrophages and monocytes are potential producers of these cytokines and may influence immune response to control virus replication of HB171/11 in the periphery.

Because HB171/11 caused only mild gastrointestinal and constitutional symptoms without specific neurologic symptoms, we hypothesized that HB171/11 might be less neuroinvasive than Torö-2003. To assess this hypothesis, we infected mice subcutaneously and isolated different parts of the CNS (olfactory bulb, cerebrum, cerebellum, brain stem, and spinal cord) at different times after infection and analyzed viral load in the CNS. At 6 dpi, we detected Torö-2003 virus in almost all the CNS regions; replication was highest in the olfactory bulb. At 10 dpi, Torö-2003 virus replication further increased in all parts except the olfactory bulb, where viral burden was maintained. For HB171/11, we detected no viral RNA at 6 dpi and only low levels of virus in most CNS parts at 10 dpi (Figure 2, panels A–E), indicating delayed neuroinvasiveness of HB171/11.

Next, we investigated the neurovirulence of the different strains. We injected mice with 100 focus-forming units of virus directly into cerebral cortex through the intracranial route. Torö-2003 was highly pathogenic, leading to 100% deaths; median survival was 7 days.

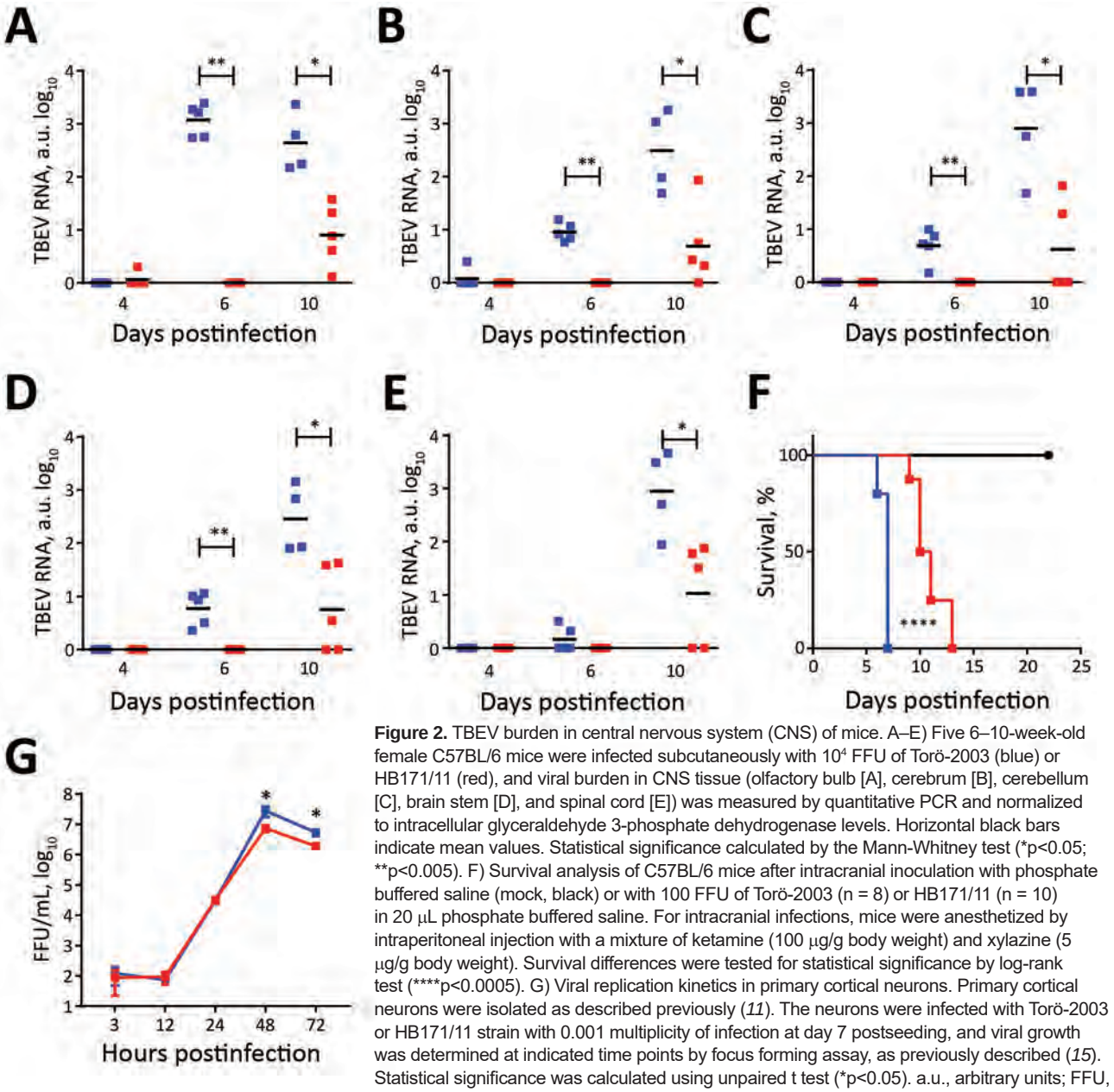

Figure 2. TBEV burden in central nervous system (CNS) of mice. A–E) Five 6–10-week-old female C57BL/6 mice were infected subcutaneously with 10^4 FFU of Torö-2003 (blue) or HB171/11 (red), and viral burden in CNS tissue (olfactory bulb [A], cerebrum [B], cerebellum [C], brain stem [D], and spinal cord [E]) was measured by quantitative PCR and normalized to intracellular glyceraldehyde 3-phosphate dehydrogenase levels. Horizontal black bars indicate mean values. Statistical significance calculated by the Mann-Whitney test (*p<0.05; **p<0.005). F) Survival analysis of C57BL/6 mice after intracranial inoculation with phosphate buffered saline (mock, black) or with 100 FFU of Torö-2003 (n = 8) or HB171/11 (n = 10) in 20 μL phosphate buffered saline. For intracranial infections, mice were anesthetized by intraperitoneal injection with a mixture of ketamine (100 μg/g body weight) and xylazine (5 μg/g body weight). Survival differences were tested for statistical significance by log-rank test (****p<0.0005). G) Viral replication kinetics in primary cortical neurons. Primary cortical neurons were isolated as described previously (*11*). The neurons were infected with Torö-2003 or HB171/11 strain with 0.001 multiplicity of infection at day 7 postseeding, and viral growth was determined at indicated time points by focus forming assay, as previously described (*15*). Statistical significance was calculated using unpaired t test (*p<0.05). a.u., arbitrary units; FFU, focus-forming units; TBEV, tick-borne encephalitis virus.

We observed lower neurovirulence for HB171/11, and survival was a median of 10.5 days.

To explore the relationship between mice pathogenicity and viral replication in target cells, we analyzed replication in neurons. We isolated primary cortical neurons from C57BL/6 mice infected with 0.001 multiplicity of infection and measured progeny particles by focus-forming assay (*11*). Both strains replicated to the same level at early time points; later in infection (48 and 72 h), Torö-2003 replicated to higher levels compared with HB171/11 (Figure 2, panel G).

Taken together, HB171/11 shows lower neurovirulence in mice, probably because of reduced replication in neurons. However, we cannot exclude that other cell types within the CNS (astrocytes and microglia) also could contribute to the lower neurovirulence. The low neurovirulence in combination with the slower neuroinvasiveness, resulting from low replication in the periphery and high up-regulation of proinflammatory cytokines, make the HB171/11 less pathogenic in the mouse model.

Conclusions

TBEV is spreading into new regions in Europe: Sweden, Norway, Finland, France, the Netherlands, Italy, and Switzerland (*1*). The typical symptoms of infection are meningitis, encephalitis, and paralysis. However, recent reports also indicate gastrointestinal problems (*8*). Such new strains that cause these rare symptoms complicate the diagnosis of TBEV infection and raise the question of the number of unrecognized TBE cases. We characterized the pathogenesis and immune response of 2 European isolates of TBEV from infection foci that coincide with human cases displaying completely different disease symptoms. To characterize these clinical manifestations of disease, we used C57BL/6 mice to study TBEV pathogenesis. These mice are susceptible to infection and develop encephalitis without the need for adaptation of the virus isolates. The pathogenicity of the 2 virus strains clearly differed. We could not detect gastrointestinal symptoms in the HB171/11-infected mice, but the low-virulence phenotype of HB171/11 could be mimicked. The mice showed a strong cytokine response in periphery, low and delayed neuroinvasiveness, and low neurovirulence that, when translated into humans, might explain the lack of neurologic symptoms. Because genetic changes in TBEV could affect pathogenicity (*12*,*13*), future molecular studies are needed to determine the low pathogenicity of and enhanced immune response against low-virulence strain HB171/11.

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About the Author

Mr. Kurhade is a PhD student at the Department of Clinical Microbiology, Virology, Umeå University. His research focuses on TBEV.

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Address for correspondence: Anna K. Överby, Umeå University, Department of Clinical Microbiology, Virology, S-90185 Umeå, Sweden; email: anna.overby@umu.se

Increasing Prevalence of *Borrelia burgdorferi* sensu stricto–Infected Blacklegged Ticks in Tennessee Valley, Tennessee, USA

Graham J. Hickling, Janetta R. Kelly, Lisa D. Auckland, Sarah A. Hamer

In 2017, we surveyed forests in the upper Tennessee Valley, Tennessee, USA. We found *Ixodes scapularis* ticks established in 23 of 26 counties, 4 of which had *Borrelia burgdorferi* sensu stricto–infected ticks. Public health officials should be vigilant for increasing Lyme disease incidence in this region.

In the United States, Lyme disease caused by tickborne bacterium *Borrelia burgdorferi* sensu stricto occurs primarily in the Northeast and upper Midwest (*1*). In eastern Tennessee, which is considered nonendemic for Lyme disease, most of the human population resides in a low-elevation swath of the Tennessee Valley bordered to the west by the Cumberland Plateau and the east by the Great Smoky Mountains. The vector of Lyme disease, the blacklegged tick *Ixodes scapularis*, was unreported in this area before 2006; in this year, uninfected adult ticks were collected from hunter-harvested deer in 8 Tennessee Valley counties (Figure 1, panel A) (*2*). This finding, plus uninfected *I. scapularis* ticks detected in Knox County in 2013, were later incorporated into the national distribution map for *I. scapularis* ticks (*3*).

During 2000–2014, human Lyme disease cases expanded southward along the eastern foothills of the Appalachian Mountains in nearby Virginia (*4*). In the winters of 2012 and 2013, *B. burgdorferi*–infected adult *I. scapularis* ticks were detected in Pulaski County, Virginia (*5*). This report of abundant infected *I. scapularis* ticks only 100 km from the Tennessee border motivated us to investigate whether *Borrelia*-infected ticks might now be present in the Tennessee Valley.

The Study

In late 2017, we sampled host-seeking *I. scapularis* ticks at 70 forested sites in 26 low-elevation counties in the upper Tennessee Valley (Figure 1, panel B). To find tick habitats (hardwood or conifer forests <800 m in elevation) accessible for sampling (i.e., trails through public forests or margins of public roads through private forests), we reviewed Google Earth (https://www.google.com/earth/) satellite imagery. We sampled each site once during the peak of adult *I. scapularis* tick activity (late October–January). We recorded site elevation and geocoordinates and collected host-seeking ticks using a standardized drag-cloth method; in brief, we dragged a 1-m^2 white corduroy cloth across leaf litter and checked every 10 paces for attached ticks. We dragged cloths 30–60 minutes per site and described tick tallies as number collected per hour to correct for variations in effort per site. We did not conduct drag-cloth collections during periods of rain, strong wind, low air temperatures (<8°C), or low relative humidity (<40%).

We placed ticks in 70% ethanol, identified species using a morphologic key (*6*), and tested ticks for *Borrelia* spirochete infection by DNA extraction and quantitative multiplex real-time PCR using differential probes targeting the 16S rDNA of Lyme group *Borrelia* and relapsing fever group *Borrelia* (*7*). We then subjected a random subset of negative samples and samples positive by the 16S assay (maximum 6 samples/site) to PCR amplification of the 16S–23S rDNA intergenic spacer region (*8*) and Sanger sequencing for species-level identification.

No previous tick drag-cloth counts existed for the counties in our survey area, except for a 1,050-m transect of land in a forest in Anderson County, which we have drag-cloth sampled annually each December since 2012. To assess a trend in adult *I. scapularis* tick abundance, we applied linear regression modeling to the tick tallies from that transect of land.

In late 2017, we collected 479 adult *I. scapularis* ticks from 49 of 70 sites in the upper Tennessee Valley. Two adult *Amblyomma americanum* ticks collected during the survey were excluded from analysis. We detected *I. scapularis* ticks in all 26 counties surveyed, 23 of which met the criterion used by Eisen et al. for established *I. scapularis* populations (Figure 1, panel B) (*3*). Site elevations were 210–730 m; the highest elevation at which *I. scapularis* ticks were found was 570 m. The average number of adult ticks collected per hour during drag-cloth surveys was 8.8 (range 0–48). At the Anderson County site that had been

Author affiliations: University of Tennessee Institute of Agriculture, Knoxville, Tennessee, USA (G.J. Hickling, J.R. Kelly); Texas A&M University, College Station, Texas, USA (L.D. Auckland, S.A. Hamer)

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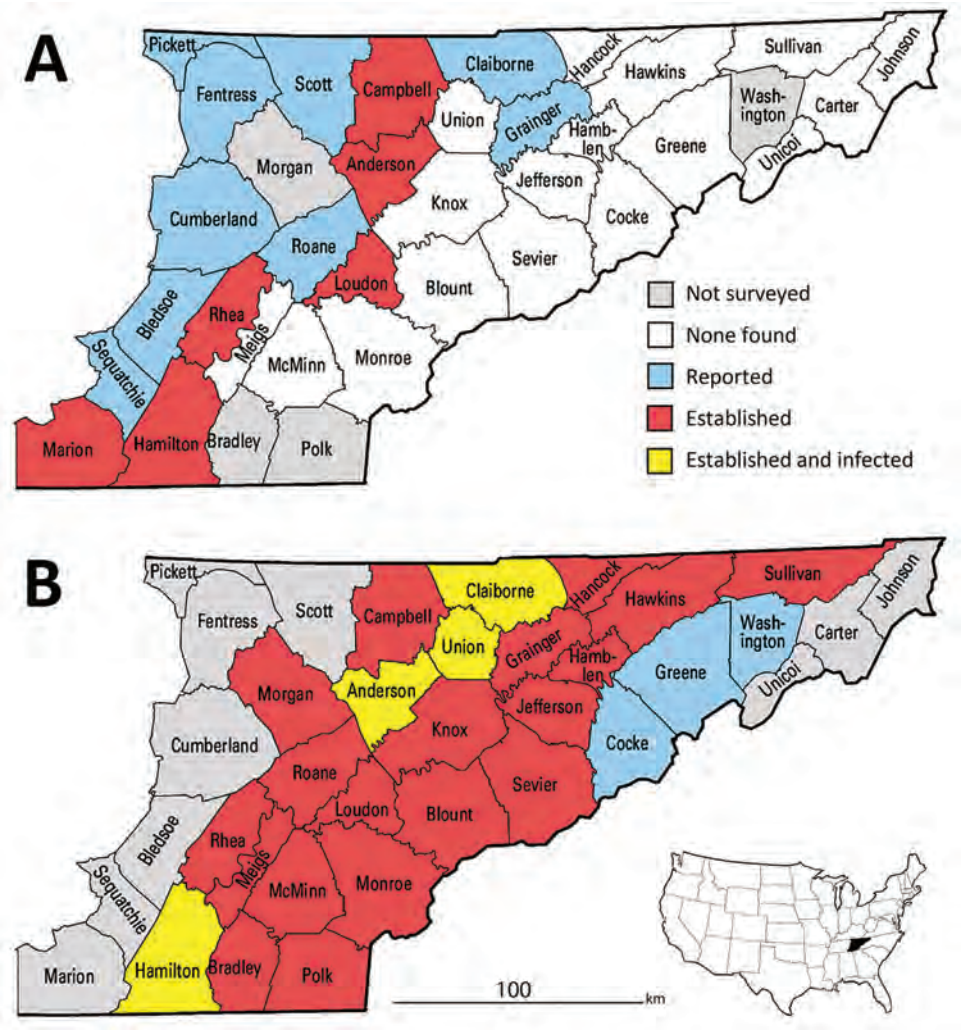

Figure 1. County-level distribution of *Ixodes scapularis* ticks and *Borrelia burgdorferi*–infected *I. scapularis* ticks in upper Tennessee Valley, USA, 2006 and 2017. A county was classified as having an established *I. scapularis* population if ≥6 *I. scapularis* adult ticks or ticks of 2 life stages were collected in that county. A county was classified as having *I. scapularis* ticks reported if 1–5 *I. scapularis* ticks of a single life stage were collected in that county. A county was classified as infected if *I. scapularis* ticks infected with *B. burgdorferi* were detected in that county. A) *I. scapularis* ticks in 2006 (*2*), determined by collecting ticks from hunter-harvested deer. B) *I. scapularis* ticks in 2017 determined by drag-cloth surveying during the peak of adult tick activity (late October–January).

drag-cloth sampled annually, a highly significant increasing trend in *I. scapularis* ticks was evident (p = 0.003; Figure 2); the count in 2017 (24.8 ticks/hour) was 3.5× higher than that in 2012.

We tested all *I. scapularis* ticks collected (N = 479) for *Borrelia* spp. infection; 46 ticks (9.6%) from 7 sites in 4 counties (Anderson, Claiborne, Hamilton, and Union; Figure 1, panel B) tested positive for Lyme group *Borrelia* by 16S rDNA PCR screening. We tested 26 samples for the intergenic spacer region by PCR; all were positive for this sequence and identified as *B. burgdorferi* sensu stricto by sequencing. Most infected ticks came from 2 Union County sites, which had prevalences of 44% (14/32) and 78% (18/23). No ticks were found to be infected with *B. miyamotoi* or other relapsing fever group borreliae.

Conclusions

In eastern Tennessee, public awareness and concern about ticks focuses primarily on the abundant lone star ticks (*Amblyomma americanum*) and American dog ticks (*Dermacentor variabilis*) encountered during the spring and summer. Both species can spread pathogens (*9*), but neither are vectors of *B. burgdorferi* spirochetes. Immature *I. scapularis* ticks are similarly active in the summer, but in southern states, these ticks typically avoid host-seeking above leaf litter and are rarely seen on humans or drag-cloths (*10*). For this reason, assessment of *I. scapularis* distribution in southern states is best achieved by acquiring adult life-stage ticks during cool season drag-cloth surveys (as reported here) or by collecting ticks from deer harvested in the fall. Inspection of hunter-harvested deer is efficient for the detection of low-density *I. scapularis* ticks (*11*). Thus, our drag-cloth sampling for *I. scapularis* ticks in 14 counties where none were found on deer a decade ago (Figure 1, panels A, B) suggests that tick abundance in these counties has increased. This suggestion is supported by a >3-fold increase in *I. scapularis* tick counts at the Anderson County site where we have 6 consecutive years of drag-cloth counts.

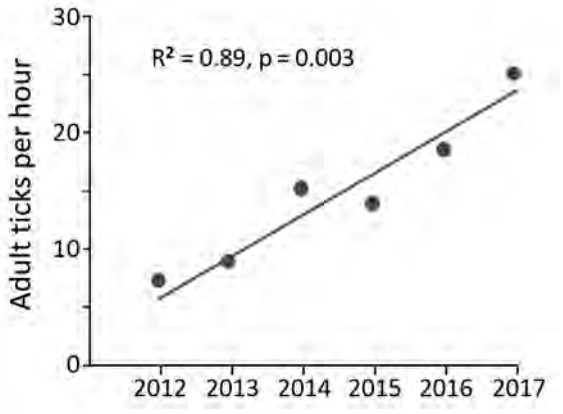

Figure 2. Six-year trend in adult *Ixodes scapularis* tick counts at Forest Resources Research and Education Center (36.00°N, 84.22°W; elevation 298 m), Anderson County, Tennessee, USA, 2012–2017. We collected host-seeking *I. scapularis* adult ticks by drag-cloth sampling vegetation on a 1,050-m transect of mixed hardwood forest once each December.

This study documents emergence of *B. burgdorferi* senso stricto in tick populations in eastern Tennessee. Infected ticks were predominantly found in high-prevalence hot spots in Union County (36.39°N). Relative to Lyme disease–endemic areas in the north, *B. burgdorferi* prevalence in the study area was low (10%) and had a patchy distribution (7/49 sites had positive ticks). This distribution could reflect host barriers of *B. burgdorferi* transmission in the South (*12*), or more concerning, the hot spots in Union County might reflect the beginning of an infection surge, similar to that seen in southwestern Virginia during the past decade (*4*).

In the United States, Lyme disease is primarily a summertime disease associated with bites from nymphal *I. scapularis* ticks. In southern states, detection of *B. burgdorferi* bacteria in adult ticks does not necessarily imply risk to humans; for example, *B. burgdorferi* cycles in *I. scapularis* populations on the Outer Banks of North Carolina, yet nymphs in that area cannot be collected on drag-cloths and no locally acquired cases of Lyme disease have been reported (*13*). In contrast, infected nymphs have been found on drag-cloths from surveys in Virginia, where Lyme disease incidence has spiked (*14*). We speculate that *Borrelia*-infected *I. scapularis* populations emerging in southwestern Virginia include immigrant ticks from the North, with some nymphs in these populations exhibiting host-seeking behaviors that lead to contact with humans. A similar invasion process might be under way in eastern Tennessee; the surveillance data reported here provide a baseline for investigating this possibility. Health officials and practitioners need to be vigilant for increasing Lyme disease incidence in Tennessee.

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About the Author

Dr. Hickling is professor in the Center for Wildlife Health at the University of Tennessee, Knoxville, Tennessee, USA. His research focuses on the eco-epidemiology of tickborne diseases in the southeastern United States.

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Address for correspondence: Graham J. Hickling, University of Tennessee Institute of Agriculture, Center for Wildlife Health, 274 Ellington Bldg, 2431 Joe Johnson Dr, Knoxville, TN 37996, USA; email: ghicklin@utk.edu

Susceptibility of White-Tailed Deer to Rift Valley Fever Virus

William C. Wilson, In Joong Kim, Jessie D. Trujillo, Sun Young Sunwoo, Leela E. Noronha, Kinga Urbaniak, D. Scott McVey, Barbara S. Drolet, Igor Morozov, Bonto Faburay, Erin E. Schirtzinger, Tammy Koopman, Sabarish V. Indran, Velmurugan Balaraman, Juergen A. Richt

Rift Valley fever virus, a zoonotic arbovirus, poses major health threats to livestock and humans if introduced into the United States. White-tailed deer, which are abundant throughout the country, might be sentinel animals for arboviruses. We determined the susceptibility of these deer to this virus and provide evidence for a potentially major epidemiologic role.

Rift Valley fever virus (RVFV) is a zoonotic, arthropodborne RNA virus (order *Bunyavirales*, family *Phenuiviridae*, genus *Phlebovirus)* (*1*,*2*). The virus is maintained in nature in a mosquito–vertebrate host cycle and is endemic to sub-Saharan Africa where epidemics have great consequences for livestock and human health. There is potential for RVFV incursions into neighboring regions or introductions into other continents, including North America, which has mosquito species capable of harboring and transmitting RVFV (*3*).

Although domestic cattle, sheep, and goats are susceptible to RVFV and function as amplification hosts during epidemics, the potential role of wildlife host species, such as white-tailed deer (*Odocoileus virginianus*) is unknown. RVFV is capable of infecting a range of cell lines from wildlife in North America, including white-tailed deer (*4*), suggesting in vivo susceptibility. White-tailed deer might be good sentinel animals for various arboviruses because of their abundance and wide geographic distribution in the United States (*5*).

A serious concern is that white-tailed deer could serve as reservoir or amplification hosts for RVFV (*6*–*9*). Furthermore, modeling suggests that these deer as reservoir hosts would enhance spillover of RVFV into human populations because of overlap of mosquitoes, humans, and wildlife in urban and periurban areas (*7*). Therefore, we determined the susceptibility of white-tailed deer to RVFV infection and described the potential role of white-tailed deer populations in RVFV epidemiology.

Author affiliations: US Department of Agriculture, Manhattan, Kansas, USA (W.C. Wilson, L.E. Noronha, D.S. McVey, B.S. Drolet, E.E. Schirtzinger); Kansas State University College of Veterinary Medicine, Manhattan (I.J. Kim, J.D. Trujillo, S.Y. Sunwoo, K. Urbaniak, I. Morozov, B. Faburay, T. Koopman, S.V. Indran, V. Balaraman, J.A. Richt)

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The Study

Wild-type RVFV (Kenya 2006 strain 128B-15, KEN06) was propagated in C6/36 mosquito cells and prepared as inoculum (1×10^6 PFU/animal). Five 5-month-old male white-tailed deer from the US Department of Agriculture, Agricultural Research Service, National Animal Disease Center (Ames, IA, USA) captive herd were acclimated to Biosafety Level 3 conditions and housed in such a facility with specifically designed paneling.

After sedation and blood collection (0 days postinoculation [dpi]), 4 animals were injected subcutaneously in the neck with virus inoculum; 1 contact control animal was sham inoculated with cell culture medium. To minimize stress, 2 animals in the virus-inoculated group were sedated on alternating days (2–6 dpi) and then at 10 and 14 dpi for blood collection and physical examination. The control was sedated and sampled at 2, 4, 6, and 7 dpi. Animals were initially monitored 2 times/day, then 3 times/day after development of fever.

We determined blood levels of albumin, alkaline phosphatase, γ-glutamyl transferase, aspartate aminotransferase, and blood urea nitrogen by using a VetScan VS2 Analyzer (Abaxis, Union City, CA, USA). We used a quantitative reverse transcription PCR (qRT-PCR) to detect RVFV RNA (*10*). We performed humane euthanasia and necropsy when deer were moribund or at the end of the study (14 dpi). All animal work was performed at the Biosecurity Research Institute, Kansas State University (Manhattan, KS, USA), in compliance with Institutional Animal Care and Use Committee protocol no. 3518.

The 5 deer adapted well to the specifically designed room. Rectal temperatures were in the standard range (37.5°C–40.1°C) (*11*) at 0 dpi (Table). On dpi 2, clinical assessment of 2 infected deer (nos. 43 and 44) and the control (no. 41) showed that 1 inoculated animal (no. 44; 41.3°C) and the control (41.2°C) had increased body temperatures. Also, the control was highly agitated during capture.

Table. Assessment of Rift Valley fever virus infection in 5 white-tailed deer at selected days postinfection*

Group, animal no.	Day postinoculation, real-time qRT-PCR/virus isolation results, temperature, °C							
	0	2	3	4	6	7	10	14
Mock								
41	–/–, 39.2	–/–, **41.0**	NC	+/+, **39.6**	+++/+++, 40.7	+++/+++, **41.2**, **e**uthanized	NA	NA
Ken 06								
43	–/–, 39.3	++/++, **40.2**	NC	+/+, 39.0	–/–, **40.6**	NC	–/–, 39.1	–/–, 39.0
44	–/–, 39.4	+++/+++, **41.3**	+++/+++, died	NA	NA	NA	NA	NA
47	–/–, 39.5	NC	+++/+++, died	NA	NA	NA	NA	NA
52	–/–, 39.2	NC	NC	+/+, 39.3	–/–, 39.9	NC	–/–, 39.5	–/– , 39.1

*Bold indicates increased body temperature. NA, not available; NC, not collected; qRT-PCR, quantitative reverse transcription PCR; –, negative; +, cycle threshold range 31–35, 1 × 10^1–1 × 10^3 PFU/mL; ++, cycle threshold range 25–30, 1 × 10^4–1 × 10^6 PFU/mL; +++, cycle threshold <25, 1 × 10^7–1 × 10^8 PFU/mL.

qRT-PCR analysis of RNA isolated from serum samples obtained 2 dpi showed high concentrations of circulating virus RNA in deer no. 44 (8.15 × 10^{10} copies/mL) and high concentrations in deer no. 43 (3.0 × 10^7 copies/mL). We did not detect RVFV RNA in serum from the control at 2 dpi. Later that day, 2 deer (nos. 44 and 47) were less active, and diffuse bilateral hyperemia of the ocular sclera developed in deer no. 44. At 3 dpi, bloody diarrhea developed in these 2 deer, and they died suddenly. qRT-PCR showed high serum levels of RVFV RNA (1 × 10^{11} copies/mL in deer no. 44 and 1 × 10^{12} copies/mL in deer no. 47).

Necropsy findings were similar for both animals and included severe, multifocal, hemorrhagic hepatic necrosis; moderate to severe segmental to diffuse hemorrhagic enteritis; moderate pulmonary edema; and moderate to severe hemorrhagic lymphadenopathy (Figure). Hepatic necrosis and petechiae have also been found in cattle and sheep with acute RVFV infections (*12*,*13*). Enteric lesions appeared to be severe and unique to white-tailed deer. Bloody fecal material covered the perineum, ventral tail, and hind limbs. Segmental hemorrhage of gastrointestinal mucosa was most severe in deer no. 44. We found watery and bloody gastrointestinal contents from the abomasum to the rectum (deer no. 44) or small intestine to the rectum (deer no. 47). Mesenteric and gastrohepatic lymph nodes of both animals were edematous and had multifocal hemorrhagic foci. We observed diffuse thymic hemorrhage in deer no. 44.

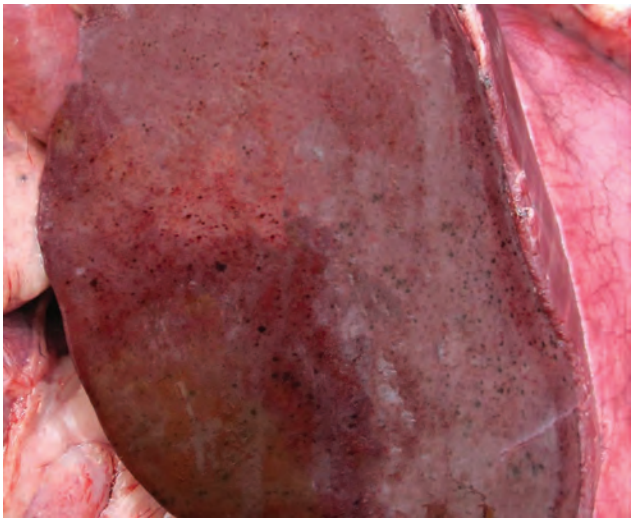

Figure. Gross pathologic view of liver of white-tailed deer no. 44 after experimental infection with Rift Valley fever virus inoculum. The animal died at day 3 postinoculation; at necropsy, the liver showed severe, multifocal, hemorrhagic hepatic necrosis attributed to acute infection with Rift Valley fever virus.

The remaining animals were bright, alert, and responsive at 3 dpi. However, 2 deer (nos. 43 and 52) had transient diarrhea with loose feces persisting until 6 dpi. qRT-PCR of serum showed moderate levels of RVFV RNA in deer no. 43 and deer no. 52. We also detected a low concentration (1 × 10^3 copies/mL) of RVFV RNA in the control by 4 dpi, indicative of horizontal transmission. On day 6, the control and 1 inoculated deer (no. 43) had slightly increased body temperatures (40.7°C for the control and 40.6° for no. 43). By day 6, serum RVFV RNA concentration for the control had increased to ≈1 × 10^{10} copies/mL, and concentrations of virus RNA in deer no. 47 and no. 52 had decreased to 1 × 10^2–1 × 10^3 copies/mL. By 7 dpi, the control was recumbent and febrile (41.2°C) and marked swelling of the left hind limb had developed, which warranted euthanasia.

At necropsy for the control, hepatic and gastrointestinal lesions attributed to RVFV infection were similar to those in deer no. 44 and no. 47, albeit much less severe. Examination of the markedly swollen left hind limb showed marked expansion of subcutis and fascia with hemorrhage and emphysema but definite diagnosis is pending further investigation. RVFV infection of the control was supported by the high serum level of RVFV RNA at 7 dpi (8.1 × 10^8 copies/mL). Serum qRT-PCR showed RVFV RNA in deer no. 43 (1 × 10^3 copies/mL) but not in deer no. 52 at 10 dpi. By 14 dpi (end of the study), we did not detect RVFV RNA in serum of either remaining animal. We did not observe gross lesions in the remaining deer (nos. 43 and 52) at the end of the study. However, we detected RVFV RNA in liver, kidneys, spleen, and lymph nodes from both animals.

Conclusions

Clinical signs, gross pathology, and qRT-PCR–determined virus RNA loads demonstrated that white-tailed deer are highly susceptible to RVFV infection, causing hepatic necrosis and hemorrhage. Supporting this conclusion, we found that levels of aspartate aminotransferase increased in serum of all animals when blood was collected at the time of clinical illness (range 91–153 U/L at 0 dpi and 629–3,543 U/L at the time of clinical illness). Similar results were reported for previous experimental RVFV infections of domestic cattle and sheep (*12*,*13*).

In addition, and unique to this study, RVFV infection in white-tailed deer resulted in development of hemorrhagic enteritis and bloody diarrhea at the time of peak viremia in 2 infected deer (nos. 44 and 47), which likely enabled horizontal transmission of RVFV to the control animal. Additional laboratory analysis is ongoing. However, our results clearly indicate that white-tailed deer in North America are susceptible to RVFV infection. Infected white-tailed deer died from the infection (n = 2), might survive the infection (n = 2), and can transmit the virus through direct contact (n = 1), presumptively by the fecal–oral route.

This study indicates that white-tailed deer in North America are highly susceptible to RVFV and capable of horizontal virus transmission. The potential role of other wildlife in the epidemiology of RVFV should be evaluated.

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About the Author

Dr. Wilson is a research microbiologist at the US Department of Agriculture, Manhattan, KS. His research interests are understanding virus–vector–host interactions and developing means to detect and control arboviruses.

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Address for correspondence: William C. Wilson, US Department of Agriculture, Agricultural Research Service, Center for Grain and Animal Health Research, Arthropod-Borne Animal Diseases Research Unit, 1515 College Ave, Manhattan, KS 66502, USA; william.wilson@ars.usda.gov

Outbreak of Pneumococcal Meningitis, Paoua Subprefecture, Central African Republic, 2016–2017

Matthew E. Coldiron, Oumar Touré, Thierry Frank, Nathalie Bouygues, Rebecca F. Grais

We report a pneumococcal meningitis outbreak in the Central African Republic (251 suspected cases; 60 confirmed by latex agglutination test) in 2016–2017. Case-fatality rates (10% for confirmed case-patients) were low. In areas where a recent pneumococcal conjugate vaccine campaign was conducted, a smaller proportion of cases was seen in youngest children.

In early January 2017, an abnormally large number of pneumococcal meningitis cases was reported at Paoua Subprefectural Hospital in northwestern Central African Republic. This region is at the southern edge of the traditional meningitis belt in Africa (*1*), and the hospital has been supported by the international medical humanitarian organization Médecins Sans Frontières since 2007. Routine data collected since 2012 showed a weekly maximum of 3 cases of pneumococcal meningitis (confirmed by latex agglutination test) and never >29 reported cases in any given 25-week period. A case-based meningitis surveillance system, including latex agglutination testing, was implemented in Paoua Subprefectural Hospital. All suspected cases in peripheral health centers were referred free of charge. We provide an epidemiologic description of this outbreak.

The Study

The Central African Republic has experienced a series of crises over the past several decades. The most recent acute crisis began in 2013, when a series of armed rebellions led to multiple changes of power at the central level; a newly elected government took office in 2016, but many areas are still not secure. Thus, health systems, particularly in the rural periphery, are particularly weak.

Vaccination coverage remains low: nationwide administrative coverage for the first dose of 13-valent pneumococcal conjugate vaccine (PCV13) was 77% in 2016 and 52% for the third dose. PCV13 was introduced in the Paoua Subprefecture (population 236,000) in 2012. A series of multiantigen catch-up vaccination campaigns for children <5 years of age that included PCV13 was conducted by Médecins Sans Frontières in 2016. Several areas were inaccessible because of security concerns and were not included in the vaccination campaign.

Outbreaks of pneumococcal meningitis have been reported in Africa before and after introduction of pneumococcal vaccine. There was a recent large outbreak in Ghana (*2*) and several other smaller outbreaks in the traditional meningitis belt (*3*,*4*). Pneumococcal meningitis has case-fatality rates (CFRs) of 36%–66% depending on age, and the risk for sequelae is high (*5*). Outbreaks generally occur during the dry season (typical meningitis season), but these outbreaks are usually smaller than meningococcal outbreaks (*6*). Unlike meningococcal meningitis, there is no formal epidemic definition for pneumococcal meningitis, although a provisional definition was recently issued: a district or subdistrict with a weekly incidence of ≥5 suspected cases/100,000 population with ≥60% of confirmed meningitis cases caused by *Streptococcus pneumoniae* and ≥10 confirmed cases of pneumococcal meningitis (*7*,*8*).

During October 10, 2016–April 9, 2017 (epidemiologic week 41 in 2016 through epidemiologic week 14 in 2017), 251 suspected cases of meningitis were reported at Paoua Subprefectural Hospital: 200 cases from Paoua Subprefecture (attack rate 85 cases/100,000 population), 40 cases from outside Paoua Subprefecture, and 11 cases from villages that could not be identified (Figure). Lumbar puncture and latex agglutination testing were performed for 110 patients, of whom 101 had not received antimicrobial drugs before lumbar puncture.

Of 110 samples, 60 (55%) were positive for *S. pneumoniae* by latex agglutination test, 1 for *Neisseria meningitidis* strain NmW/Y, and 2 for *Haemophilus influenzae*. Two other samples showed a positive result, but the causative organism could not be identified. The remaining 45 samples were negative by latex agglutination test. Ten samples positive for *S. pneumoniae* by latex agglutination test were sent to the national reference laboratory (Institut Pasteur, Bangui, Central African Republic) where 6 were confirmed as serotype 1 *S. pneumoniae* by PCR, and 4 showed weak positive results for *S. pneumoniae* by PCR. Two

Author affiliations: Epicentre, Paris, France (M.E. Coldiron, R.F. Grais); Epicentre, Paoua, Central African Republic (O. Touré); Institut Pasteur, Bangui, Central African Republic (T. Frank); Médecins Sans Frontières, Paris (N. Bouygues)

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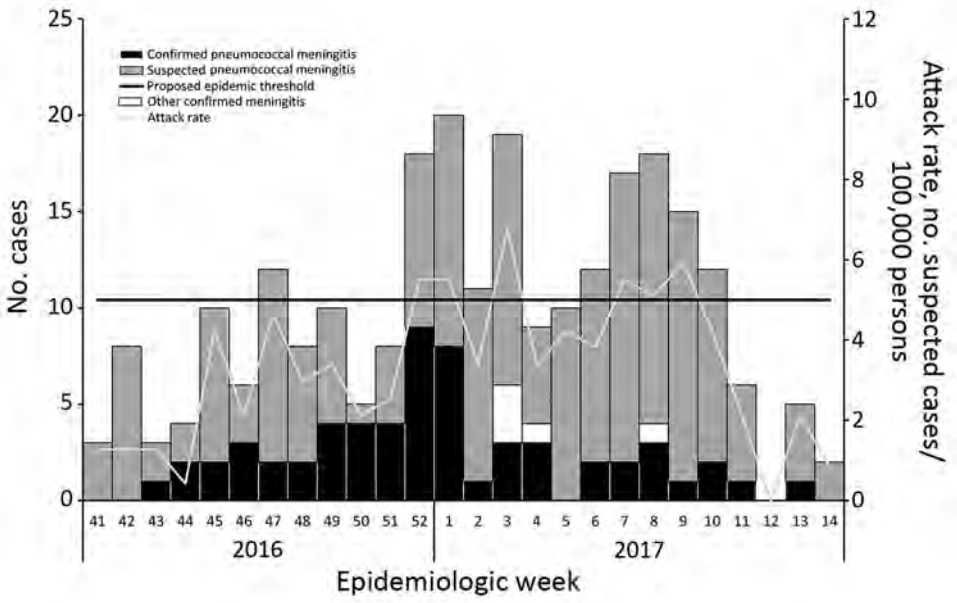

Figure. Cases of meningitis and weekly attack rate, Paoua Subprefecture, Central African Republic, 2016–2017.

samples negative by latex agglutination were also negative by PCR. *S. pneumoniae* represented 60 (92%) of 65 of all samples positive by latex agglutination during this period.

Overall, 9 patients died (CFR 3.6%). For case-patients with confirmed pneumococcal meningitis, 6 patients died (CFR 10.0%). For all case-patients, median length of treatment with ceftriaxone was 4 days (interquartile range 3–6 days). For case-patients with pneumococcal meningitis, median length of treatment was 10 days (interquartile range 9–12 days). Although information was incomplete, 25 case-patients with pneumococcal meningitis had documented evidence of treatment with dexamethasone.

Attack rates were highest for children <2 years of age (Table) when we considered all suspected cases and confirmed cases of pneumococcal meningitis. Areas targeted for the 2016 PCV13 vaccination campaign did not necessarily correspond to established political divisions. Thus, we were unable to calculate attack rates in vaccinated areas versus nonvaccinated areas because of lack of precise denominators. Nonetheless, in vaccinated areas, 5 (17%) of 30 confirmed cases were in children <5 years of age. In unvaccinated areas, 10 (36%) of 28 confirmed cases were in children <5 years of age. PCV13 vaccination status of case-patients was not recorded.

At a district level, this outbreak seems to have met the provisional definition of a pneumococcal meningitis outbreak, at least during epidemiologic weeks 52 in 2016 and weeks 1 and 3 in 2017, although it is unclear whether the criterion of ≥10 confirmed pneumococcal meningitis cases refers to a single week or overall during the outbreak. At a subdistrict level, only 2 subdistricts (Bah-Bessar, population 33,820; and Mia-Pendé, population 35,261) met the provisional definition at any point during the outbreak.

Conclusions

We describe a pneumococcal meningitis outbreak in the Central African Republic in 2016–2017. This outbreak was not large, but it clearly was an abnormal event. Although pneumococcal outbreaks have been reported more frequently in recent years, outbreak definitions and guidance remain provisional and have been based on scanty data. We have highlighted a potential clarification that could be used in outbreak definitions in terms of the overall number of confirmed cases of pneumococcal meningitis.

We report low CFRs for case-patients with confirmed pneumococcal meningitis, which is in contrast to results of previous reports (*9*). These differences might have been

Table. Attack rates for pneumococcal meningitis, Paoua Subprefecture, Central African Republic, 2016–2017

Patient age, y	Overall cases of pneumococcal meningitis		Confirmed cases of pneumococcal meningitis	
	No. cases	Attack rate, no. cases/100,000 population	No. cases	Attack rate, no. cases/100,000 population
<2	61	301	12	59
2–4	22	82	4	15
5–14	38	62	24	39
15–29	79	124	11	17
30–44	37	96	7	18
≥45	14	54	2	8

caused by an extended duration of antimicrobial drug therapy and, at least for some case-patients, the addition of corticosteroids. Our small-scale observational data should not be overinterpreted, but length of therapy and utility of adjuvant corticosteroids were both highlighted as knowledge gaps in the provisional guidance document of the World Health Organization (*8*).

One limitation of our work was the level of biologic confirmation. However, the Pastorex Latex Agglutination Test Kit (Bio-Rad Laboratories, Marne-la-Coquette, France) we used has shown good performance in detecting *S. pneumoniae* (*10*). These kits were shipped and stored according to manufacturer's instructions, and positive and negative controls were tested regularly according to standard procedures (*11*). We are reassured that at least a few samples underwent PCR confirmation and serotyping. Given that it appears that *S. pneumoniae* serotype 1 was the circulating serotype, differences in age distribution of case-patients seen between areas targeted and not targeted by the 2016 PCV13 catch-up vaccination campaign were likely caused by this intervention.

This outbreak highlights some of the difficulties inherent with performing surveillance in complex and insecure settings. The lack of infrastructure and laboratory capacity remain major obstacles to more precise characterizations of similar events. During this outbreak, it was not possible to perform cell counts or biochemical testing, which could have been useful. Increasing PCV13 coverage in the routine vaccination programs is the most efficient way to prevent future outbreaks, but given the overall context in the Central African Republic and other areas of the traditional meningitis belt, it would be prudent to consider formally evaluating (either by modeling or in real-life situations) the potential effects of reactive vaccination as an outbreak response.

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About the Author

Dr. Coldiron is a medical epidemiologist at Epicentre–Médecins Sans Frontières, Paris, France. His primary research interests are meningitis and malaria in the Sahel region of Africa and neglected tropical diseases.

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Address for correspondence: Matthew E. Coldiron, Epicentre, 8 Rue Saint-Sabin, 75011 Paris, France; email: matthew.coldiron@epicentre.msf.org

Molecular Confirmation of Rocky Mountain Spotted Fever Epidemic Agent in Mexicali, Mexico

Luis Tinoco-Gracia, Moisés Rodríguez Lomelí, Sawako Hori-Oshima, Nicole Stephenson, Janet Foley

Since 2008, a large epidemic of Rocky Mountain spotted fever has been emerging among humans and dogs in Mexicali, adjacent to the United States in Baja California, Mexico. We molecularly confirmed the causative agent; this information can be used to study the origin and dynamics of the epidemic.

Rocky Mountain spotted fever (RMSF), caused by the bacteria *Rickettsia rickettsii*, is responsible for more human deaths than any other tickborne disease in North America (*1*). During 1999–2007, a total of 80 fatal cases were reported from Sonora, Mexico, alone (*2*). Recent epidemics in Arizona (USA) and Sonora have been associated with the brown dog tick (*Rhipicephalus sanguineus*) (*3*,*4*), whereas most cases in the United States have been transmitted by bites of infected *Dermacentor* spp. ticks (*5*). The risk to humans is heightened by the epidemiologic cycle of the brown dog tick, a cosmopolitan tick that prefers the dog as its host and can live its entire life cycle in a periurban setting, often spending its off-host time indoors. *R. sanguineus* ticks, in addition to being vectors of *R. rickettsii*, are probable or confirmed vectors of *Leishmania*, *Coxiella burnetii*, and *R. conorii* (*6*).

The Study

In 2008, an epidemic of RMSF began in Mexicali, adjacent to the US border in Baja California, Mexico. In 2015, the Mexican Ministry of Health declared the epidemic an epidemiologic emergency, which as of 2018 has affected ≈4,000 persons. In 2014, a fatal human case in Imperial County, CA, USA, was probably associated with the Mexicali epidemic. Overall, since 2000, in the United States, the incidence of RMSF has reportedly increased ≈4-fold (*7*); this dramatic increase may be caused in part by increased transmission via the brown dog tick but also by changes in reporting and inclusion of false-positive test results in case diagnoses.

Local response to the ongoing epidemic in Mexicali has involved the Secretariat of Health and doctors and researchers at the Universidad Autónoma de Baja California schools of medicine and veterinary medicine. During 2008–2009, in the impoverished neighborhood of Los Santorales in Mexicali, at least 13 persons died of RMSF. Under agreement with the Sector Salud de Mexicali, the Universidad Autónoma de Baja California veterinary team documented 81% seroprevalence among local dogs and confirmed active *R. rickettsii* infection in a human resident by conducting PCR of kidney tissue (*8*). Of 120 persons from Mexicali with clinical signs compatible with RMSF, 30 were positive by PCR for the gene *gltA*, according to an unpublished method (*9*). In 2014, the local team partnered with researchers at the University of California, Davis (Davis, California, USA), to further molecularly characterize the strains of *R. rickettsii* and *R. sanguineus* ticks from Mexicali. We provide definitive molecular confirmation of the identity of the disease agent causing the Mexicali epidemic.

The University of California, Davis, laboratory received DNA extracted by use of QIAGEN Blood and Tissue Kits (Valencia, CA, USA) from 16 cases from Mexico. Initial *R. rickettsia*–specific real-time PCR for the citrate synthase gene (*10*) was positive for 10 samples. To obtain products for DNA sequencing, we performed traditional PCR for the *ompA* and *17kDa* genes as published (*11*,*12*). Sequence-confirmed positive DNA and water-containing negative control reactions were incorporated in each PCR run. Results were assessed by electrophoresis and UV-transillumination of 1% agarose gels stained with Gelstar (Lonza, Rockland, ME, USA). Bands of the expected size were excised and cleaned with a QIAquick Gel Extraction Kit (QIAGEN) according to the manufacturer's instructions. Products were sequenced in the forward and reverse directions in an ABI Prism 3730 Genetic Analyzer at the UCDNA Sequencing Facility at the University of California, Davis. Sequences were manually trimmed and corrected if the nucleotide could be unambiguously determined, then aligned by using CLC Main Workbench 6 (CLC bio, Waltham, MA, USA).

We successfully obtained *ompA* and *17kDa* products from 5 samples and compared the sequences with those in

Author affiliations: Universidad Autónoma de Baja California, Mexicali, Mexico (L. Tinoco-Gracia, M. Rodríguez Lomelí, S. Hori-Oshima); University of California, Davis, California, USA (N. Stephenson, J. Foley)

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the GenBank database by using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). For *ompA*, the resulting 472-bp amplicons from the 5 products from Mexicali were 100% similar. For this gene, numerous accessions in GenBank also have 100% homology with 100% coverage, including strains Sheila Smith, Hauke, Hilo, Colombia, and Arizona. Sequences of *17kDa* spanned 206 bps and were also completely homologous among them. This gene did not differentiate to species but was 100% homologous with *R. rickettsii*, *R. parkeri*, and others in the database. Representative sequences from Mexicali were submitted to GenBank (accession nos. KY689935 for *ompA* and KY824575 for *17kDa*).

Among sequence-confirmed samples, data were not available for 1 sample. The other 4 samples were collected in June, July, and September 2013 and April 2014. Two samples were from men (41 and 25 years of age) and 2 from women (18 and 29 years of age); all patients had dogs with ticks. Signs and symptoms were fever and headache for all; for 1 patient, a rash and convulsions also developed. The 2 men died and the 2 women survived with treatment. All patients had home addresses in various parts of Mexicali, including central west, southwest, and southeast bordering agricultural land. Clinical data were not available for patients for whom samples were considered PCR positive but not sequence confirmed, although inclusion of such clinical data and risk factors could bias interpretation if they were false positive or only weakly positive.

Conclusions

The RMSF epidemic in Mexicali has not been contained and may be spreading to other parts of Baja California and into the United States. More data are needed before we can understand why this epidemic emerged, where the specific areas of high risk for exposure to infected ticks are located, and whether the particular *R. rickettsii* strain or relationship with this *R. sanguineus* tick strain is likely to be particularly invasive or virulent. Pockets of RMSF have occurred in Mexico since at least 1947, when cases attributable to the brown dog tick in Sonora, Sinaloa, Coahuila, and Durango were described (*13*). Given the very limited phylogeographic resolution available for *R. rickettsii* in many of the commonly used PCR products (*14*), it is not known whether the bacteria in the Mexicali epidemic originated from Sonora or more distantly. Next steps include obtaining a culture of the bacteria from Mexicali, studying bacterial virulence in vitro or in animal models, and assessing vector competence of the Mexicali *R. sanguineus* tick strain for *R. rickettsii*. Epidemiologic data on the spatial distribution and prevalence of infection in dogs are needed.

Aggressive intervention achieved partial and temporary resolution of the Arizona and Sonora epidemics, which were localized and relatively small; these interventions included dog spay and neuter programs, treatment of houses against ticks, and use of a long-acting tick collar (Seresto; Bayer, Shawnee Mission, KS, USA) directly on the dogs (*15*). However, the dog collars were initially donated and are prohibitively expensive and not feasible for the scope of the Mexicali epidemic. This large epidemic in a major city will require a far greater and more creative public health response. Studying this epidemic offers an opportunity to understand the origin and dynamics of this epidemic and can inform response to emerging tickborne diseases in general.

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About the Author

Dr. Tinoco-Gracia is a research professor in the School of Veterinary Medicine at Universidad Autonoma de Baja California and director of the Laboratory of Veterinary Public Health Sciences. He studies zoonotic diseases of dogs and humans in Mexicali and leads community education programs of Universidad Autónoma de Baja California.

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Address for correspondence: Janet Foley, University of California, Department of Medicine and Epidemiology, 1320 Tupper Hall, Davis, CA 95616, USA; email: jefoley@ucdavis.edu

Fatal Tickborne Phlebovirus Infection in Captive Cheetahs, Japan

Keita Matsuno, Noriyuki Nonoue, Ayako Noda, Nodoka Kasajima, Keita Noguchi, Ai Takano, Hiroshi Shimoda, Yasuko Orba, Mieko Muramatsu, Yoshihiro Sakoda, Ayato Takada, Shinji Minami, Yumi Une, Shigeru Morikawa, Ken Maeda

Two captive cheetahs from a zoo in Japan died of a severe fever with thrombocytopenia syndrome–like illness. Severe fever with thrombocytopenia syndrome virus, an endemic tickborne phlebovirus, was detected systemically with secretion of infectious viruses into the saliva. These cases highlight the risk for exposure of captive animals to endemic arthropodborne pathogens.

An emerging tickborne virus, severe fever with thrombocytopenia syndrome (SFTS) virus (SFTSV; genus *Phlebovirus*, family *Phenuiviridae* [previously known as family *Bunyaviridae*]) (*1*,*2*), causes severe and often fatal febrile illness in humans, especially in elderly patients. SFTS cases have been identified in East Asia countries (e.g., China, South Korea, and Japan), where the virus also was detected in multiple species of ticks (*3*,*4*) and in domestic and wild animals (*4*,*5*). Ticks and animals play a central role in maintaining the life cycle of SFTSV in the environment and in the occasional transmission of SFTSV to humans. The pathogenesis of SFTSV has been studied in human (*1*,*6*) and animal models using immunocompromised mice that show a lethal SFTS-like illness (*7*,*8*).

In humans, SFTS begins with a high fever, marked thrombocytopenia and leukocytopenia, and a high serum viral load, followed by multiorgan dysfunction, which may be a consequence of systemic inflammatory responses and disseminated intravascular coagulation (*9*,*10*). Gastrointestinal symptoms, such as nausea and vomiting in the early phase and bloody diarrhea in the later phase of the disease, have been frequently reported (*11*). The serum viral load, which can be a prognostic marker associated with a fatal outcome, remains high in fatal cases but decreases in convalescent patients. Here we report 2 fatal SFTS cases in cheetahs in a zoo in the endemic area.

Author affiliations: Hokkaido University, Sapporo, Japan (K. Matsuno, Y. Sakoda, A. Takada); Hiroshima City Asa Zoological Park, Hiroshima, Japan (N. Nonoue, A. Noda, S. Minami); Hokkaido University Research Center for Zoonosis Control, Sapporo (N. Kasajima, Y. Orba, M. Muramatsu, A. Takada); Yamaguchi University, Yamaguchi, Japan (K. Noguchi, A. Takano, H. Shimoda, K. Maeda); Okayama University of Science, Imabari, Japan (Y. Une); National Institute of Infectious Diseases, Tokyo, Japan (S. Morikawa)

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The Study

In July 2017, anorexia was first recognized in a 7-year-old female cheetah (cheetah 1) in a group of 4 cheetahs sharing the same outside enclosure; she was anesthetized for medical examination on day 3. Laboratory studies showed extremely low leukocyte and low platelet counts and slightly elevated aspartate aminotransferase, alanine aminotransferase (ALT), and total bilirubin levels (Table). The animal was confirmed negative for feline leukemia virus, feline immunodeficiency virus, and feline panleukopenia virus using rapid test kits (Checkman FIV, SNAP FIV/FeLV Combo, Checkman FeLV, and Checkman CPV; Kyoritsu Seiyaku Corporation, Tokyo, Japan). On day 4, cheetah 1 started vomiting with hemorrhage and then died after generalized convulsion. Pathologic analysis identified 4 ulcers in the digestive tract, bleeding in the esophagus, and swollen spleen with white nodules.

Slightly abnormal behavior of a 6-year-old male cheetah (cheetah 2) was first observed at 10 and 15 days after the death of cheetah 1. Obvious anorexia in cheetah 2 was recorded at 20 days after cheetah 1 died (hereafter referred to as day 1). Dragging of hind limbs was observed on day 2, and hematologic tests revealed a moderately low platelet count and elevated ALT (Table). A fecal sample was negative for *Helicobacter pylori* antigen, enteric bacteria, and ova-parasite. On day 3, slightly decreased leukocyte count along with continued low platelet count and high liver aspartate aminotransferase levels were detected. On day 4, the gastroscopy test showed erosion and petechiae in the stomach, and a blood-feeding tick was found and removed from the ear. In addition to the low leukocyte and platelet counts, elevated ALT, creatine phosphokinase, and lactate dehydrogenase levels were revealed by laboratory tests on day 6. Cheetah 2 vomited with hemorrhage on days 6 and 7 and died on day 7. Similar to cheetah 1, cheetah 2 had a swollen spleen with white nodules and ulcers in the stomach. No clinical signs were observed in the 2 other cheetahs.

Table. Hematology and blood chemistry parameters in 2 fatal cases of severe fever with thrombocytopenia syndrome in cheetahs, Japan, 2017

Laboratory value	Normal (± SD)*	Cheetah 1, day 3†	Cheetah 2‡ Day 2	Day 3	Day 4	Day 6	Day 7§
Leukocytes/μL	10,350 (3,500)	1,700	12,500	10,700	6,900	3,900	4,800
Erythrocytes, × 10^3 cells/μL	684 (106)	707	845	756	834	722	952
Platelets, × 10^3/μL¶	349 (119)	1	12.7	9.1	5.9	0.9	1.3
Hemoglobin, g/L	12.5 (1.9)	13.9	16.7	15	15.7	14	18.3
Hematocrit, %	37.9 (5.8)	38.4	58.6	43.1	47.4	39.5	54.4
Mean cell volume, fL	55.6 (5.5)	54.3	69.3	57	56.8	54.7	57.1
Aspartate aminotransferase, U/L	52 (35)	161	119	162	145	492	500
Alanine aminotransferase, U/L	98 (71)	157	412	377	284	501	471
Creatine phosphokinase, U/L	296 (311)	262	200	915	746	>2,000	>2,000
Lactate dehydrogenase, U/L	92 (87)	273	203	574	174	684	906
Total bilirubin, mg/dL	0.3 (0.2)	2.7	0.6	2.9	1.2	5.4	12.3

*Numbers are obtained from (*12*).
†After illness onset.
‡Day 1 was 20 days after cheetah 1 died.
§Blood was collected from the carcass.
¶Possible lower platelet counts due to blood collection using heparin.

We performed laboratory tests for virus detection using plasma from cheetah 1 and serum, spleen, and mesenteric lymph node samples from cheetah 2. SFTSV RNA genomes were detected using a quantitative reverse transcription PCR (RT-PCR) targeting the S (small) segment RNA in the spleen and lymph node but not in plasma and serum (Figure 1, panel A). Quantitative RT-PCR showed intensive replication of viral RNA in the popliteal lymph nodes and salivary gland and moderate replication in the brain and spleen. In addition to these tissues, the livers, kidneys, and small intestines of both animals were positive for SFTSV RNAs by conventional RT-PCR. The tissues were negative for flaviviruses, alphaviruses, and canine distemper virus using conventional RT-PCR targeting these viruses.

We isolated infectious viruses using Huh-7 cells and Vero E6 cells from the plasma and popliteal lymph node of cheetah 1 and the spleen, lymph nodes, and brain of cheetah 2 but not from the serum of cheetah 2. Infected cells were clearly stained in an immunofluorescence assay with a monoclonal antibody YG1-7-3-3-4 raised against the recombinant SFTSV nucleoprotein of the Japanese prototype strain YG1. Conditions of the plasma and serum samples may result in negative RT-PCR results in the plasma and negative RT-PCR results and virus isolation in the serum because both samples were collected from animals supposed to cause viremia.

To assess the potential for virus shedding into the secretions of infected animals, infectious SFTSV of the salivary gland, oral, nasal, and rectal swabs from cheetah 2 were titrated using Huh-7 cells. Swab samples were collected from the carcass after freeze and thaw. We detected virus titers of ≈10^5 50% tissue culture infectious dose ($TCID_{50}$)/g or $TCID_{50}$/mL in the salivary gland and oral swab samples, respectively; however, virus was not detected in the nasal and rectal swab samples (Figure 1, panel B).

We determined genomic sequences of 2 isolates (SkrP/2017 from the plasma of cheetah 1 and ArtSp/2017

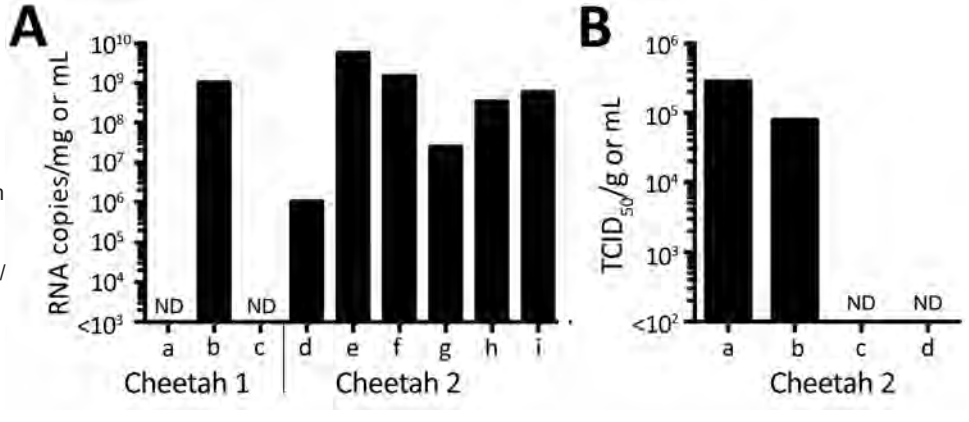

Figure 1. Detection of severe fever with thrombocytopenia syndrome virus (SFTSV) in samples from 2 cheetahs, Japan, 2017. A) RNA was extracted from tissues, plasma, and serum and subjected to quantitative reverse transcription PCR (RT-PCR). The amounts of SFTSV RNA were quantified, with a reference, as RNA copies/mg for tissues and RNA copies/mL for plasma and serum. The mean of duplicate results is shown in the graph. a, plasma; b, popliteal lymph node (left); c, serum; d, brain; e, salivary gland; f, spleen; g, mesentric lymph node; h, popliteal lymph node (left); i, popliteal lymph node (right). B) The $TCID_{50}$ of salivary gland (per mg) and swab specimens (per mL) for cheetah 2 was determined using Huh-7 cells. Virus proteins were detected by an immunofluorescence assay with an anti-SFTSV N monoclonal antibody. a, salivary gland; b, oral swab sample; c, nasal swab sample; d, rectal swab sample. ND, not done; $TCID_{50}$, 50% tissue culture infectious dose.

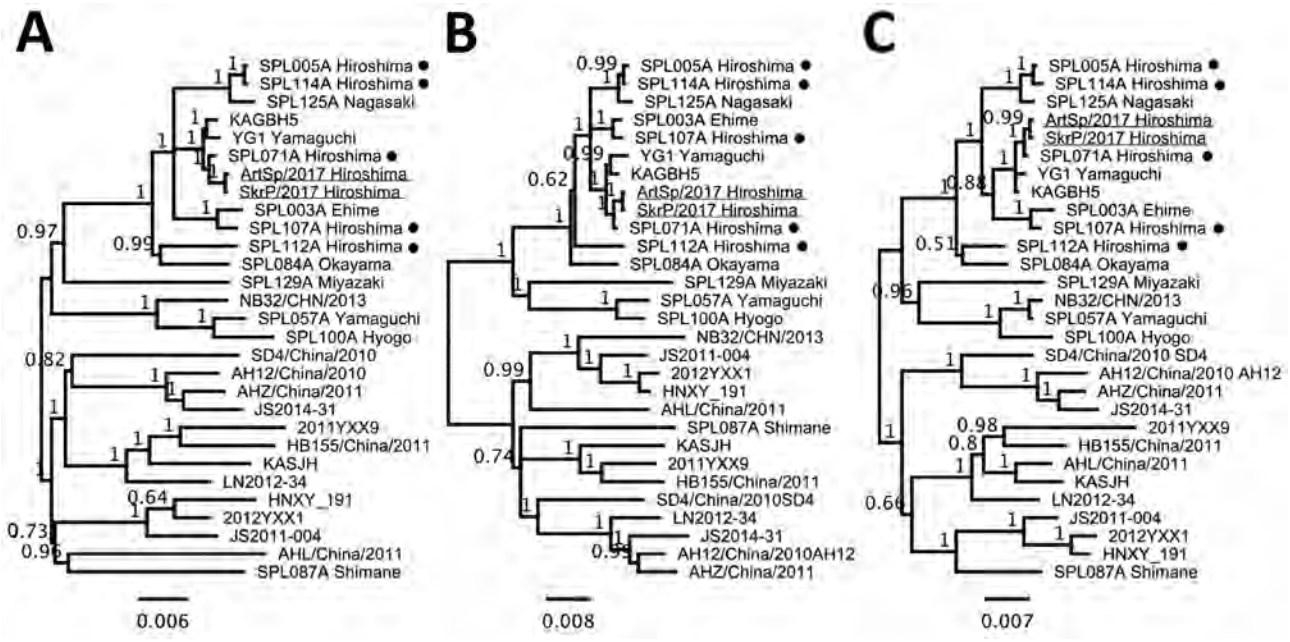

Figure 2. Phylogenetic analyses of severe fever with thrombocytopenia syndrome virus (SFTSV) isolates from 2 cheetahs, Japan, 2017. The phylogenetic trees constructed based on large (A), medium (B), and small (C) segment RNA nucleotide sequences of isolates SkrP/2017 from cheetah 1 and ArtSp/2017 from cheetah 2 (underlined) with representative SFTSV isolates. Isolates from human cases reported in the same prefecture as the zoo are indicated with black dots. The trees were calculated using MrBayes version 3.2.6 (http://mrbayes.sourceforge.net) with the general time reversible plus gamma plus invariate sites substitution model. Numbers beside nodes indicate posterior probabilities. Scale bars indicate nucleotide substitutions per site.

from the spleen of cheetah 2) using MiSeq (Illumina, San Diego, CA, USA) with NEBNext-Ultra RNA Library Prep kit (NEB). De novo assembly on CLC Genomics Workbench (QIAGEN, Hilden, Germany) determined virtually full-length sequences of all 3 RNA segments of 2 SFTSV isolates. We manually edited the termini and remapped virus reads to contigs to define the complete full-length genome sequences (sequences deposited into GenBank under accession nos. LC325234–9). We found only 1 synonymous nucleotide difference on the coding region of the L (large) segment between the 2 isolates. Phylogenetic analyses of 3 RNA segments revealed that both cheetah isolates were clustered together with an SFTSV isolate, SPL071A, which had been reported in a human in the same prefecture as the zoo (Figure 2) (*13*).

Conclusions

We found a fatal SFTS-like illness of 2 cheetahs naturally infected with an endemic tickborne virus, SFTSV. Disease progression of cheetah 2 was carefully tracked by daily monitoring, providing important clinical information on fatal SFTSV infection in animals. Because the genome sequences of 2 SFTSV isolates were almost identical to each other, and closely related to those of a local isolate from a human case, SFTSV circulating among ticks and wild animals in the area may intrude into the zoo. That 2 cheetahs sharing the same outside enclosure were successively infected with SFTSV within a month of each other and that they had the potential to shed infectious SFTSV into their saliva indicates the virus might have been independently transmitted to 2 cheetahs by ticks; however, the possibility of horizontal transmission through a bite of the animal is undeniable. Further investigation on ticks and animals around the zoo is ongoing. Our study highlights the zoonotic risk for SFTSV infection and the importance of monitoring this endemic arthropodborne disease in zoo animals, as well as livestock, pets, and wildlife.

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About the Author

Dr. Matsuno is a lecturer with the Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido, Japan. His research interests are the epidemiology, ecology, and molecular virology of tickborne viruses.

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Address for correspondence: Keita Matsuno, Hokkaido University, Department of Disease Control, Graduate School of Veterinary Medicine, N18 W9, Sapporo 060-0818, Japan; email: matsuno@vetmed.hokudai.ac.jp

Elizabethkingia anophelis and Association with Tap Water and Handwashing, Singapore

Chee-Fu Yung, Matthias Maiwald, Liat H. Loo, Han Y. Soong, Chin B. Tan, Phaik K. Lim, Ling Li, Natalie WH Tan, Chia-Yin Chong, Nancy Tee, Koh C. Thoon, Yoke H. Chan

We report an *Elizabethkingia anophelis* case cluster associated with contaminated aerators and tap water in a children's intensive care unit in Singapore in 2017. We demonstrate a likely transmission route for *E. anophelis* to patients through acquisition of the bacteria on hands of healthcare workers via handwashing.

Elizabethkingia anophelis is an emergent pathogen first described from midgut specimens of the *Anopheles gambiae* mosquito (*1*). To date, there have been 2 reported confirmed *E. anophelis* outbreaks in humans. One occurred in an adult critical care unit in Singapore; the second was a large community outbreak in the United States (Wisconsin, Michigan, and Illinois) (*2*–*5*). Water sources have been identified to harbor members of the genus *Elizabethkingia*, but the source of the community outbreak in the United States remains unknown (*3*,*6*). Effective interventions for outbreak control and transmission routes of *E. anophelis* remain unclear (*3*).

KK Women's and Children's Hospital (KKH) is the single largest public tertiary-care specialist women's and children's hospital in Singapore. The Children's Intensive Care Unit (CICU) is a 16-bed unit that provides advanced monitoring and therapeutic technologies for critical pediatric cases. On May 30, 2017, an alert was triggered due to the detection of 3 patients with *Elizabethkingia* spp. within 13 days in the unit. The incidence rate of the cluster, 2.87/1,000 bed-days, was ≈4 times higher than the average rate in the previous 5 years, 0.63/1,000 bed-days (2012 through 2016). Initially, the strains were reported as *E. meningoseptica*, but subsequent testing confirmed the cluster to be associated with *E. anophelis*. We conducted an epidemiologic investigation to identify the source of the cluster. We also conducted a pragmatic experiment to test our hypothesis that *E. anophelis* could be transmitted by healthcare workers during handwashing with water contaminated with *E. anophelis.*

Author affiliation: KK Women's and Children's Hospital, Singapore.

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The Study

We collated clinical and epidemiologic data using a standardized spreadsheet for all patients testing positive for *Elizabethkingia* species in the KKH CICU in 2017. We also performed environmental sampling on all tap outlets and sinks in the clinical areas. For each tap, we swabbed the aerator and collected a water sample for culture. The water source of KKH has no supplemental treatments and meets WHO guidelines for drinking-water quality (*7*). To test our transmission hypothesis, we had 2 volunteer nurses place their hands on agar plates at 3 stages: before handwashing; after handwashing with chlorhexidine soap (4% wt/vol chlorhexidine gluconate; Microshield 4 Chlorhexidine Surgical Handwash; Schülke, Norderstedt, Germany) and tap water from the tap outlet in CICU known to be positive for *E. anopheles*; and finally after hand hygiene using alcohol-based hand rub (ABHR) (70% vol/vol ethanol and 0.5% wt/vol chlorhexidine gluconate; Microshield Handrub; Schülke).

We tested samples using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (VITEK MS; bioMérieux, Marcy-l'Étoile, France). We retested all samples positive for *Elizabethkingia* spp. by using 16S rDNA PCR: we extracted bacterial DNA and amplified 16S rDNA using primers 27f and 1492r (*8*). We performed sequencing using standard protocols and used BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for comparison with database sequences.

The 3 cluster cases were the only patients positive for *Elizabethkingia* species in the CICU in 2017. All were detected from blind bronchial sampling (BBS) via endotracheal tube (ETT). (Table 1) Patient 3's isolate was confirmed as *E. anophelis.* Unfortunately, the samples from the first 2 cases were not available for follow-up confirmatory testing. The patients were 2.8 months, 4.9 months, and 4.8 years of age, and all had significant underlying medical conditions. The average number of days in CICU before detection of *Elizabethkingia* species was 36 (range 11–66). None of the patients had been moved since admission, and 2 were cared for in single rooms.

Of the 27 environmental samples collected from 9 tap outlets or sinks in the unit, 10 samples were positive for *E. anophelis* and 1 positive for *E. meningoseptica.* Only 1 room (single bed) in the unit was negative for

Table 1. Characteristics of *Elizabethkingia* cases in children admitted to the Children's Intensive Care Unit, KK Women's and Children's Hospital, Singapore, May 2017*

Category	Patient 1	Patient 2	Patient 3
Sample date	2017 May 15	2017 May 22	2017 May 28
Sample type	ETT, BBS	ETT, BBS	ETT, BBS
Bacterial identification			
MALDI-TOF mass spectrometry	*E. meningoseptica*	*E. meningoseptica*	*E. meningoseptica*
16S rDNA	Isolate not available	Isolate not available	*E. anophelis*
Sex	M	F	F
Age, mo	4.9	2.8	57.9
Preterm birth	No	No	No
Underlying clinical condition	Duodenal atresia; small atrial septal heart defect	Pulmonary atresia; Large ventral septal heart defect; large patent ductus arteriosus	Thoracic tumor
Outcome	Discharged	Discharged	Deceased
Days in hospital	11	83	33
CICU bed type	Single room	4-bed cubicle	Single room
Other beds used	No	No	No
Antimicrobial drug treatment within 72 h before detection	Piperacillin/tazobactam, ceftriaxone	Clindamycin	Piperacillin/tazobactam
History of immunosuppressive medication	No	No	Yes (chemotherapy)
On ECMO at time of detection	Yes	No	Yes

* BBS, blind bronchial sampling; CICU, Children's Intensive Care Unit; ECMO, extracorporeal membrane oxygenation; ETT, endotracheal tube; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight.

Elizabethkingia bacteria. All 3 *Elizabethkingia* case-patients' rooms or cubicles were confirmed positive for *E. anophelis* from their respective tap outlets (aerator or water or both). The tap outlet from 1 cubicle not associated with any of the cases was positive for both *Elizabethkingia* species, *E. meningoseptica* in water and *E. anophelis* in the aerator. The Figure illustrates the spatial distribution of *Elizabethkingia* bacteria detected in tap outlets stratified by aerator, water, or sinks in the unit.

Our transmission experiment found that 1 staff member (staff B) acquired *E. anophelis* on her hands after handwashing (Table 2). After hand hygiene using ABHR,

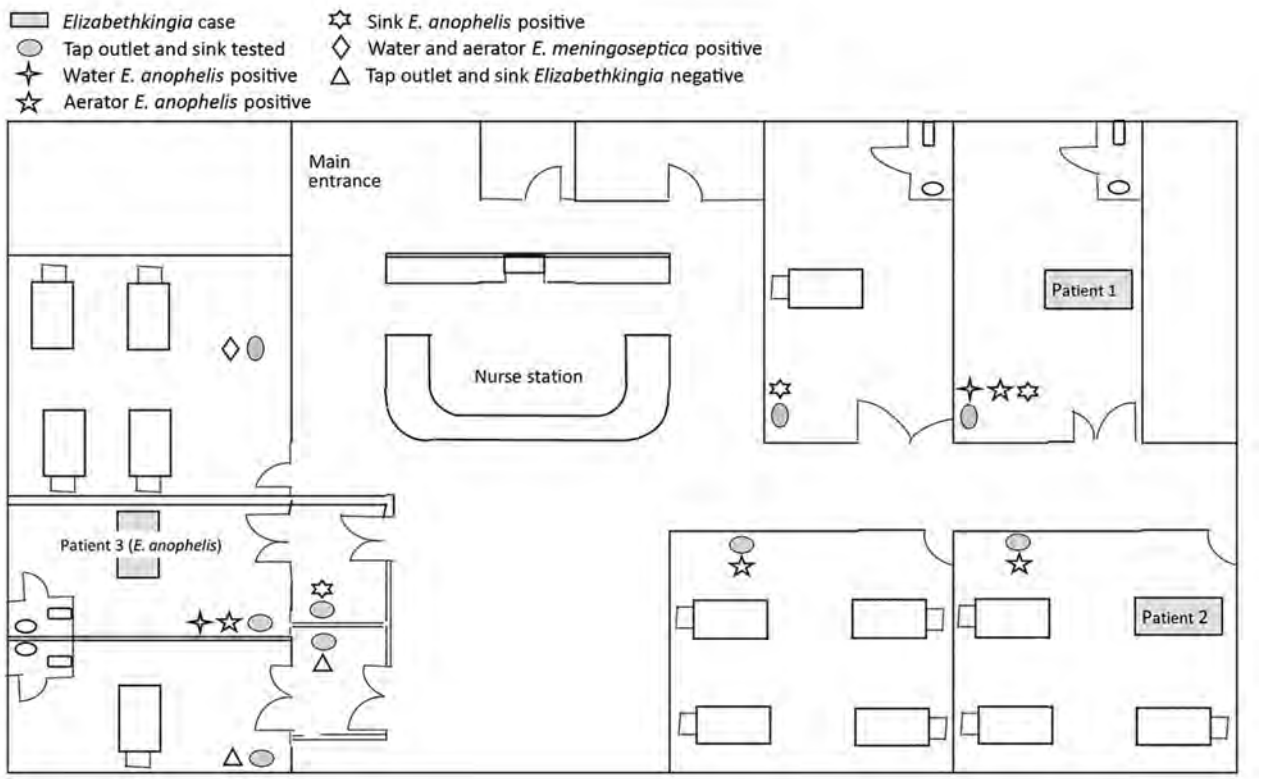

Figure. Spatial distribution of *Elizabethkingia* isolates by location (patients, tap water, aerators, and sinks) in children's intensive care unit, KK Women's and Children's Hospital, Singapore, May 2017.

Table 2. Potential transmission route of *E. anophelis* via handwashing for 2 hospital staff, Children's Intensive Care Unit, KK Women's and Children's Hospital, Singapore, May 2017

Procedure	Hands culture result	
	Staff A	Staff B
Before handwashing	Coagulase-negative *Staphylococcus* sp.	Coagulase-negative *Staphylococcus* sp.
After handwashing with chlorhexidine soap	Coagulase-negative *Staphylococcus* sp.	*E. anophelis*
After use of alcohol-based hand rub	No growth	No growth

both staff members had no detectable microbial growth on their hands.

Upon detection of the case cluster, we reinforced standard precautions, specifically hand hygiene compliance, and implemented environmental and patient-care equipment cleaning. We had all aerators permanently removed from the tap outlets in the CICU following confirmation of *Elizabethkingia* bacteria. The water from all 5 tap outlets previously found to be positive for *Elizabethkingia* bacteria in aerator or water was negative upon repeat testing after the intervention. We also recommended prioritizing hand hygiene using ABHR over handwashing unless hands were visibly soiled. All staff were reminded not to dispose of body fluids from patients into sinks used for handwashing because this was previously identified to be associated with *Elizabethkingia* tap colonization (*2*). In addition, we ended the use of tap water for patient care and allowed only sterile water. After these interventions, no additional cases of *Elizabethkingia* occurred in the unit for ≥4 months.

Conclusions

We report a confirmed *E. anophelis* case cluster affecting infants and children in the CICU of a pediatric hospital. Our investigation identified the likely source of *E. anophelis* to be tap outlets with aerators. We confirmed that removal of the aerators was effective in eliminating *E. anophelis* from tap water sources. We also demonstrated a likely transmission route for *E. anophelis* to patients through acquisition of the bacteria on hands of healthcare workers via handwashing. Subsequent use of ABHR was effective in eliminating the acquired *E. anophelis* from workers' hands.

Although 2 patients' isolates were not available for confirmatory testing, we detected *E. anophelis* in the tap outlets where they were cared for, suggesting that the *Elizabethkingia* species detected in their samples was highly likely to be *E. anophelis.* Isolates were initially misidentified as *E. meningoseptica* by MALDI-TOF mass spectrometry because *E. anophelis* was not represented in our routine database and only present in research databases of MALDI-TOF mass spectrometry systems (*9*). This discrepancy means that *E. anophelis* is probably overlooked in most diagnostic microbiology laboratories. There is a clinical need to differentiate these species in light of observations that *E. anophelis* infections tend to be more severe and associated with more deaths than are *E. meningoseptica* infections (*10*).

We showed how handwashing, despite the use of chlorhexidine soap, is a possible vehicle of transmission for *E. anophelis* from an affected tap outlet via the hands of healthcare workers to patients. Perinatal transmission of *E. anophelis* was previously documented to have occurred from a mother with chorioamnionitis to her neonate (*11*). We confirmed that hand hygiene using ABHR was effective in removing *E. anophelis* from hands of healthcare workers, which has implications for infection control. Although current hand hygiene guidelines prioritize ABHR over handwashing when hands are not visibly soiled, there is no requirement to perform ABHR in addition to handwashing (*12*). Therefore, most staff consider handwashing as complying with hand hygiene requirements. Our findings support using ABHR as the primary hand-hygiene method in clinical care, especially in critical care units and in outbreak situations involving waterborne organisms such as *E. anophelis*.

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About the Author

Dr. Yung is a consultant with the Infectious Disease Service, KK Women's and Children's Hospital, Singapore. He has a keen interest in infectious diseases, vaccines, outbreak control, epidemiology, and public health.

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Address for correspondence: Chee Fu Yung, KK Women's and Children's Hospital, 100 Bukit Timah Rd, 229899, Singapore; email: cheefu.yung@gmail.com

Maripa Virus RNA Load and Antibody Response in Hantavirus Pulmonary Syndrome, French Guiana

Séverine Matheus, Hatem Kallel, Alexandre Roux, Laetitia Bremand, Bhety Labeau, David Moua, Dominique Rousset, Damien Donato, Vincent Lacoste, Stéphanie Houcke, Claire Mayence, Benoît de Thoisy, Didier Hommel, Anne Lavergne

We report viral RNA loads and antibody responses in 6 severe human cases of Maripa virus infection (2 favorable outcomes) and monitored both measures during the 6-week course of disease in 1 nonfatal case. Further research is needed to determine prevalence of this virus and its effect on other hantaviruses.

Hantaviruses are members of the genus *Orthohantavirus* (family *Hantaviridae*) and are carried by various rodent species, depending on the strain. Humans can be infected by inhalation of aerosolized viruses excreted in the urine or feces of infected rodents. New World hantaviruses in the Americas cause hantavirus pulmonary syndrome (HPS) in humans, characterized by fever, headache, cough, myalgia, and nausea, evolving rapidly to pulmonary edema (*1*,*2*). This respiratory insufficiency is associated with death in 26%–39% of cases, depending on the New World hantavirus species (*3*,*4*).

Following the identification of Sin Nombre virus (SNV) as the etiologic agent of HPS in the United States in 1993, many other hantaviruses have been identified in the Americas (*3*–*6*). In French Guiana, a laboratory-confirmed case of hantavirus infection was reported in a hospitalized patient in 2008; the complete sequence analysis showed that this was a novel hantavirus closely related to the Rio Mamore species called Maripa virus (*7*,*8*).

We describe antibody responses to Maripa hantavirus infection and viral RNA loads in the 6 laboratory-confirmed human cases in French Guiana, measured at admission to the hospital. We also report how these 2 markers evolved during the course of the disease in the most recent hospitalized case-patient, who had a favorable clinical outcome.

Author affiliations: Institut Pasteur de la Guyane, Cayenne, French Guiana (S. Matheus, L. Bremand, B. Labeau, D. Moua, D. Rousset, D. Donato, V. Lacoste, B. de Thoisy, A. Lavergne); Centre Hospitalier de Cayenne, Cayenne (H. Kallel, A. Roux, S. Houcke, C. Mayence, D. Hommel)

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The Study

Since the time hantavirus diagnostic tools were set up at French Guiana's Institut Pasteur in 2008, a total of 6 severe human cases of infection by native hantavirus have been reported. All the patients were male; the mean age was 54.6 years (range 38–71 years). The mean time from onset of the disease until admission to the hospital was 4.6 days (range 2–7 days). The clinical outcome was favorable for 2 of the patients; 4 died (Table 1). The clinical and biologic parameters of the first 5 confirmed hantavirus cases were reported previously (*9*). The sixth patient was a 47-year-old man who complained of fever, cough, myalgia, and sweating that had been developing over 6 days. He was admitted to the Andrée Rosemon General Hospital in Cayenne, French Guiana, on August 31, 2017. He experienced respiratory failure, requiring rapid transfer to the intensive care unit for intubation and mechanical ventilation. Thoracic radiography revealed bilateral diffuse alveolar pulmonary infiltrates. The patient remained under mechanical ventilation for 18 days and was discharged from the hospital after 23 days with complete clinical recovery. The clinical symptoms of the patient, and his outdoor activities making the contact with rodents possible, led to suspicion of acute hantavirus infection, which was confirmed by molecular and serologic tests. The complete RNA coding sequence of the S RNA segment (GenBank accession no. MG785209) was also generated and compared with those of the other 5 previous hantavirus cases, showing that it corresponded to a Maripa virus infection (*9*).

We tested serum samples from the 6 HPS case-patients that were collected on admission at the intensive care unit and the other 7 sequential serum samples provided from case-patient 6 (6 samples during the hospitalization and 1 after discharge). We performed serologic IgM and IgG tests and assayed them for viral RNA quantification (Tables 1, 2). We obtained informed consent from the patients, their representatives, or both at admission and before discharge.

We assayed all serum samples by IgM capture and IgG ELISA using the protocol described by Ksiazek et al. (*10*). We tested samples against SNV antigen and control antigen using 4-fold dilutions, from 1:100 to 1:6,400. Because

Table 1. Immune response and viral loads on admission in 6 confirmed hantavirus cases, French Guiana*

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Year case reported	2008	2009	2010	2013	2016	2017
Age, y	38	56	49	67	71	47
Days of disease at admission	7	4	2	4	4	7
SNV IgM	Positive	Positive	Positive	Positive	Positive	Positive
IgM sum OD†	1.02	1.70	4.72	0.92	2.23	1.79
SNV IgG	Negative	Negative	Negative	Negative	Positive	Negative
IgG sum OD†	0.05	0.01	0.01	0.01	2.05	0.73
Serum viral RNA load‡	5.8	6.6	6.4	5.9	6.0	6.4
Clinical evolution	Favorable	Death	Death	Death	Death	Favorable

*OD, optical density; SNV, Sin Nombre virus.
†Adjusted sum OD values (dilution 1:100, 1:400, 1:1,600, and 1:6,400).
‡Virus copy number was determined as $\log_{10}$ copies/mL. Primers and TaqMan probe for quantitative PCR were Maripa_qRT2F 5'-GCAGCTGTGTCTACATTGGAGAA-3', Maripa_qRT2R 5'-CCACCAGATCCGCCAACT-3', and Maripa_Probe2 5'-FAM-AAACTTGCAGAACTCA-MGB-3'.

of antibody cross-reactivities, positive ELISA findings with SNV antigens indicated infections with New World hantaviruses. The positive criteria were similar to those described by MacNeil et al. (*11*).

The serologic investigations showed that all samples collected at admission had detectable amounts of hantavirus IgM: minimum IgM titers ≥400 for patients 1, 2, 4, 5, and 6 and a maximum titer of ≥16,00 for patient 3 (Table 1). These data were similar to those reported in previous work (*11*,*12*). Only patient 5, who died 24 hours after admission, had serum samples positive for hantavirus IgG (titer ≥6,400). Although the time from the onset of disease and sample collection at admission was different for each of the 6 patients, this single positive hantavirus IgG case may be explained in part by the longer viral incubation period, resulting in the induction of IgG before the appearance of symptoms. A previous study reported that the presence of hantavirus IgG during the first week of infection might be a predictor of survival, but we found no evidence supporting this view (*11*).

To determine the viral RNA load in each serum sample, we performed real-time PCR. Each reaction was performed in duplicate. For absolute quantification, we calculated the exact number of copies of the gene of interest using a standard curve established with plasmid DNA at dilutions from 5 to 5×10^7 copies/mL. The viral RNA loads in the samples collected on admission were 5.8–6.6 $\log_{10}$ copies/mL (mean 6.2 ± 0.3 $\log_{10}$ copies/mL) (Table 1). These values were similar to those observed in patients infected by other hantaviruses, including patients with mild or moderate symptoms (*13*–*15*). We also observed that the viral RNA load in the 4 fatal cases was 6.2 $\log_{10}$ copies/mL, whereas in the 2 nonfatal cases it was 6.1 $\log_{10}$ copies/mL. A correlation between hantavirus RNA loads in the serum during the acute phase of disease and the clinical outcome has been hypothesized (*14*,*15*); however, although our study includes only a small number of cases and only severe cases, it provides no evidence supporting this possibility. Presumably, the fatal or nonfatal outcome depends not only on the hantavirus viral load but also on other pathogenic or host factors.

The progression of these antibody responses and viral RNA loads was also followed during the course of disease for patient 6, from admission to the hospital (day 7) until day 46 after the onset of disease (Table 2). IgM titers were high at admission but decreased to become undetectable by day 46. Conversely, seroconversion (IgM to IgG) was observed between day 7 and day 12; these hantavirus IgG titers then increased to 4.4 by day 46. Likewise, viral RNA load evaluated in these 7 sequential serum samples showed a high value at admission (6.4 $\log_{10}$ copies/mL), declining by 7 days later to 4.7 $\log_{10}$ copies/mL (Table 2). Viral load then remained around 4 $\log_{10}$ copies/mL in samples collected on days 20, 25, and 30 and was undetectable on day 46.

Conclusions

Although limited in sample size, this study found similar results for viral load and immune response in the first 6 cases of Maripa virus infection reported in French Guiana after laboratory-based surveillance began in 2008. Further

Table 2. Monitoring of hantavirus antibodies and viral RNA load in sequential serum samples from patient 6, French Guiana*

	Days after symptom onset						
Characteristic	Day 7	Day 12	Day 15	Day 20	Day 25	Day 30	Day 46
SNV IgM	Positive	Positive	Positive	Positive	Positive	Positive	Negative
IgM sum OD†	1.79	1.56	1.50	1.34	1.01	0.72	0.42
SNV IgG	Negative	Positive	Positive	Positive	Positive	Positive	Positive
IgG sum OD†	0.73	1.83	2.20	3.08	4.21	4.71	4.40
Serum viral RNA load‡	6.4	5.4	4.7	4.1	4.0	4.1	0

*OD, optical density; SNV, Sin Nombre virus.
†Adjusted sum OD values (dilution 1:100, 1:400, 1:1,600, and 1:6,400).
‡Virus copy number was determined as $\log_{10}$ copies/mL. real-time PCR Primers and TaqMan probe for quantitative PCR were Maripa_qRT2F 5'-GCAGCTGTGTCTACATTGGAGAA-3', Maripa_qRT2R 5'-CCACCAGATCCGCCAACT-3', and Maripa_Probe2 5'-FAM-AAACTTGCAGAACTCA-MGB-3'.

work is needed to determine the overall prevalence of this hantavirus in French Guiana and also the possible undetected mild or moderate cases induced by Maripa virus infection as reported for other New World hantaviruses (*13–15*). Moreover, it would be informative to determine the infectious potential of the virus in the sequential samples to provide a better understanding of the pathophysiology of this infection. Investigations of the immune response to hantavirus, consequences of different viral loads, and the pathologic characteristics of different hantavirus strains would help identify the determinants of disease outcome.

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About the Author

Dr. Matheus is a research assistant at the Institut Pasteur de la Guyane, Cayenne, French Guiana. Her research interests are the diagnosis and pathophysiology of arboviruses, with special interest in hantavirus circulation in French Guiana.

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Address for correspondence: Séverine Matheus, Centre National de Référence des Hantavirus, Institut Pasteur de la Guyane, 23 avenue Pasteur, BP 6010 – 97306 Cayenne CEDEX, French Guiana; email: smatheus@pasteur-cayenne.fr

Severe Manifestations of Chikungunya Fever in Children, India, 2016

Pradeep K. Sharma, Maneesh Kumar, Girraj K. Aggarwal, Virender Kumar, R.D. Srivastava, Ashish Sahani, Rohit Goyal

Chikungunya is a relatively benign disease, and a paucity of literature on severe manifestations in children exists. We describe a cohort of pediatric chikungunya fever patients in New Delhi, India, who had severe sepsis and septic shock, which can develop during the acute phase of illness.

Chikungunya fever, regarded as a benign disease with infrequent severe manifestations, has caused epidemics in many countries in the past decade. The worst epidemic of chikungunya fever in Delhi, India, occurred in 2016 (*1*). Severe manifestations of chikungunya fever in adults have been reported recently (*2*–*7*); however, there is a paucity of similar data in children. The objective of our study was to describe the characteristics of pediatric patients who had atypical or severe forms of the disease and to search for predictive factors for severe forms.

The Study

We conducted a retrospective, observational study in the pediatric intensive care unit (PICU) and pediatric high-dependency unit of a tertiary care hospital in New Delhi, India. We included patients whose chikungunya infection was diagnosed by positive real-time reverse transcription PCR (RT-PCR) during September–December 2016. The RT-PCR was done using a Gene Finder DENV/CHKV Real*Amp* Kit (Osang Healthcare, Gyeonggi-do, South Korea) at Oncquest Laboratories (New Delhi, India). This qualitative assay uses a 1-tube RT-PCR technique with internal control for amplification and detection of chikungunya virus RNA. The study protocol was approved by the hospital's Institutional Research Council.

The information recorded consisted of demographic features, clinical features, laboratory parameters, course, organ dysfunction, ventilation days, inotropic days, hospital stay, and whether the patient died. We classified the disease as severe in the presence of severe sepsis, septic shock, or organ dysfunction, which were defined according to standard definitions (*8*).

Author affiliation: Sri Balaji Action Medical Institute, New Delhi, India

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A total of 49 children had chikungunya fever; 36 had nonsevere disease and 13 had severe disease. All patients with severe disease were admitted to the PICU; 11 had illness consistent with the case definition of severe sepsis and septic shock, and 2 had acute liver failure. Of the 36 patients with nonsevere disease, 16 were admitted to the PICU (11 had seizures, 4 had fluid-responsive shock, 1 had peripheral cyanosis and mottling) and 20 were admitted to the pediatric high-dependency unit (3 had bleeding manifestations, 4 had severe abdominal pain, 2 had underlying cyanotic congenital heart disease, 2 had body temperature >40.3°C with irrelevant talking, 7 had dehydration, and 2 had severe rash). The median age was 12 years for patients with severe disease and 6.5 years for patients with nonsevere disease; male sex predominated in both groups (Table). Frequency of fever, body ache, arthralgia, and vomiting were similar for both groups. Peripheral cyanosis, along with mottling of skin and encephalopathy, was significantly higher in the group with severe disease. Serum albumin was significantly lower in the group with severe disease (3 vs. 3.75 g/dL).

Of the 11 children with septic shock, 8 were admitted to the hospital within 24 hours of developing fever; 9 had hypotensive shock, and 2 had compensated shock. In this group, 6 children required 1 vasoactive agent, 3 children required 2 vasoactive agents, and 2 children required 3 vasoactive agents. Dopamine was used in 8 patients, dobutamine in 5 patients, epinephrine in 2 patients, and norepinephrine in 2 patients. The median duration of vasoactive support was 56 hours (range 31–114 hours), and the median vasoactive inotropic score in the first 24 hours was 10 (range 5–90; score >15–20 is considered serious). A vasoactive inotropic score >20 was seen in 2 children. Mean pH was 7.26 (reference range 7.35–7.45), mean lactate 5.1 mmol/L (reference range <2 mmol/L), mixed venous saturation 55% (reference range 70%–80%), and mean base excess at admission –7.7 mEq (reference range –2 to 2 mEq). Of the 2 children with acute liver failure with encephalopathy, 1 had dengue virus (positive dengue IgM by enzyme immunoassay) and the other had hepatitis E virus (reactive anti–hepatitis E IgM by enzyme immunoassay) co-infection.

The usual symptoms of chikungunya are fever, rash, and joint pain. Children can have features distinct from adults, such as more frequent dermatological and hemorrhagic manifestations and less frequent rheumatologic manifestations (*7*). Most patients with symptomatic disease have mild to moderate illness. A recent pediatric study

Table. Demographic, clinical, and laboratory features at admission of pediatric patients with severe and nonsevere chikungunya fever, New Delhi, India, 2016*

Parameters	Nonsevere disease, n = 36	Severe disease, n = 13	p value†
Median age, y (range)	6.5 (0.75–15)	12 (0.5–14)	0.28
Male:female ratio	3.5:1	2.25:1	0.53
Age group			
Infant, 1 mo–1 y	3 (8.3)	4 (30.7)	0.06
Toddler, 2–5 y	13 (36)	1 (7.7)	
School age, 6–12 y	3 (8.3)	0	
Adolescent, 13 to <18 y	17 (47.2)	8 (61.5)	
Clinical profile			
Fever	36 (100)	13 (100)	
Body ache	10 (27.7)	4 (30.7)	0.83
Rash	15 (41.6)	8 (61.5)	0.22
Arthralgia	5 (13.9)	3 (23)	0.44
Vomiting	15 (41.6)	5 (38.4)	0.84
Seizures	11 (30.5)	1 (7.7)	0.14
Bleeding	3 (8.3)	4 (30.7)	0.16
Abdominal pain	4 (11)	5 (38.4)	0.25
Peripheral cyanosis and mottling of skin	2 (5.5)	10 (76.9)	**0.00**
Encephalopathy	0	3 (23)	**0.01**
Laboratory test results, median (range)			
Hemoglobin, g/dL	12.2 (6.6–17.5)	11.6 (8–13.5)	0.08
White cell count/μL	8,195 (3,700–15,200)	11,200 (4,100–44,800)	0.058
Platelet count, $\times 10^3$/μL)	203 (25–362)	192 (13–362)	0.61
AST, IU/L	44 (22–174)	43 (16–8,837)	0.96
ALT, IU/L	20 (9–96)	24 (8–2,311)	0.26
Albumin	3.75 (3.5–4)	3.3 (1.6–3.5)	**0.006**
Urea, mg/dL	21 (11–55)	36 (13–87)	0.094
Creatinine, mg/dL	0.4 (0.3–1.2)	0.6 (0.2–1.3)	0.37
APTT, s, control 28.4 s	NA	41.7 (27–247)	
PT, s, control 13.3 s	NA	22.3 (18.5–117)	
International normalized ratio	NA	1.77 (1.3–12.4)	
Organ dysfunction			
Cardiovascular	0	11 (84.6)	
Respiratory	0	3 (23)	
Hematological	0	5 (38.4)	
Neurologic	0	3 (23)	
Renal	0	2 (15.3)	
Hepatic	0	3 (23)	
Course and outcome			
Mechanical ventilation	0	3 (23)	
Inotropic support	0	11 (84.6)	
Renal replacement	0	2 (15.3)	
Hospital stay, d (range)	3 (2–7)	5 (2–13)	**0.0015**
Death	0	1 (7.7)	

*Values are no. (%) patients except as indicated. ALT, alaninine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; NA, not applicable; PT, prothrombin time.
†Categorical variables were compared using the χ^2 test or Fisher exact test, as appropriate, and continuous variables were compared by using the nonparametric Mann-Whitney test. p values <0.05 were considered statistically significant and are shown in bold type.

reported severe disease in infants and neonates; however, septic shock was not well defined (*9*). Recently, sepsis and septic shock in adults have been described in the literature, with relatively high death rates (36%–100%) (*2*–*6*).

Conclusions

Our study reports a cohort of pediatric chikungunya fever patients who had severe sepsis and septic shock. In our study, children <1 year of age and 11–14 years of age were more likely to have septic shock. Most of these children were admitted to the hospital within 24 hours of developing fever, with peripheral cyanosis and cold extremities. Although children can have cold extremities during high fever, in our cohort central capillary refill time was also prolonged, and generalized skin mottling was present. In addition, these children had hypotension or metabolic evidence of poor perfusion (high lactate and low mixed venous saturation). Children with early shock had generalized erythema and diffuse edema.

Although dopamine was the most used inotropic agent overall, in infants dobutamine was more helpful in improving shock, both clinically and metabolically. Of the infants, 3 of 4 required only dobutamine; 1 required dopamine as well.

Shock in children who were admitted early usually resolved around the time of mitigation of fever. All 3 children who were admitted late had multiorgan failure and required mechanical ventilation. Of these, 2 had myocardial

dysfunction and required multiple inotropic agents and renal replacement therapy. Both of these children had severe ascites; the child who died had bilateral pleural effusion, pericardial effusion, generalized confluent ecchymosis, and pregangrenous changes at peripheral sites.

Our cohort of septic shock patients did not reveal clinical or microbiological evidence of other infections. Results of tests for dengue nonstructural protein 1 antigen, dengue IgM and IgG (by enzyme immunoassay), leptospira IgM and IgG (by immuno-chromatographic assay), Weil Felix serology (by latex agglutination), blood cultures, and other relevant cultures were all negative.

Although chikungunya usually has a mild course, severe life-threatening manifestations can occur. Clinicians should be aware that these manifestations can develop within 24 hours of the onset of illness, and a high index of suspicion is required to establish diagnosis. In our study, age <1 year and 11–14 years were predictive of severe disease. Further studies are required to clarify the clinical spectrum and risk factors associated with severe disease.

About the Author

Dr. Sharma is senior consultant and in charge of the pediatric critical care and pulmonology unit at Sri Balaji Action Medical Institute, Paschim Vihar, New Delhi, India. His research interests are infectious diseases, antimicrobial drug resistance, and antibiotic stewardship, especially in pediatric intensive care.

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Address for correspondence: Pradeep K. Sharma, Sri Balaji Action Medical Institute, Pediatric Critical Care and Pulmonology, A-4, Paschim Vihar, New Delhi, 110063, India; email: drsharma025@gmail.com

Zika Virus Seropositivity in 1–4-Year-Old Children, Indonesia, 2014

R. Tedjo Sasmono, Rama Dhenni, Benediktus Yohan, Paul Pronyk, Sri Rezeki Hadinegoro, Elizabeth Jane Soepardi, Chairin Nisa Ma'roef, Hindra I. Satari, Heather Menzies, William A. Hawley, Ann M. Powers, Ronald Rosenberg, Khin Saw Aye Myint, Amin Soebandrio

We assessed Zika virus seroprevalence among healthy 1–4-year-old children using a serum sample collection assembled in 2014 representing 30 urban sites across Indonesia. Of 662 samples, 9.1% were Zika virus seropositive, suggesting widespread recent Zika virus transmission and immunity. Larger studies are needed to better determine endemicity in Indonesia.

Zika virus, first isolated in 1947 (*1*), is a flavivirus phylogenetically related to dengue virus (DENV) that is, like DENV, also transmitted by *Aedes* mosquitoes. Because of the epidemic that swept through the Americas in 2016, Zika virus infection is known to cause microcephaly, as well as other congenital defects and Guillain-Barré syndrome (*2*).

Zika virus has long been known to be endemic in Southeast Asia (*3*,*4*), but laboratory confirmation of infection can be challenging. Acute infections are often asymptomatic. In those who are symptomatic, viral RNA typically persists in blood <7 days and in urine <10 days after symptom onset, limiting the usefulness of nucleic acid testing (*5*). Zika virus antibody cross-reacting with DENV can confuse results of tests conducted in regions where the viruses co-circulate (*6*). Virus-specific neutralization assays can more accurately detect and measure Zika virus antibody, but because of their complex requirements, these tests have seldom been used in epidemiologic studies (*7*).

Acute Zika virus cases have been reported in Indonesia (*8*), Singapore (*9*), Malaysia (*10*), Vietnam (*11*), and Thailand (*12*). However, little is known about Zika virus prevalence in the region. Limited retrospective testing of archived specimens collected from clinically ill patients in Thailand (*12*) and Cambodia (*13*) suggest that incidence in these countries is low. However, given the limited number of samples tested and lack of confirmatory testing in these studies, information on prevalence and distribution is challenging to assess. Likewise, little is known about the prevalence and geographic distribution of Zika virus in Indonesia, the biggest country in Southeast Asia.

DENV and chikungunya virus, also transmitted by *Aedes* mosquitoes, are endemic throughout Indonesia, suggesting the ecologic conditions exist for Zika virus transmission as well. An estimated 80% of the population in Indonesia is infected with ≥1 DENV by the age of 10 years (*14*). In our study, we assessed Zika virus seroprevalence among healthy 1–4-year-old children to determine the prevalence and distribution of Zika virus in Indonesia.

The Study

We used serum samples collected during October–November 2014 for a previous population-based, cross-sectional cluster survey conducted to assess DENV seroprevalence; in the study, 3,312 samples were collected from 1–18-year-old children in 30 urban districts in 14 provinces of Indonesia (*14*). In our study, we assessed only the children 1–4 years (range 12–59 months) of age because these children were least likely to have cross-reactive DENV antibodies. Ethics clearance was obtained from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia, and the US Centers for Disease Control and Prevention (CDC; Atlanta, Georgia, USA).

Plaque reduction neutralization tests (PRNTs) that could differentiate Zika virus neutralizing antibodies from those produced in response to DENV infection were adapted from protocols developed by the CDC (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/24/9/18-0582-Techapp1.pdf). The challenge virus used in the PRNT was Zika virus JMB-185, acquired from a patient in 2014 (*8*). Convalescent serum from this same patient was used as a PRNT positive control. We subjected all specimens

Author affiliations: Eijkman Institute for Molecular Biology, Jakarta, Indonesia (R.T. Sasmono, R. Dhenni, B. Yohan, C.N. Ma'roef, K.S.A. Myint, A. Soebandrio); UNICEF Indonesia, Jakarta (P. Pronyk); University of Witwatersrand School of Public Health, Johannesburg, South Africa (P. Pronyk); Universitas Indonesia Medical School, Jakarta (S.R. Hadinegoro, H.I. Satari); Cipto Mangunkusumo Hospital, Jakarta (S.R. Hadinegoro, H.I. Satari); Ministry of Health of the Republic of Indonesia, Jakarta (E.J. Soepardi); Centers for Disease Control and Prevention, Atlanta, Georgia, USA (H. Menzies, W.A. Hawley); Centers for Disease Control and Prevention, Fort Collins, Colorado, USA (A.M. Powers, R. Rosenberg)

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to 2 tiers of testing by $PRNT_{90}$ (i.e., a PRNT in which serum samples suppressing ≥90% of challenge virus were considered positive for neutralizing antibody). In the first tier, we tested serum samples diluted 1:10. Samples that suppressed ≥90% of Zika virus PFUs were considered potentially positive for Zika virus antibodies because DENV-specific antibodies, if present, could have cross-reacted and neutralized Zika virus. We then subjected the specimens considered potentially positive to a second $PRNT_{90}$, in which we tested serum samples against Zika virus and all 4 DENV serotypes (online Technical Appendix). Specimens that tested positive for Zika virus neutralizing antibody and negative for DENV neutralizing antibody by $PRNT_{90}$ were classified as Zika virus seropositive, as were specimens that had Zika virus $PRNT_{90}$ titers ≥4-fold higher than all DENV $PRNT_{90}$ titers. We categorized specimens as flavivirus seropositive when Zika virus neutralizing antibodies were present but at titers <4-fold higher than any DENV neutralizing antibody titer (online Technical Appendix Table). We also tested a subset of samples for Japanese encephalitis virus antibody by $PRNT_{90}$; none of the samples tested had a titer >20, and none of the sample classifications were changed after testing.

In the initial $PRNT_{90}$ screening, we detected possible Zika virus antibody in 73 (11.0%) of the 662 serum samples (Table). Of these, 72 had a sufficient volume to undergo second-tier testing; 60 (83.3%) of 72 samples were Zika virus seropositive, and 12 (16.7%) were flavivirus seropositive. Serum samples from 11 of 14 provinces were Zika virus seropositive, and the collections from the provinces ranged from ≈4.5% seropositive (North Sumatra, Banten, East Kalimantan) to >18% seropositive (Central Java, Jambi; Figure). Overall, Zika virus seroprevalence in the 1–4-year-old cohort was 9.1% (95% CI 3.95%–11.01%).

Our assessment, involving use of the $PRNT_{90}$, which is highly specific for Zika virus antibodies, indicates widespread, recent Zika virus infection in much of western and central Indonesia. Our criterion for confirmed Zika virus antibodies (i.e., $PRNT_{90}$ titer for Zika virus ≥4-fold higher than that for any DENV in the same specimen) is the international standard. In just 2% (12/662) of specimens, we could not determine whether the antibodies were Zika virus or DENV specific. When using the more conservative criterion of only classifying a sample as positive for Zika virus antibodies if no DENV-specific neutralizing antibodies are detected, the number of Zika virus antibody–positive samples decreases by only 6, leaving 54 samples still classified as Zika virus seropositive. Further evidence for the validity of the $PRNT_{90}$ was that DENV neutralizing antibody–positive samples were negative for the presence of Zika virus neutralizing antibodies across a range of titers (R.T. Sasmono, unpub. data).

Although our data provide some evidence regarding geographic distribution, no information is presented regarding a specific threshold titer associated with clinical illness or a correlation between geography and titer. Further studies involving larger sample sets would be necessary to address these topics. The single age group, relatively small number of specimens, and limited number of sites, particularly from eastern parts of the country, do not give a comprehensive picture of endemicity throughout Indonesia. The small numbers of specimens available from most localities did not enable accurate estimation of the proportional differences between localities. We could perform $PRNT_{90}$ with samples from the remaining cohort (the 5–18-year-olds), but we expect higher percentages of nonspecific flavivirus seropositivity in the samples from this older age group.

Table. Seropositivity of 1–4-year-old urban children for Zika virus and other flaviviruses, by province, Indonesia, October–November 2014*

Province	Serologic status, % (no. positive samples/total samples)		
	Suspected Zika virus seropositive†	Confirmed Zika virus seropositive‡	Flavivirus seropositive§
Aceh	0 (0/22)	0 (0/22)	0 (0/22)
North Sumatra	9.1 (2/22)	4.5 (1/22)	4.5 (1/22)
West Sumatra	18.2 (4/22)	13.6 (3/22)	4.5 (1/22)
Jambi	18.2 (4/22)	18.2 (4/22)	0 (0/22)
Lampung	8.7 (2/23)	8.7 (2/23)	0 (0/23)
Banten	4.4 (2/45)	4.4 (2/45)	0 (0/45)
DKI Jakarta	10.6 (7/66)	10.6 (7/66)	0 (0/66)
West Java	11.1 (17/153)	8.5 (13/153)	2.0 (3/153)
Central Java	20.5 (18/88)	18.2 (16/88)	2.3 (2/88)
East Java	11.7 (13/111)	9.0 (10/111)	2.7 (3/111)
Bali	0 (0/22)	0 (0/22)	0 (0/22)
East Kalimantan	4.5 (1/22)	4.5 (1/22)	0 (0/22)
South Sulawesi	0 (0/22)	0 (0/22)	0 (0/22)
Southeast Sulawesi	13.6 (3/22)	4.5 (1/22)	9.1 (2/22)
All provinces	11.0 (73/662), 95% CI 5.34–13.32	9.1 (60/662), 95% CI 3.95–11.01	1.8 (12/662), 95% CI 0.23–3.35

*DENV, dengue virus; $PRNT_{90}$, plaque reduction neutralization test with neutralization defined as ≥90% reduction in challenge virus PFUs.
†Serum samples that neutralized ≥90% of the challenge virus at a 1:10 dilution on initial Zika virus $PRNT_{90}$ screening.
‡Serum samples that neutralized Zika virus only or had a $PRNT_{90}$ titer ≥4-fold higher for Zika virus than for any DENV.
§Serum samples that neutralized Zika virus and DENV and had a $PRNT_{90}$ titer for Zika virus that was <4-fold higher than that for any DENV.

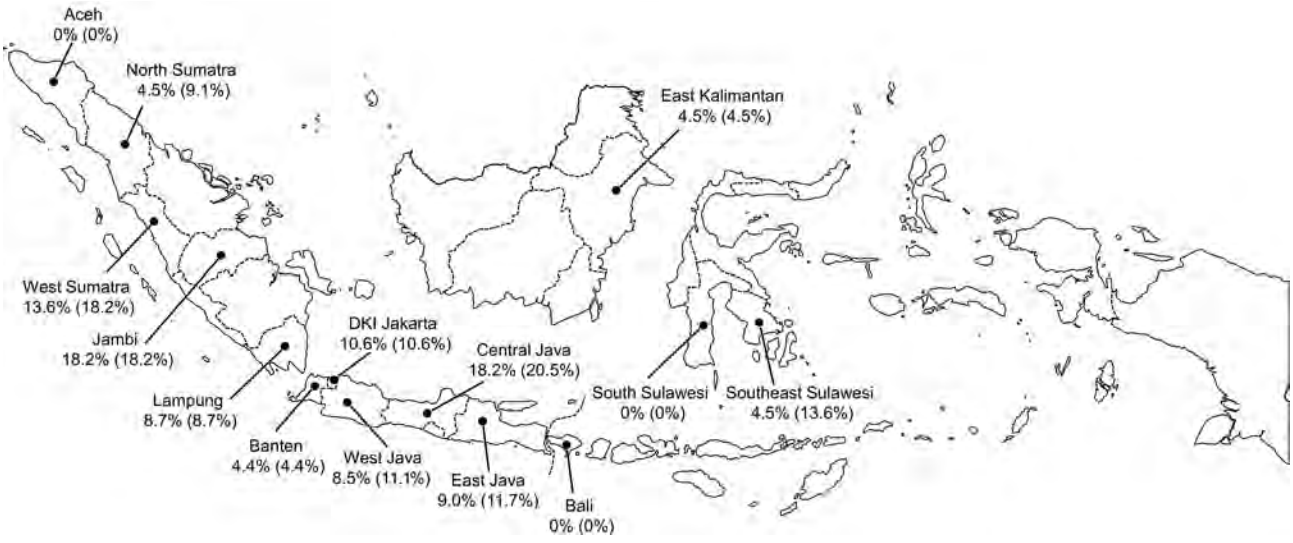

Figure. Geographic distribution of Zika virus–seropositive 1–4-year-old children, Indonesia, October–November, 2014. The values listed for each province indicate the percentage of serum samples confirmed Zika virus seropositive (percentage serum samples suspected to be Zika virus seropositive). Samples suspected to be Zika virus positive were those that were positive on initial Zika virus $PRNT_{90}$ (plaque reduction neutralization test with neutralization defined as ≥90% reduction in challenge virus PFUs) screening when using a 1:10 serum sample dilution. Serum samples confirmed as Zika virus seropositive were those that neutralized Zika virus only or had a $PRNT_{90}$ titer for Zika virus that was ≥4-fold higher than the $PRNT_{90}$ titer for any DENV.

Conclusions

Much has been published on epidemic Zika virus, but little is known about the effect of Zika virus in endemic areas. Determining the prevalence of Zika virus in Indonesia can provide clues to its potential long-term public health significance in endemic settings. Mild or asymptomatic infection is common, and confusion with dengue during diagnosis probably accounts for how long Zika virus was unrecognized in Indonesia and other areas of Southeast Asia. Besides the need to better evaluate Zika virus incidence and distribution, a high priority for future investigations will be determining the extent of Zika virus–related birth defects. If, like other flaviviruses, a primary Zika virus infection results in lifelong immunity, infections during childhood could reduce a person's risk for infection later in life and thus the incidence of Zika virus–related birth defects. This knowledge provides clues for understanding future patterns of Zika virus transmission in the Americas.

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About the Author

Dr. Sasmono is a senior research fellow at the Eijkman Institute for Molecular Biology, Jakarta, Indonesia. His primary research interests are dengue and other arboviral diseases.

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Address for correspondence: R. Tedjo Sasmono, Eijkman Institute for Molecular Biology, Jl Diponegoro 69, Jakarta 10430, Indonesia; email: sasmono@eijkman.go.id

Trichodysplasia Spinulosa Polyomavirus in Respiratory Tract of Immunocompromised Child

Arwa A. Bagasi, Tasneem Khandaker, Gemma Clark, Terry Akagha, Jonathan K. Ball, William L. Irving, C. Patrick McClure

Author affiliations: King Saud University, Riyadh, Saudi Arabia (A.A. Bagasi); University of Nottingham, Nottingham, UK (A.A. Bagasi, T. Khandaker, T. Akagha, J.K. Ball, W.L. Irving, C.P. McClure); Nottingham University Hospitals NHS Trust, Nottingham (G. Clark, W.L. Irving)

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Trichodysplasia spinulosa polyomavirus causes trichodysplasia spinulosa, a skin infection, in immunocompromised persons, but the virus is rarely detected in respiratory samples. Using PCR, we detected persistent virus in respiratory and skin samples from an immunocompromised boy with respiratory signs but no characteristic skin spicules. This virus may play a role in respiratory illness.

Trichodysplasia spinulosa is a rare skin disease that occurs exclusively in immunocompromised persons. It is characterized by facial keratotic spicules formed by trichohyalin accumulation in the inner root sheath cells of affected hair follicles. In 1999, electron microscopy identified a novel polyomavirus, subsequently named trichodysplasia spinulosa polyomavirus (TSPyV) or human polyomavirus 8, in sections of skin spicules of a solid organ transplant patient (*1*); in 2010, the virus was more completely characterized (*2*). TSPyV is 1 of 5 polyomaviruses associated with human diseases, particularly those that affect immunocompromised persons (*3*). Although worldwide seroprevalence of TSPyV antibodies among the general population is estimated at 70% (*4*) and a respiratory route of infection has been hypothesized (*5*,*6*), as of 2015, only 32 cases of trichodysplasia spinulosa had been reported (*7*), suggesting that other pathology caused by TSPyV may have gone undiagnosed. We describe PCR detection of TSPyV in an immunocompromised boy with respiratory signs and symptoms.

To elucidate potential causes of undiagnosed viral respiratory infection, during January 2015–February 2016, we used a panpolyomavirus degenerate primer PCR to screen archived samples for polyomavirus. The archived samples were nucleic acid of respiratory specimens from 218 children 6 months to 5 years of age, previously negative for typical respiratory viruses in a panel used for routine diagnosis (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/24/9/18-0829-Techapp1.pdf). Of the 218 samples screened in 22 pools, we obtained positive results for polyomavirus in 1 pool and, subsequently, 1 sample (from the patient reported here). Subsequent Sanger sequencing and BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) analysis of the 274-bp degenerate primer PCR product indicated that the sample contained TSPyV. The complete genome of this TSPyV strain was amplified in 4 overlapping PCR fragments and Sanger sequenced (online Technical Appendix). Phylogenetic analysis of the assembled complete 5,232-nt genome with all available 23 reference sequences revealed that the TSPyV strain was most closely related to TSPyV 1312, which had been isolated in 2012 in Dallas, Texas, USA (online Technical Appendix), but bootstrap support was limited because of the highly conserved nature of TSPyV genomes.

The patient from whom this TSPyV-positive sample was collected was a 4-year-old boy in Nottinghamshire, United Kingdom, who had common acute lymphoblastic leukemia and was receiving maintenance chemotherapy during the study period. Retrospective clinical analysis for March 2014–February 2016 revealed that the child had had frequent cough with fever and coryzal symptoms of varying severity (Table). Concurrently collected nasopharyngeal aspirate and throat swab specimens were negative for bacterial and viral pathogens routinely tested for, except at the start of the study period, when rhinovirus and adenovirus were detected, and the end of the period, when rhinovirus and respiratory syncytial virus were detected (Table). No bacteria were cultured from paired specimens. On this basis, in conjunction with unremarkable physical examination and radiologic findings and stable neutrophil and leukocyte counts (data not shown), the patient's respiratory signs were treated conservatively on an outpatient basis. However, on 2 occasions (August and November 2015), the child required hospital admission, without and with co-infection, respectively.

Further retrospective laboratory investigation found that all 11 additional samples collected from this patient during November 2014–2015 were positive for TSPyV, with co-infection at the 4 time points (November and December 2014, September and November 2015); testing showed fluctuating cycle threshold (C_t) levels on quantitative PCR (Table; online Technical Appendix). Of note, various forms of rashes appeared in different anatomic regions of the patient but did not resemble the characteristic appearance of trichodysplasia spinulosa and, thus, did not raise any clinical suspicion for this condition. Indeed, retrospective testing found that a single skin swab sample taken from a suspected viral rash (site undocumented) that looked like blisters and

Table. Clinical and laboratory data from TSPyV-positive patient, Nottinghamshire, United Kingdom, November 2014–2015*

Collection date	Sample type	Signs and symptoms at time of sample collection	Documented skin lesion	Hospital admission	Viral/bacterial co-infection	TSPyV C_t value
2014						
Nov	Throat swab	Cough, sore throat, fever	Tiny skin colored pustules on hand	Not required	Rhinovirus	31.83
Dec	NPA	Cough, fever	None	Not required	Adenovirus	31.17
2015						
Jan	Skin swab	None recorded	Suspected varicella zoster virus rash	Not required	None	24.97
Feb	NPA	Dry cough, fever	None	Not required	None	31.23
Mar	Throat swab	Dry cough, coryzal symptoms	None	Not required	None	21.43
Jul†	Throat swab	Cough with runny nose	None	Not required	None	23.90
Jul†	NPA	Cough with runny nose	None	Not required	None	22.70
Jul‡	NPA	Dry cough, fever	Few blisters on fingers	Not required	None	22.30
Jul‡	Throat swab	Dry cough, fever	Few blisters on fingers	Not required	None	25.37
Aug	Throat swab	Cough, fever (high)	Erythematous rash with tiny white center on face	Hospitalized 4 d	None	21.47
Sep	NPA	Cough	None	Not required	Rhinovirus	26.87
Nov	Throat swab	Cough, wheeze, fever (high), coryzal symptoms	Rash across chest	Hospitalized 5 d	Respiratory syncytial virus	23.45

*C_t, cycle threshold; NPA, nasopharyngeal aspirate; TSPyV, trichodysplasia spinulosa polyomavirus; VZV, varicella zoster virus.
†Collected on the same date.
‡Collected on the same date.

was queried as chickenpox was positive for TSPyV with a low C_t value of 24.97 (Table). Thus, it is conceivable that this rash represented the early papular stages of a trichodysplasia spinulosa lesion that did not progress to the characteristic spicules.

Previously, TSPyV has almost exclusively been associated with pathology of the skin (*4*); but 4 reports indicate its isolation from blood (*6*) and respiratory samples, suggesting a potential transmission route (*5*,*8*–*10*). However, respiratory signs and symptoms were observed only in patients co-infected with another virus. In contrast, the patient we report had persistent respiratory signs and symptoms and concomitant TSPyV-positive (by PCR) respiratory samples in conjunction with varying forms of skin lesion lacking the characteristic spicule form of trichodysplasia spinulosa. However, it is difficult to assess the virus pathogenicity in the absence of any supportive cell culture results. Hence, the potential of TSPyV to cause respiratory signs and symptoms needs further investigation and surveillance. The relatively low C_t values (and thus high viral loads) of TSPyV DNA obtained from this patient in the absence of positive results for any other microbial agents may suggest an etiologic role of the TSPyV in respiratory pathogenesis. The fact that TSPyV skin disease can be effectively treated with antiviral medication, such as cidofovir (*6*), presents potential for treatment of respiratory manifestations of TSPyV infection.

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About the Author

Ms. Bagasi is a PhD student at the University of Nottingham. Her research interests are epidemiology and cellular entry of viral infections.

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Address for correspondence: C. Patrick McClure, University of Nottingham, School of Life Sciences, Queen's Medical Centre, West Block A Floor, Nottingham, NG7 2UH, UK; email: patrick.mcclure@nottingham.ac.uk

Wohlfahrtiimonas chitiniclastica Bacteremia in Hospitalized Homeless Man with Squamous Cell Carcinoma

Yuichi Katanami, Satoshi Kutsuna, Maki Nagashima, Saho Takaya, Kei Yamamoto, Nozomi Takeshita, Kayoko Hayakawa, Yasuyuki Kato, Shuzo Kanagawa, Norio Ohmagari

Author affiliation: National Center for Global Health and Medicine, Tokyo, Japan

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We report a case of *Wohlfahrtiimonas chitiniclastica* bacteremia in an elderly man in Japan who had squamous cell carcinoma. Blood cultures were initially negative for *W. chitiniclastica* but were positive on day 20. Careful attention needs to be paid to this organism in patients who have chronic wounds with maggots.

We report *Wohlfahrtiimonas chitiniclastica* bacteremia in a 75-year-old man in Japan who had squamous cell carcinoma on his shoulder. In September 2016, an unidentified patient was found unconscious on the ground by a passerby and admitted to the emergency department of the National Center for Global Health and Medicine (Tokyo, Japan). He had a necrotic lesion on his left shoulder with maggots. Blood analysis showed leukocytosis (26.61 × 10^9 cells/L [reference range 3.30–8.60 × 10^9 cells /L]), thrombocytosis (626 × 10^9/L [reference range 158–348 × 10^9/L]), anemia (hemoglobin, 9.6 g/dL [reference range 13.7–6.8 g/dL]), and elevated C-reactive protein (87.9 mg/L [reference range 0.00–1.40 mg/L]). Albumin was 2.4 g/dL (reference range 4.1–5.1 g/dL) and calcium was 12.6 mg/dL (reference range 8.8–10.1 mg/dL). He was diagnosed with disturbance of consciousness caused by hypercalcemia and was hospitalized.

After saline infusion and intravenous cefazolin (3 g/d) were initiated, the patient's condition improved. A blood culture taken at the time of admission grew *Peptoniphilus harei*. A swab culture of the ulcer site grew *Proteus mirabilis*, *Morganella morganii*, and *Kerstersia gyiorum*. A biopsy was performed on day 3, and the patient was diagnosed with squamous cell carcinoma. Enhanced computed tomography scanning revealed an ulcer and ring-enhancing lesion on his left shoulder (which was suspected of being a tumor or abscess) and multiple enlarged lymph nodes and 10-mm pulmonary nodules in the right lung.

On day 20, the patient had fever and disturbance of consciousness; therefore, he was transferred to the Infectious Disease department of the hospital. Intravenous therapy with vancomycin (1.5 g/d), cefepime (3 g/d), and metronidazole (1,500 mg/d) was initiated, and the patient's fever and consciousness improved. Two cultures of blood taken on day 20 grew *P. mirabilis*, *M. morganii*, *Streptococcus anginosus*, *Streptococcus agalactiae*, *Bacteroides fragilis*, and gram-negative rods. After we obtained the culture results, vancomycin was stopped in accordance with the susceptibility test results. We identified the gram-negative rods as *W. chitiniclastica* by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonics, Billerica, MA, USA), which showed scores of 2.239. We further confirmed the isolate to be *W. chitiniclastica* by using 16S rRNA sequencing; the isolate was 99.08% identical to strain S5 (GenBank accession no. AM397063). We assessed the isolate's antimicrobial susceptibility profile (Table). The patient improved and was later discharged to another hospital.

W. chitiniclastica is a gram-negative, short, facultative anaerobic, straight-rod gammaproteobacterium that was first isolated from the parasitic fly *Wohlfahrtia magnifica* (*1*). This fly has not been reported in Japan. However, *W. chitiniclastica* has also been isolated from the *Chrysomya megacephala* fly, and this species has been reported in Japan (*2*), and from from the *Musca domestica* housefly, which is widely distributed all over the world (*3*). Campisi et al. reported that the *Lucilia sericata* fly might be a vector for *W. chitiniclastica* (*4*); this fly is common and widely distributed throughout Japan, and a

Table. Antimicrobial susceptibility profile of *Wohlfahrtiimonas chitiniclastica* from a blood culture from a patient treated at the National Center for Global Health and Medicine, Tokyo, Japan, 2016.

Antimicrobial drug	MIC mg/L, susceptibility profile
Piperacillin	≤8, S
Piperacillin/tazobactam	≤8, S
Ceftazidime	≤4, S
Cefepime	≤2, S
Aztreonam	≤4, S
Imipenem/cilastatin	≤1, S
Meropenem	≤1, S
Amikacin	≤8, S
Gentamicin	≤2, S
Tobramycin	≤2, S
Minocycline	≤2, S
Levofloxacin	1, S
Ciprofloxacin	1, S
Trimethoprim/sulfamethoxazole	≤2, S

*S, susceptible.

case of cutaneous myiasis on skin cancer was reported (*5*). Unfortunately, we could not collect maggots from this patient because they were rapidly discarded at the emergency department.

Worldwide, few human cases of *W. chitiniclastica* infection have been documented. *W. chitiniclastica* has been described as a zoonotic pathogen (*6*) and reported from Hungary, Egypt, Niger, Germany, India, France, China, Argentina, Estonia, the United Kingdom, and the United States (*4*,*7*,*8*). Rebaudet et al. (*9*) described the first human case of bacteremia attributable to *W. chitiniclastica*, which occurred in a 60-year-old homeless woman from southeastern France who had a history of alcoholism. Other human cases of *W. chitiniclastica* bacteremia were reported from Argentina (*10*) and the United Kingdom (*4*). Recently, a bacteremia case in a 72-year-old man was reported from Hawaii, USA (*1*).

Risk factors for *W. chitiniclastica* infection are poor personal hygiene, alcoholism, peripheral vascular disease, and chronic open wound (*8*). The patient we describe had a chronic wound because of squamous cell carcinoma, and the associated maggots were thought to be the transmission route. At admission, blood and swab cultures grew polymicrobial isolates without *W. chitiniclastica*, as confirmed by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. However, a blood culture on day 20 was positive for *W. chitiniclastica*. The patient probably was infected with *W. chitiniclastica* during hospitalization. Cases of *W. chitiniclastica* infection (with or without bacteremia) were reported as parts of polymicrobial infections (*1*,*4*,*7*,*8*). *W. chitiniclastica* might have first infected this patient's ring-enhancing lesion as part of a polymicrobial infection. Because *W. chitiniclastica* was undetected in blood and swab cultures at admission, the organism might have entered the bloodstream during hospitalization.

This patient improved after intravenous therapy with cefepime and metronidazole. Previously reported *W. chitiniclastica* bacteremia cases were treated with combination antimicrobial therapies, including cefuroxime plus metronidazole plus clarithromycin (*4*), ceftazidime plus amikacin (*10*), piperacillin–tazobactam plus clindamycin plus vancomycin (*1*), ceftriaxone monotherapy (*9*), and meropenem monotherapy (*1*). Two of the 5 cases were fatal (*1*,*10*).

Clinicians should be attentive to the possibility of *W. chitiniclastica* infection in patients who have chronic wounds with maggots and poor hygiene. Clinical suspicion is warranted even if blood and swab cultures are initially negative for *W. chitiniclastica*.

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About the Author

Dr. Katanami is a medical doctor at the National Center for Global Health and Medicine, Disease Control Prevention Center. His main research interest is tropical infectious diseases.

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Address for correspondence: Yuichi Katanami, National Center for Global Health and Medicine, Disease Control and Prevention Center, 1-21-1, Toyama, Shinjuku, Tokyo 162-8655, Japan; email: yuichi.katanami@gmail.com

Symptomatic Dengue during Pregnancy and Congenital Neurologic Malformations

Enny S. Paixão, Maria Glória Teixeira, Maria da Conceição N. Costa, Mauricio L. Barreto, Laura C. Rodrigues

Author affiliations: London School of Hygiene and Tropical Medicine, London, United Kingdom (E.S. Paixão, L.C. Rodrigues); Instituto de Saúde Coletiva, Salvador-Bahia, Brazil (E.S. Paixão, M.G. Teixeira, M. da Conceição N. Costa, M.L. Barreto); Center of Data and Knowledge Integration for Health (CIDACS), Salvador-Bahia (M.L. Barreto, L.C. Rodrigues)

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Dengue virus infection during pregnancy increased the risk for any neurologic congenital anomaly in the infant by roughly 50% and for other congenital malformations of brain 4-fold. Our results show an association between dengue during pregnancy and congenital anomalies of the brain, suggesting that flaviviruses other than Zika virus are associated with such malformations.

Before the causal relationship between Zika virus and neurologic congenital anomalies (*1*), especially microcephaly (*2*), was established, no evidence associated flavivirus with congenital malformations in humans, although postnatal complications have been described (*3*). We investigated whether dengue virus (DENV) infection during pregnancy could be associated with neurologic defects in the infant at birth.

We conducted a population-based study using routinely collected data from live births and from women who were notified and confirmed to have DENV infection during 2006–2012 in Brazil, before the introduction of Zika virus. We probabilistically linked records of mothers of live births with records of dengue notification to identify women who were reported as having dengue during pregnancy. We excluded records with missing or implausible names, multiple pregnancies, and births in municipalities with no dengue notifications. We obtained ethics approval from Federal University of Bahia, Salvador, Brazil (CAAE: 26797814.7.0000.5030) and from London School of Hygiene and Tropical Medicine (Ethics Ref:10269).

In the matching process, we used name, age, and place of residence of the mother at time of delivery and notification. We included only links and nonlinks with a high degree of certainty. We validated the linkage process in a study that demonstrated 62% sensitivity (*4*).

We used an outcome definition of congenital malformation of the nervous system coded as Q00-Q07 in International Classification of Diseases, 10th Revision (ICD-10). We defined dengue as a confirmed case of DENV infection notified during a pregnancy that resulted in a live birth. We estimated the association between symptomatic dengue during pregnancy and neurologic congenital malformations using the Firth method to reduce the small sample bias in maximum-likelihood estimation.

The study parameters encompassed 16,103,312 live births. Neurologic congenital anomalies are rare; they occurred in 13,634 (0.08%) live births. Dengue during pregnancy increased the odds of a neurologic congenital anomaly by 50% (Table), but this result was not statistically significant (95% CI 0.97–2.27). We split the neurologic congenital defects into ICD-10 categories; the 95% CI around the estimated odds ratios (ORs) was not statistically significant in 7 categories, including microcephaly (OR 1.7, 95% CI 0.33–8.32). Two other types of neurologic congenital anomalies were >4 times more frequent in women who had DENV infection during pregnancy: other congenital malformations of spinal cord (OR 5.4, 95% CI 1.0–26.9) and other congenital malformations of brain (OR 4.5, 95% CI 1.7–11.3, which was statistically significant). We found no sign of space-time clusters or recording errors suggestive of a coding artifact in the 4 records of other congenital malformations of brain wherein the mother had DENV infection (online Technical Appendix Table, https://wwwnc.cdc.gov/EID/article/24/9/17-0361-Techapp1.pdf)

Symptoms of DENV infection occurred in the first trimester in 50% of patients. The specific diagnosis of those among the nonexposed group were congenital malformation of corpus callosum (9%; 81/943), holoprosencephaly (24%; 225/943), and septooptic dysplasia (0.6%; 6/943).

Our study showed an association between DENV infection during pregnancy and congenital anomalies of the

Table. Association between congenital anomalies and symptomatic dengue virus infection during pregnancy, Brazil, 2006–2012

Congenital anomaly	Odds ratio (95% CI)
Neurologic congenital anomalies	1.5 (0.9–2.2)
Anencephaly	1.9 (0.8–4.4)
Encephalocele	1.4 (0.3–6.9)
Microcephaly	1.7 (0.3–8.3)
Congenital hydrocephalus	1.6 (0.8–3.2)
Other congenital malformations of brain	4.5 (1.7–11.3
Spina bifida	0.8
Other congenital malformations of spinal cord	5.4
Other congenital malformations of nervous system	Not available

brain. Congenital anomalies of the brain detectable by routine examination at birth are so rare (6/100,000 live births by our data) that it was necessary to assemble a cohort of >16 million live births to detect an effect of dengue; even then, we did not have sufficient power to confidently exclude associations with other neurologic abnormalities.

Because DENV infection had not been associated with congenital anomalies, there is no established biologic mechanism for its teratogenicity. However, there is evidence for postnatal neurotropism and virus isolation from brain tissue (*5*) and for dengue virus crossing the blood–brain and placental barriers (*6*,*7*). The pattern of anomalies we described has similarities with congenital Zika syndrome. Brain images and autopsies from infants with Zika and other infectious diseases have revealed abnormalities similar to those we described (*8*,*9*).

Our study has limitations inherent to the linkage process. Rigorous evaluation of the linkage process showed that it is unlikely to introduce bias and that it did not affect the magnitude of the association (*4*). Another potential limitation was diagnosis of DENV infection. In notifiable epidemics, not all cases are tested after the cause is established. DENV infection in Brazil is notified for the presence of clinical criteria, laboratory confirmation, or both. Only ≈30% of notified DENV infections are laboratory confirmed, which could lead to bias if unconfirmed cases are not dengue. However, a previous article found no difference in pregnancy outcomes for women with notified DENV infection with and without laboratory confirmation (*10*). We did not control for potential confounders, so confounders such as maternal illness or environmental exposures may have contributed to the association between dengue infection and neurologic malformations.

The association of symptomatic dengue during pregnancy and congenital anomalies of the brain in the infant, while not as high frequency as the linkage with Zika, opens the possibility of other flaviviruses causing congenital malformations and raises questions about policy implications. We recommend careful observation and recording of DENV infection in antenatal records and full investigation of live births with neurologic malformations, as well as animal and in vitro research of teratogenic effects of dengue.

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Authors' contributions: E.S.P. carried out the analysis and wrote the first draft of the article. L.C.R. and M.G.T. conceived the study. M.da C.N.C. and M.L.B. contributed to the study design and interpretation. All authors revised the manuscript and approved the final version.

About the Author

Ms. Paixão is a PhD student at London School of Hygiene and Tropical Medicine. Her main research interests are congenital infections and use of routinely collected data.

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Address for correspondence: Enny S. Paixão, London School of Hygiene and Tropical Medicine, Keppel St, London WC1E 7HT, UK; email: enny.cruz@lshtm.ac.uk

Travelers' Actual and Subjective Knowledge about Risk for Ebola Virus Disease

Isabelle Régner, Oana Elena Ianos, Loucy Shajrawi, Philippe Brouqui, Philippe Gautret

Author affiliation: Aix-Marseille Université, Marseille, France (I. Régner); Méditerranée Infection Institute, Marseille (O.E. Ianos, L. Shajrawi, P. Brouqui, P. Gautret)

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To determine travelers' actual and subjective knowledge about risk for Ebola virus disease, we surveyed travelers from France. Actual knowledge did not prevent irrational perceptions or promote safe behavior. Rather, readiness to adopt protective behavior depended on subjective knowledge and overconfidence in ability to self-protect.

The 2014–2016 epidemic of Ebola virus disease (EVD) in West Africa was the largest ever recorded. As for many other infectious diseases (*1*,*2*), surveys of knowledge, attitudes, and practices report suboptimal knowledge and misperceptions of risk for EVD among various populations (*3*–*6*). Recommendations typically emphasize the need to increase actual knowledge (what persons really know) to reduce irrational beliefs and risky behavior. However, subjective knowledge (what persons think they know), which has been overlooked in EVD surveys, can lead to the erroneous feeling that one has the requisite knowledge to avoid adverse events, resulting in a higher risk of experiencing negative outcomes (*7*). To determine if actual and subjective knowledge about EVD would lead to differing perceptions of risk, we surveyed travelers from France who had visited the International Vaccination Center at North Hospital in Marseille, France, for pretravel consultation during May 2015–February 2016.

A sample of 189 participants (93 women, 96 men; mean age ± SD 37.78 ± 14.50 years) anonymously completed a questionnaire about their knowledge and perceptions of risk of acquiring EVD. Respondents reported their sociodemographic characteristics, destination, purpose of travel, date of departure, and date of return. Questions about EVD actual knowledge included preventive measures, transmission routes, epidemic status, affected countries, and presence of EVD in the destination country. We used correct responses to compute final scores (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/24/9/17-1343-Techapp1.pdf). We used 5-point Likert scales (1 = strongly disagree to 5 = strongly agree) to record travelers' self-reports pertaining to their subjective knowledge (*7*) and several risk perceptions about EVD (*6*,*8*,*9*): perceived seriousness of EVD, awareness of EVD risk in the destination country, perceived effectiveness of protective measures, fear of contracting EVD in the country of destination, fear of contracting EVD in Europe, and intentions to adopt preventive behavior. Personal control and unrealistic optimism were assessed as key measures of positive illusions that typically lead persons to overestimate their capabilities to protect themselves against adverse events (*8*,*9*) (online Technical Appendix).

Among the 189 participants, 25.9% planned to travel to West Africa (2.6% to an affected country, Guinea), 21.7% to other African countries, and 52.4% to other countries worldwide. Only 10.6% were able to correctly report the 3 countries affected by the EVD epidemic (Liberia, Sierra Leone, Guinea), and many were unaware of preventive measures (45%) and modes of Ebola virus transmission (39.1%). The most frequent answers for preventive measures were practice careful hygiene (24.34%), avoid contact with infected persons (23.28%), and wear protective equipment (21.16%). Answers about modes of Ebola virus transmission were body contact (31.22%), body fluids (30.16%), and aerosol (12.17%; this answer is wrong). Overall, the actual knowledge about EVD was very low (mean 3.57 correct responses; maximum possible score = 16). Simultaneously, subjective knowledge was low (mean ± SD 2.39 ± 1.00; maximum possible score = 5.00) (online Technical Appendix Table 3 for bivariate intercorrelations).

To go beyond bivariate correlations and to estimate the associations between risk perceptions and each type of knowledge, we used multiple regression analyses (Table). Findings showed that actual knowledge was far from being as effective, as typically thought from knowledge,

Table. Results of multiple regression analyses for variables predicting actual and subjective knowledge of risk for Ebola virus disease*

	Actual knowledge		Subjective knowledge	
Risk perception variable	*b*	95% CI	*b*	95% CI
Perceived seriousness	0.12 (p<0.001)	0.05 to 0.20	0.08	–0.08 to 0.24
Risk awareness	–0.19 (p<0.001)	–0.26 to –0.11	–0.16 (p<0.05)	–0.33 to –0.01
Perceived effectiveness of protective measures	0.04	–0.02 to 0.10	0.22 p<0.01)	0.09 to 0.35
Positive illusions	–0.07	–0.14 to 0.01	0.16 (p<0.05)	0.01 to 0.33
Fear of contracting EVD in destination country	0.03	–0.07 to 0.12	0.02	–0.19 to 0.22
Fear of contracting EVD in Europe	–0.01	–0.09 to 0.07	-0.09	–0.26 to 0.08
Behavioral intention	0.04	–0.03 to 0.11	0.16 (p<0.05)	0.01 to 0.32
% variance explained by the model	Adj R^2 = 0.32 (p<0.001)		Adj R^2 = 0.21 (p<0.001)	

*All regression coefficients are unstandardized coefficients that were adjusted for participants' destination (Africa vs. other countries). Adj, adjusted; *b*, unstandardized regression coefficients; EVD, Ebola virus disease.

attitudes, and practices studies (*3*–*5*). Actual knowledge was associated only with higher perceived seriousness of the disease and lower awareness of risk for EVD in the country of destination, which reflects some rational perceptions (EVD is indeed serious, and most destination countries for this sample population were not affected by the epidemic). However, travelers with greater actual knowledge were not more likely to view protective measures as efficient, to avoid positive illusions, or to intend to engage in protective behavior. On the contrary, travelers with higher subjective knowledge reported confidence in preventive measures and intention to adopt safe behavior, while indicating illusions of having personal control and unrealistic optimism. Results of a further analysis (online Technical Appendix) revealed that positive illusions and subjective knowledge were positively associated with behavioral intentions.

Our observations of suboptimal actual knowledge about EVD replicated findings of past knowledge, attitudes, and practices studies (*3*–*6*); however, we went further by showing that relationships between actual versus subjective knowledge and perceptions of risk for EVD differed. The fact that subjective knowledge and positive illusions, but not actual knowledge, were associated with protective behavior intentions is problematic, especially because actual knowledge was low. Persons' belief that they know how to protect themselves when they actually do not and the feeling of knowing added to a feeling of overconfidence in how to self-protect might result in risky rather than safe behavior (*7*).

Our results indicate that not considering subjective knowledge and positive illusions can lead to the erroneous conclusion that increasing actual knowledge will necessarily translate into behavioral change and good practices. EVD communication would benefit from research showing that promoting behavioral change requires changing subjective evaluations of risk to make it self-relevant and to induce a reappraisal of the perceived benefits of (or costs of not) performing safe behavior (*10*).

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About the Author

Dr. Régner is assistant professor at the Cognitive Psychology Laboratory and head of the Centre of Social Sciences at the Faculty of Sciences, Aix-Marseille Université, France. Her research interests include social cognition and risk perceptions.

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Address for correspondence: Isabelle Régner, Aix-Marseille Université, CNRS, LPC, Site Saint-Charles, Case D, 3 Place Victor Hugo, 13331 Marseille CEDEX 3, France; email: isabelle.regner@univ-amu.fr

Spread of *mcr-1*–Driven Colistin Resistance on Hospital Surfaces, Italy

Elisabetta Caselli, Maria D'Accolti, Irene Soffritti, Micol Piffanelli, Sante Mazzacane

Author affiliation: Università Degli Studi di Ferrara, Ferrara, Italy

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Plasmid-mediated colistin resistance driven by the *mcr-1* gene is of great clinical concern. Its diffusion in the hospital environment is unknown. We detected *mcr-1*–driven resistance in 8.3% of *Enterobacteriaceae* isolates from hospital surfaces in Italy, which might represent a reservoir of threatening nosocomial pathogens.

The rapid and continuous growth of drug resistances is of global concern and one of the most severe threats for human health. Among those detected in recent years, a plasmid-mediated colistin resistance, driven by the *mcr-1* gene (*1*), represents a serious clinical concern because colistin was considered a last-resort drug against multidrug-resistant (MDR) gram-negative bacteria.

Since its original isolation in an *Escherichia coli* strain in China in 2016, the *mcr-1* gene has been detected almost globally in ≈10% of animal isolates (*2*) and in 0.1%–2% of human isolates (*3*), suggesting that this plasmid-mediated resistance spread efficiently from animals (where colistin has been used for years as a therapeutic drug or food supplement) to humans through horizontal gene transfer. Furthermore, the *mcr-1* gene was found in different gram-negative bacteria, including *Klebsiella pneumoniae*, *Enterobacter*, *Salmonella* (*1*,*4*,*5*), and recently *Citrobacter* (*6*). The emergence of *mcr-1* in clinical *Enterobacteriaceae* isolates appears particularly alarming because it frequently occurs in MDR strains, further limiting current treatment options for lethal infections sustained by carbapenem-resistant *Enterobacteriaceae*.

In Italy, *mcr-1*–driven colistin resistance was first reported in an *E. coli* strain in 2016 (*7*). However, colistin resistance already had been reported previously in carbapenem-resistant *Enterobacteriaceae* from different peripheral laboratories in Italy (*8*).

The *mcr-1* gene has been detected in infected persons, but its epidemiology is poorly described, and data are lacking about its presence in the microbial population that persistently contaminates hospital environments. Surface contamination is known to contribute to the onset of healthcare-associated infections, which are often sustained by MDR or even pan–drug-resistant strains. Thus, based on the need for information about this aspect, we aimed to determine the diffusion of *mcr-1*–driven colistin resistance in the hospital environment.

We searched for the presence of *mcr-1* gene in our library of 300 *Enterobacteriaceae* samples collected from the surfaces of 8 hospitals in Italy during 2016–2017. Surface samples were collected from 3 points in hospital rooms (floor, bed footboard, and sink) as previously described (*9*), then grown in MacConkey broth for 48 h at 37°C to amplify the *Enterobacteriaceae* population. An aliquot of grown bacteria was frozen in 50% sterile glycerol for subsequent identification and functional studies. The remaining bacterial suspension was used for total DNA extraction (UCP-Pathogen Mini Kit; QIAGEN, Hilden, Germany) and analyzed for *mcr-1* gene presence by nested PCR. We conducted first-round amplifications as previously described (*1*); nested PCR amplification was carried out using the following primers and conditions: CLRn-F (5′-AAA CCT ATC CCA TCG CGG AC-3′) and CLRn-R (5′-CCG CGC CCA TGA TTA ATA GC-3′), for 35 cycles at 57°C, originating a 147-bp amplification product, subsequently confirmed by sequence analysis. Plasmid pBAD24::mcr-1 (*3*) was used as a positive control. We also conducted a universal panbacterial PCR as a control of DNA amplification (*9*). Whole-genome sequence and *mcr-1* location were not analyzed here and might deserve future study.

Of 300 *Enterobacteriaceae* isolated from hospital surfaces, 25 (8.3%) harbored the *mcr-1* gene. All positive samples were culturally isolated on MacConkey agar plates.

Table. Antimicrobial susceptibility of the *mcr-1*–carrying bacterial isolates from hospital surfaces, Italy*

Bacteria	No. isolates	Drug-resistant isolates, % (MIC, mg/L)								
		F	AK	ATM	TZP	C	SXT	NET	CTX	Col-R
Acinetobacter lwoffii	4	50	25	50	50	25	50	25	25	7 (4–8)
Citrobacter freundii	1	0	0	0	0	100	0	0	0	4
Enterobacter cloacae	3	100	100	33.3	100	33.3	33.3	100	33.3	16
Enterobacter agglomerans	3	100	0	0	100	0	0	100	0	5.3 (4–8)
Escherichia coli	4	100	50	25	100	0	25	100	50	10 (8–16)
Klebsiella pneumoniae	6	100	100	33.3	66.6	66.6	0	100	66.6	13.3 (8–16)
K. oxytoca	2	100	100	0	0	0	0	100	0	16
Pseudomonas aeruginosa	1	0	100	0	100	100	0	100	100	4
P. putida	1	0	100	0	0	100	0	100	0	8

*AK, amikacin 30 μg; ATM, aztreonam 30 μg; C, chloramphenicol 30 μg; Col-R, colistin resistant; CTX, cefotaxime 5 μg; F, nitrofurantoin 100 μg; NET, netilmicin 10 μg; SXT, trimethoprim/sulfamethoxazole 25 μg; TZP, piperacillin/tazobactam 36 μg.

We identified presumptive positive isolates at the species level by biochemical typization (API-20E) and Vitek-2 system (BioMérieux, Florence, Italy) and tested them for drug susceptibility by disc diffusion (Entero1 Multodisc; Liofilchem,Teramo, Italy) and broth microdilution (SensiTest Colistin, Liofilchem).

Identification results indicated that different species harbored the *mcr-1* gene, including *K. pneumoniae*, *K. oxytoca*, *E. coli*, *Acinetobacter Iwoffii*, *Enterobacter cloacae*, *E. agglomerans*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, and *P. putida* (Table). These results suggest that this gene is silently spreading to many gram-negative bacteria responsible for infections in clinical settings.

All *mcr-1*–carrying isolates were colistin resistant by microdilution test (MIC 4 mg/L to >16 mg/L). In addition, as judged by the results obtained by the disc-diffusion method, all colistin-resistant isolates were resistant to ≥2 antimicrobial drugs among those effective against *Enterobacteriaceae*, exhibiting a MDR phenotype.

Our data show that *mcr-1*–carrying *Enterobacteriaceae* can be detected on hospital surfaces with higher frequency than in clinical isolates, indicating that this plasmid has the ability to spread, not only in vitro (*1*), in key human pathogens. Persistent surface contamination in hospitals might thus favor colistin resistance spread among gram-negative bacteria, perhaps helped by selective pressure exerted by some antiseptics (i.e., chlorhexidine) (*10*). Although this finding might represent a potential reservoir of threatening nosocomial pathogens and favor their diffusion in hospitalized patients, currently no specific monitoring exists to control it. Thus, we suggest that surveillance for *mcr-1*–driven colistin resistance might include not only clinical samples but also environmental analyses and all clinically relevant gram-negative species to control and counteract the increase of untreatable infections.

About the Author

Dr. Caselli is a professor of microbiology and clinical microbiology at the Faculty of Medicine of the University of Ferrara. Her research interests include infectious diseases, microbiology, virology, molecular virology, antiviral immune response, autoimmunity, and healthcare-associated infections.

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Address for correspondence: Elisabetta Caselli, Universita Degli Studi di Ferrara Dipartimento di Scienze Mediche, Department of Medical Sciences, Section of Microbiology, via Borsari 46 Ferrara, Ferrara 44121 Italy; email: csb@unife.it

Transverse Myelitis and Guillain-Barré Syndrome Associated with Cat-Scratch Disease, Texas, USA, 2011

Ramia Zakhour, Pedro Mancias, Gloria Heresi, Norma Pérez

Author affiliations: American University of Beirut, Beirut, Lebanon (R. Zakhour); University of Texas UT Health, Houston, Texas, USA (R. Zakhour, P. Mancias, G. Heresi, N. Pérez)

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We describe a case of coexisting transverse myelitis and Guillain-Barré syndrome related to infection with *Bartonella henselae* proteobacterium and review similar serology-proven cases. *B. henselae* infection might be emerging as a cause of myelitis and Guillain-Barré syndrome and should be considered as an etiologic factor in patients with such clinical presentations.

A child with lower extremity weakness raises an increasingly complex diagnostic challenge; frequently no etiology is identified (*1*,*2*). We present a case of lower extremity weakness linked to cat-scratch disease (CSD, causative agent *Bartonella henselae* proteobacterium).

In 2011, a 10-year-old girl was transferred to our hospital (UT Health, Houston, Texas, USA) from another hospital, where she had been treated for 2 days for abdominal pain, vomiting, and urinary retention. Seven days before admission to UT Health, she had a left cervical lymphadenopathy. During hospitalization, the patient had urinary retention; lower extremity weakness; worsening headache; neck pain; lower back pain; and a bilateral burning sensation in the wrists, knees, ankles, and feet.

Before her illness, she was healthy and fully immunized; her exposure history only included a cat at home that frequently bit and scratched her. Physical examination revealed a palpable lymph node (3 × 4 cm) at the left cervical lymph node, lower extremity strength of 4 on a 5-point scale (https://www.ncbi.nlm.nih.gov/books/NBK436008/), and decreased deep tendon reflexes. She reported hyperalgesia in her legs.

Peripheral blood cell counts and chemistry test values were within reference ranges. Alanine aminotransferase and aspartate aminotransferase were both mildly elevated (48 U/L [0.8 μkat/L]). A magnetic resonance image (MRI) of the brain showed a focus of increased T2 signal, and an MRI of the spine showed a long centrally located segment of increased T2 signal (Figure). Cerebrospinal fluid (CSF) studies showed a leukocyte concentration of 58 cells/mm^3 (reference range ≤10 cells/mm^3), glucose of 46 mg/dL (nonfasting reference range 45–100 mg/dL), and protein of 55 mg/dL (reference range 15–45 mg/dL). We gave the patient a diagnosis of myelitis and treated her empirically with ceftriaxone and vancomycin, pending CSF culture results. On day 11 of illness, we started administering rifampin and doxycycline for a possible CSD diagnosis; the patient was positive for *B. henselae* IgG (1:152) and IgM (1:160). Increases in *B. henselae* IgG and decreases in *B. henselae* IgM were seen with subsequent serologic tests: day 27 (IgG 1:256, IgM 1:40) and day 41 (IgG 1:512, IgM 1:20). Evaluation for other etiologies included bacteria culture with urine, blood, and CSF samples; CSF latex agglutination for bacterial antigen; virus culture with nasal washes; rapid plasma reagin test; CSF venereal disease research laboratory testing; enterovirus, herpes simplex virus, and mycobacteria PCR of CSF sample; Epstein-Barr virus and

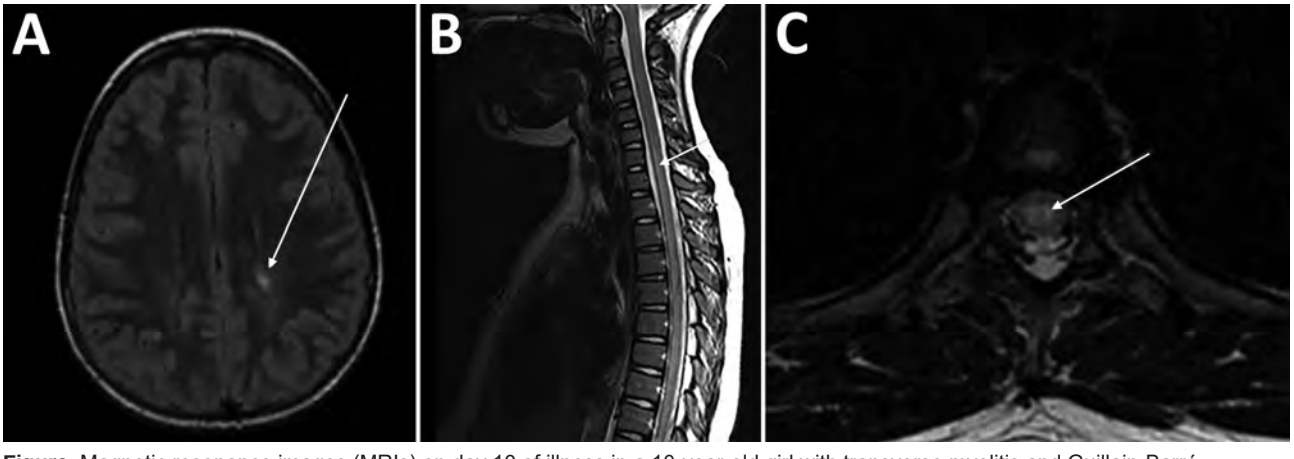

Figure. Magnetic resonance images (MRIs) on day 10 of illness in a 10-year-old girl with transverse myelitis and Guillain-Barré syndrome associated with cat-scratch disease, Houston, Texas, USA, 2011. A) Brain MRI. Arrow indicates focus of increased T2 signal in the left posterior periventricular and deep white matter. B) Sagittal spine MRI. Arrow indicates long segment of increased T2 signal centrally located within the spinal cord. C) Axial thoracic spine MRI. Arrow indicates increased central signal within the spinal cord.

cytomegalovirus PCR of serum samples; and *Mycoplasma pneumoniae*, West Nile virus, *Borrelia burgdorferi*, and human T-cell lymphotropic virus I and II antibody testing, all of which were negative for evidence of the respective microbial agents. CSF angiotensin-converting enzyme and IgG levels were in reference ranges. CSF myelin basic protein level (6.4 ng/mL [reference range ≤1.1 ng/mL]) was elevated. Vitamin B12 and folate levels were in reference ranges, and antinuclear antibody, rheumatoid factor antibody, and dsDNA antibodies were absent.

The patient completed a 14-day course of doxycycline; rifampin was discontinued after 5 days because of rising liver enzyme levels. By day 34 of illness, the patient's muscle strength substantially improved, but she continued to have difficulty voiding and severe lower extremity pain. A repeat MRI showed resolution of the increased thoracic spinal cord signal and a new enhancement of the cauda equina nerve roots. Repeat lumbar puncture indicated a leukocyte concentration of 12 cells/mm^3 (85% lymphocytes [reference range 62% ± 34%]). Nerve conduction studies revealed patchy mixed demyelinating axonal motor and sensory neuropathy. After intravenous immunoglobulin administration for possible Guillain-Barré syndrome (GBS), she showed tremendous improvement, with resolution of urinary retention and a substantial decrease in pain and weakness; 4 months later, she had only residual sensory deficits.

B. henselae proteobacterium is transmitted to humans typically through cat scratches or bites (*3*). Neurologic complications, usually self-limited, develop in 0.2%–3.0% of CSD patients (*4*). The first case of CSD with neurologic manifestations was described in 1952. By 1971, ≈40 cases had been reported (*5*), 90% involving encephalitis and a few myelopathy (*6*). The cases of myelopathy had slower recovery courses than those of encephalitis, as well as more residual deficits.

Four other serology-documented CSD-associated myelitis cases (*3*,*4*,*7*) and 1 other GBS-associated *B. henselae* infection (in a 10-year-old girl) (*8*) have been described. Carman et al. reported a case similar to the one we describe: myelitis and GBS in a 12-year-old boy (*9*).

Studies of the efficacy of treatments for CSD-associated neurologic manifestations are lacking, and thus, the optimal regimen and duration of therapy are unknown. However, we suggest that clinicians consider CSD early in disease courses involving neurologic complications; the possibility of GBS, myelitis, or both in the setting of possible CSD should prompt clinicians to initiate antimicrobial treatment early and consider steroid or intravenous immunoglobulin therapy to prevent progression of disease.

This patient had an unusual presentation of CSD, with evidence of myelitis, brain lesions, and peripheral nerve involvement. Although few cases of CSD-associated transverse myelitis and GBS have been described, clinicians should be aware of the existence of this clinical scenario and include it as a differential diagnosis for these 2 syndromes in the pediatric age group.

About the Author

Dr. Zakhour is an assistant professor at the American University of Beirut in Beirut, Lebanon. She has a special interest in immune complications of infections.

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Address for correspondence: Ramia Zakhour, American University of Beirut Medical Center, Department of Pediatrics and Adolescent Medicine, PO Box 11-0236, Riad El Solh 1107, 2020, Beirut, Lebanon; email: rz17@aub.edu.lb

Co-Circulation of 4 Dengue Virus Serotypes among Travelers Entering China from Myanmar, 2017

Binghui Wang,[1] Yuebo Liang,[1] Shuting Yang, Yirong Du, Li-Na Xiong, Ting Zhao, Fang Yang, Weihong Qin, Xueshan Xia

Author affiliations: Kunming University of Science and Technology, Kunming, China (B. Wang, S. Yang, X. Xia); Care Center for International Travel Health in Yunnan, Kunming (Y. Liang, W. Qin); Ruili Entry-Exit Inspection and Quarantine Authority, Ruili, China (Y. Du, L.-N. Xiong, F. Yang); First People's Hospital of Yunnan Province, Kunming (T. Zhao)

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We report 301 dengue virus infections among cross-border travelers entering Yunnan Province, China, from Myanmar during 2017. Phylogenetic analysis of 99 strains found all 4 serotypes co-circulating; genetic characteristics have also changed. This finding highlights the urgent need for monitoring dengue virus cross-border transmission as early warning of severe dengue fever.

Dengue virus (DENV) infection is one of the most serious threats to public health in tropical and subtropical regions worldwide (*1*). Southeast Asia is the most seriously affected region, with explosive outbreaks occurring frequently. In 2013, a small-scale DENV outbreak occurred in Yunnan, the southeasternmost province in China, as a result of imported infection from neighboring Southeast Asia countries (*2*,*3*). Ruili County is located in southwest Yunnan, bordering Myanmar on 3 sides. In this county, cross-border DENV transmission and endemic disease have become a serious challenge in the past decade.

In 2017, a total of 8.3 million cross-border travelers who entered Yunnan Province through Ruili had their body temperature measured using an intelligent infrared human body temperature measurement system. Persons with a temperature >37°C were suspected to be infected with DENV; infection was confirmed by NS1 antigen detection (*4*). In total, 1,667 travelers were screened for DENV infection because of fever symptoms. Of these, 301 were confirmed to be DENV infected. The DENV-infected travelers comprised 196 citizens of Myanmar and 105 citizens of China; median age was 27 (range 1–71) years, and male/female ratio was 1.15:1 (161:140). The occurrence of DENV infection was concentrated during August–November; 196 infections were detected in this period, accounting for 65.1% of DENV cases in 2017.

To further describe the genetic characteristics of these DENV strains, we selected 100 DENV-positive plasma samples for E gene amplification followed by phylogenetic analysis (*2*). All participants were informed and provided written consent before sample collection. This research was approved by the Institutional Ethical Committee of Kunming University of Science and Technology. We randomly enrolled 10 cases from each month; if there were <10 cases in 1 month, we included all cases from that month. As a result, 99 samples were successfully amplified; 1 sample failed, possibly because of low viral load. We identified all 4 DENV serotypes in this population, although DENV-3 (4 cases) and DENV-4 (17 cases) had previously been undiscovered in Yunnan Province (*2*). DENV-1, the dominant serotype in 2013, continued to be the most prevalent serotype in 2017 (77 cases), whereas only 1 case of DENV-2 was detected. We found no significant difference in serotype distribution based on sampling time.

We randomly selected 40 DENV-1 samples, 1 DENV-2 sample, 4 DENV-3 samples, and 17 DENV-4 samples for sequencing and phylogenetic analysis; we submitted the resulting sequences to GenBank (accession nos. MG933806–MG933867). Phylogenetically, all DENV-1 strains of the cross-border travelers in 2017 were classified as genotype I (Figure), similar to earlier reports (*2*). Specifically, most strains were closely related to the strains identified in 2013 in Dehong Prefecture and its neighboring country, Thailand (Figure), which suggests the prolonged circulation of 2013 strains or stable importing from neighboring countries. The only DENV-2 strain (D2-0022) was classified as the Asian I genotype, forming a close cluster with 2013 strains and reference sequences from Myanmar and Thailand and strains previously circulated in Yunnan Province. The DENV-3 strains detected in Ruili were classified as genotype I and genotype III, which is substantially different from the genotype II strains identified during the DENV outbreak in the southern prefecture of Xishuangbanna in 2013 (Figure) (*2*). DENV-4 genotype II was once reported as a circulating serotype in Yunnan Province in 2015 but is now a long-term epidemic in Myanmar and Thailand.

Among the travelers entering Yunnan Province from Myanmar in 2017, dengue infections showed not only inherited characteristics of previous epidemic DENV-1 and DENV-2 but also the circulation of additional serotypes and genotypes (DENV-3 genotypes I and III, DENV-4 genotype II). This importation of all serotypes of DENV may result in simultaneous or sequential epidemics of the local population in Yunnan Province. Co-circulation of the 4 serotypes, considered a key indicator of progression toward hyperendemic transmission (*5*,*6*), led to an alert for a threatening DENV pandemic. Our findings also revealed

[1]These authors contributed equally to this article.

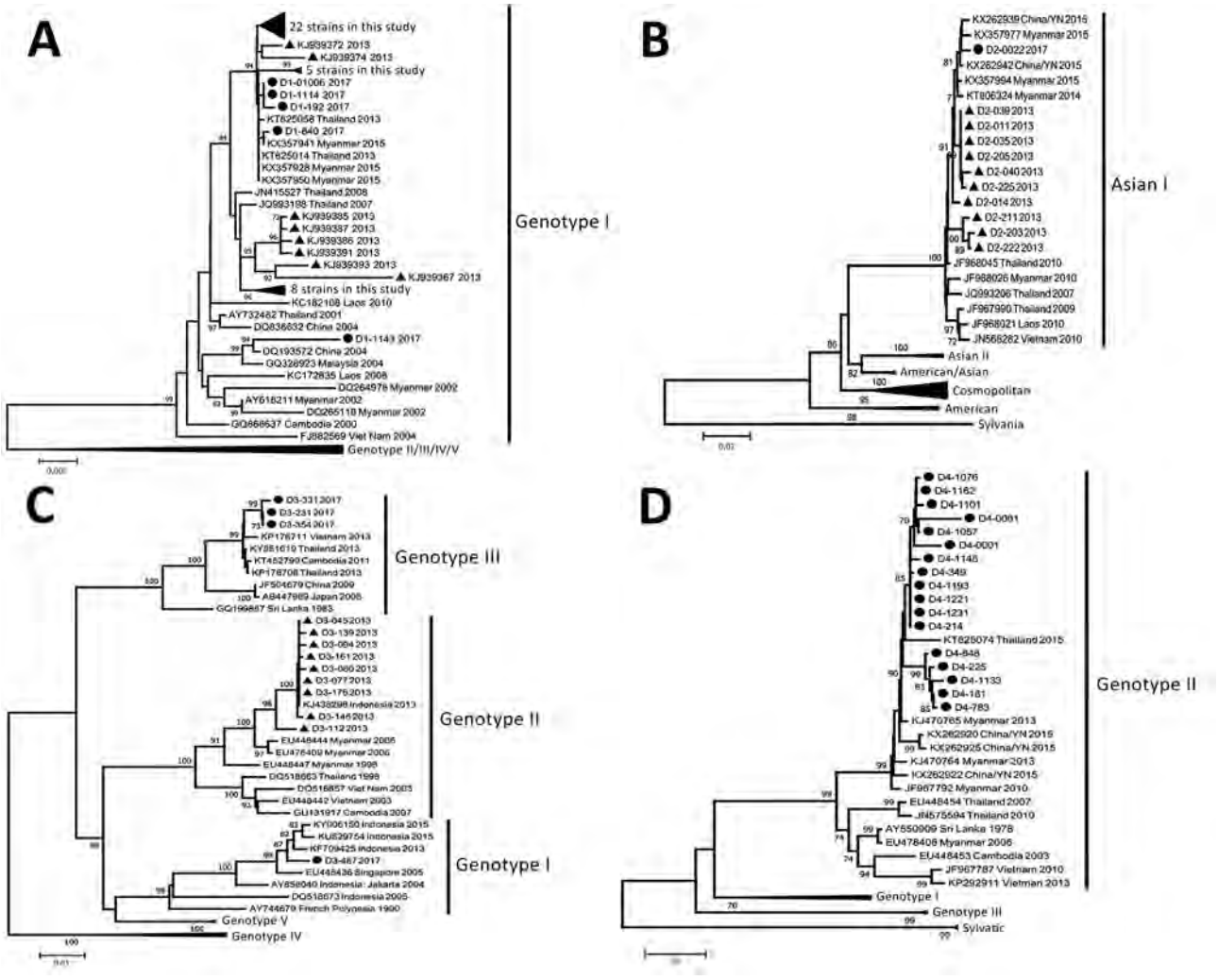

Figure. Phylogenetic tree of DENV serotypes identified in cross-border travelers entering Yunnan Province, China, from Myanmar during 2017. A) DENV-1; B) DENV-2; C) DENV-3; D) DENV-4. The phylogenetic trees were constructed by the maximum-likelihood method with a Kimura 2 parameter model using MEGA 7.0 software (https://www.megasoftware.net). Bootstrap values were set for 1,000 repetitions. Black dots denote strains from this study, and black triangles denote strains from our previous study (*2*,*3*). GenBank accession numbers for comparison isolates are provided. Scale bars indicate nucleotide substitutions per site. DENV, dengue virus.

the continued changing of DENV genetic characteristics in this float population (*5*,*7*,*8*), developed from fewer genotypes/serotypes to the co-circulation of multifarious serotypes/genotypes, from sporadic imported infection to more local infection. In the past decade, or at least since 2013, local DENV infection has occurred in border regions of Yunnan Province. Thus, DENV undoubtedly also exists in local mosquitoes. In the near future, we plan to investigate DENV infection in mosquitoes and perform genetic characterization of those strains from mosquitoes.

The cross-border population serves as a major vector for transmission of pathogens into China from neighboring countries in Southeast Asia. Because of the large number of DENV infection cases in Yunnan Province and neighboring countries, our research clearly demonstrates that surveillance and control of dengue virus is a difficult task, and south China is under the risk for an increase in dengue infections.

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About the Author

Dr. Wang is a virology researcher at Kunming University of Science and Technology. His research interests are molecular epidemiology of infectious disease, focusing especially on human immunodeficiency virus, hepatitis viruses, and dengue virus.

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Address for correspondence: Xueshan Xia, Kunming University of Science and Technology Faculty of Life Science and Technology, No 727 Jing Ming Rd, Chenggong District, Kunming City, Yunnan Province, Kunming 650500, China; email: oliverxia2000@aliyun.com; Weihong Qin, Health Care Center for International Travel in Yunnan, Kunming, Yunnan, China; email: qinwh19@sina.com

Case of Microcephaly after Congenital Infection with Asian Lineage Zika Virus, Thailand

Thidathip Wongsurawat,[1] Niracha Athipanyasilp,[1] Piroon Jenjaroenpun, Se-Ran Jun, Bualan Kaewnapan, Trudy M. Wassenaar, Nattawat Leelahakorn, Nasikarn Angkasekwinai, Wannee Kantakamalakul, David W. Ussery, Ruengpung Sutthent, Intawat Nookaew,[2] Navin Horthongkham[2]

Author affiliations: University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA (T. Wongsurawat, P. Jenjaroenpun, S.-R. Jun, D.W. Ussery, I. Nookaew); Siriraj Hospital, Mahidol University, Bangkok, Thailand (N. Athipanyasilp, B. Kaewnapan, N. Leelahakorn, N. Angkasekwinai, W. Kantakamalakul, R. Sutthent, N. Horthongkham); Molecular Microbiology and Genomics Consultants, Zotzenheim, Germany (T.M. Wassenaar)

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We sequenced the virus genomes from 3 pregnant women in Thailand with Zika virus diagnoses. All had infections with the Asian lineage. The woman infected at gestational week 9, and not those infected at weeks 20 and 24, had a fetus with microcephaly. Asian lineage Zika viruses can cause microcephaly.

Although Zika virus has circulated in Asia longer than in the Americas, only 3 confirmed cases of congenital Zika virus infection with microcephaly have been reported in Asia (2 Thailand, 1 Vietnam) (*1*). As of June 2018, the genomic sequences of the viruses from these 3 cases have not been reported; thus, whether these cases were caused by an Asian lineage or an imported American lineage is unknown.

Several mechanisms involving virus genome sequences have been proposed to explain how Zika virus might cause microcephaly (*2*). Liang et al. (*3*) showed in vitro that replication of both the African (strains MR766 and IbH30656) and American (strain H/PF/2013) lineage viruses suppress Akt phosphorylation; this suppression is caused by an accumulation of mutations in the Zika virus genome that increase the number of phosphorylation sites on virus proteins that compete with host proteins for phosphorylation. Yuan et al. proposed that a serine to asparagine substitution (S17N) in the premembrane protein (stably conserved in the American lineage but not in the

[1]These first authors contributed equally to this article.

[2]These senior authors contributed equally to this article.

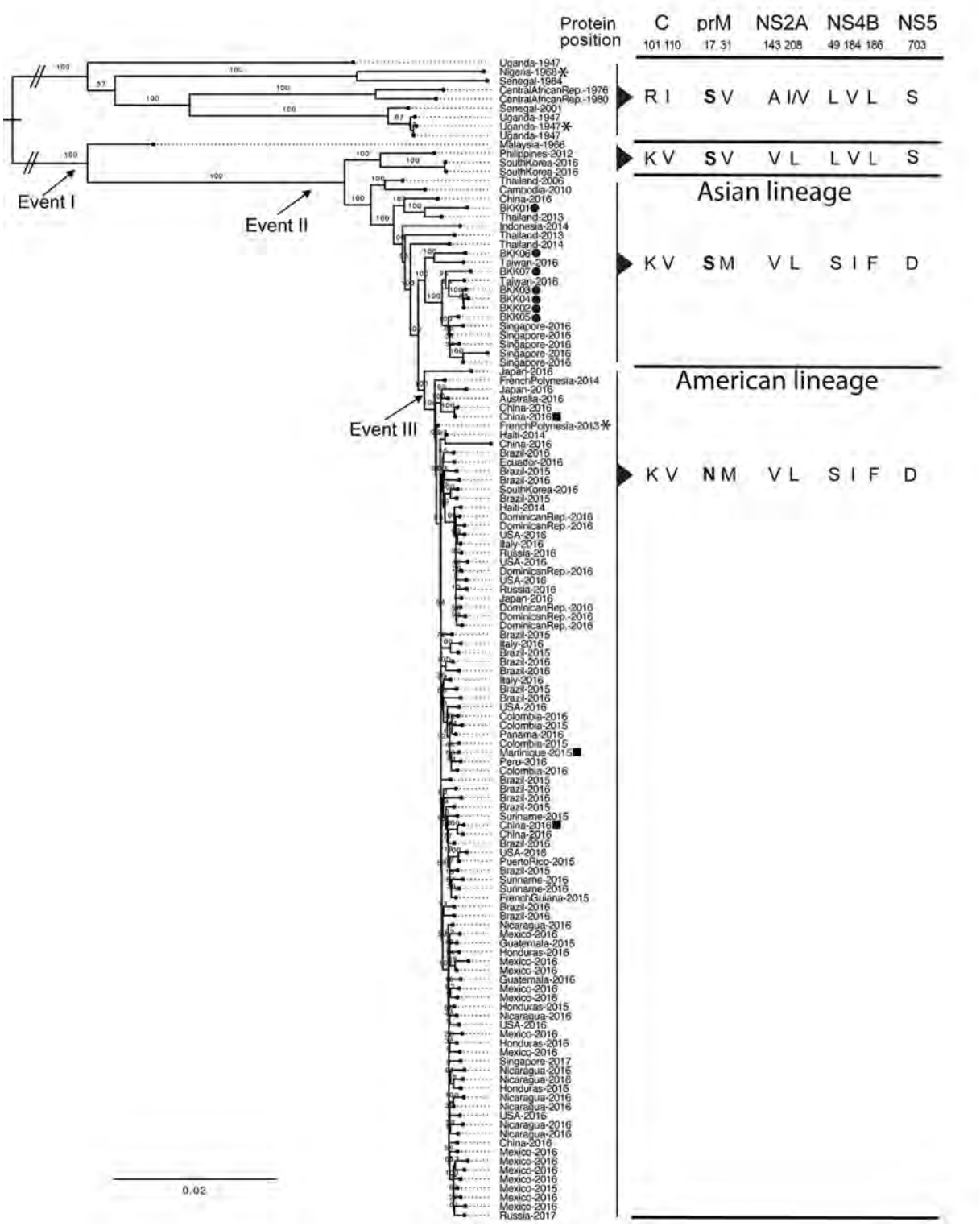

Figure. Maximum-likelihood phylogenetic analysis of nonredundant Zika virus genomes including 7 isolates from patients in Thailand, 2016–2017, and amino acid changes corresponding with 3 evolutionary events (*2*). Circles indicate the Zika virus isolates from this report; the Zika virus strains used by Liang et al. (*3*) are indicated by asterisks and Yuan et al. (*4*) by squares. The key amino acid residue changes corresponding with the 3 evolutionary events (*2*) are shown, and the conserved amino acid substitution S17N, present in the American lineage but not in the other lineages, is in bold. The amino acid residues of the 7 isolates from this report are identical to those of the other Asian lineage isolates. C, capsid; prM, premembrane; NS, nonstructural protein. Scale bar indicates nucleotide changes per basepair.

Asian) contributes to the onset of microcephaly (*4*). An increased frequency of retinoic acid response elements in the American lineage genome versus the Asian lineage genome has also been observed (*2*). We question these explanations because we report a confirmed case of congenital Zika virus infection with microcephaly in Thailand caused by an Asian lineage virus.

We sequenced 7 Zika virus genomes obtained from 5 patients, including 3 pregnant women (PW1–3), in 2016 and 2017. PW1 had fever, maculopapular rash, and mild conjunctivitis at 24 weeks of gestation. Her urine sample was positive for Zika virus (BKK05, GenBank accession no. MG807647), and she gave birth to an infant without birth defects at full term. PW2 had a suspected Zika virus infection at 9 weeks' gestation with high fever, maculopapular rash, and mild conjunctivitis. At 16 weeks, a sample of the amniotic fluid was positive for Zika virus (BKK03, GenBank accession no. MG548660). The pregnancy was terminated at 17 weeks. Autopsy of the fetus demonstrated a head circumference of 12.5 cm (less than the third percentile for this gestational age); Zika virus was detected in the brain (BKK02, GenBank accession no. MF996804) and placenta (BKK04, GenBank accession no. MG548661). No other etiologic agents associated with birth defects (cytomegalovirus, herpes simplex virus types 1 and 2, rubella virus, syphilis virus, *Toxoplasma gondii*, *Treponema pallidum*) were detectable by real-time PCR. PW2 had detectable hepatitis B viral surface antigen but no concurrent medical conditions. These findings suggest that Zika virus was the causative agent of this case of microcephaly. PW3 had a maculopapular rash without fever or conjunctivitis and received a Zika virus diagnosis at 20 weeks' gestation. Her urine sample was positive for Zika virus (BKK07, GenBank accession no. MH013290), and she gave birth to a healthy infant at full term. The last 2 samples were from a 6-year-old child with mild fever and maculopapular rash (BKK06, GenBank accession no. MG807647) and a 64-year-old man with fever and maculopapular rash (BKK01, GenBank accession no. KY272987).

We retrieved 121 nonredundant Zika virus genomes (444 viruses, 99.9% nucleic acid identity cutoff) from GenBank to compare these isolates by phylogenetic analysis. All 7 BKK Zika virus isolates grouped within the Asian lineage (Figure). Virus from the amniotic fluid (BKK03), fetal brain (BKK02), and placenta (BKK04) of PW2 closely resembled each other (5 mismatches in BKK04 and 6 in BKK03, overall 99.898% identity). These 3 isolates were separated on the tree from their closest neighbor, a 2016 isolate from Singapore, by 40 mismatches. The number of retinoic acid response elements and predicted phosphorylation sites in BKK01–BKK07 was the same as the number in other Asian lineage Zika viruses (*2*). Also, the S17N substitution in premembrane was absent in all 7 isolates. Thus, all 3 proposed mechanisms failed to explain the case of congenital Zika virus infection with microcephaly in PW2. This case clinically resembled that of a woman in Finland infected during week 11 of pregnancy while traveling in Mexico, Guatemala, and Belize (*5*); in that case, Zika virus was detected in the brain of the aborted fetus at week 21.

The 3 cases in pregnant women described here support the hypothesis that the timing of Zika virus infection during pregnancy might be a key contributor to the development of microcephaly during congenital Zika virus infection. PW2 was infected around week 9 of gestation, during embryonic neurulation and cortical neurogenesis, which lay the foundation for the developing brain. Infection during weeks 20 (for PW3) and 24 (for PW1) of gestation did not lead to microcephaly. Our observations are in agreement with reports involving American lineage Zika viruses that show a high risk for microcephaly when infection occurs before week 21 (*6*), during weeks 7–14 (*7*), or during the first trimester (*8–10*). Our findings show that Zika viruses circulating in Asia can cause microcephaly, just like American strains.

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About the Author

Dr. Wongsurawat is a postdoctoral research fellow at the Arkansas Center for Genomic Epidemiology & Medicine in Little Rock, Arkansas, USA. Her primary research interests are applying next-generation and third-generation sequencing technologies to perform DNA and RNA viral genome and metagenome sequencing.

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Address for correspondence: Intawat Nookaew, Department of Biomedical Informatics and Department of Physiology and Biophysics, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA; email: inookaew@uams.edu; Navin Horthongkham, Department of Microbiology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Wanglang Road, Bangkoknoi Bangkok, 10700 Thailand; email: navin.hor@mahidol.ac.th

Dirofilaria repens Nematode Infection with Microfilaremia in Traveler Returning to Belgium from Senegal

Idzi Potters, Gaëlle Vanfraechem, Emmanuel Bottieau

Author affiliations: Institute of Tropical Medicine Antwerp, Antwerp, Belgium (I. Potters, E. Bottieau); Centre Hospitalier Interrégional Edith Cavell Hospital Group, Brussels, Belgium (G. Vanfraechem)

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We report human infection with a *Dirofilaria repens* nematode likely acquired in Senegal. An adult worm was extracted from the right conjunctiva of the case-patient, and blood microfilariae were detected, which led to an initial misdiagnosis of loiasis. We also observed the complete life cycle of a *D. repens* nematode in this patient.

On October 14, 2016, a 76-year-old man from Belgium was referred to the travel clinic at the Institute of Tropical Medicine (Antwerp, Belgium) because of suspected loiasis after a worm had been extracted from his right conjunctiva in another hospital. Apart from stable, treated arterial hypertension and non–insulin-dependent diabetes, he had no remarkable medical history. For the past 10 years, the patient spent several months per year in a small beach house in Casamance, Senegal, and did not travel to any other destination outside Belgium. His last stay in Senegal was during October 2015–May 2016, during which time he took care of dogs roaming on the beach.

On September 30, 2016, unilateral right conjunctivitis developed in the patient, and he was referred to an ophthalmologist, who extracted a worm (length 10 cm, diameter 470 μm) (Figure, panel A). The patient did not report any previous symptoms such as itching, larva migrans, or migratory swelling.

Results of a physical examination were unremarkable. Blood analysis showed a leukocyte count of 8,330 cells/μL and 16.8% eosinophils. All other first-line laboratory parameters, including total level of IgE, were within reference ranges. A pan filaria IgG-detecting assay (*Acanthocheilonema viteae* ELISA Kit; Bordier Affinity Products SA, Crissier, Switzerland) showed a positive result. All other relevant serologic assays showed negative results. Blood smear examination after Knott concentration showed 6 microfilariae of *Dirofilaria* sp./mL of blood.

Although treatment for such infections is not well established, the patient was given ivermectin (200 μg/kg, single dose) on October 15. The patient had general itching and fever (temperature up to 40°C) the next day. Blood test results on October 26 showed a leukocyte count of 8,410 cells/μL and 27.9% eosinophils. The patient recovered uneventfully. In September 2017, the patient was free of symptoms, and his eosinophil count was 470 cells/μL.

Human dirofilariasis is a mosquitoborne zoonosis caused by filarial worms of the genus *Dirofilaria*, which has 2 subgenera: *Dirofilaria* (the most common species is *D. immitis*) and *Nochtiella* (the most common species is *D. repens*). The main clinical manifestations are subcutaneous or ocular nodules, and a diagnosis is usually made by biopsy or worm extraction. The risk for humans to acquire dirofilariasis has increased because of climate changes and larger distribution ranges of vectors (*1*).

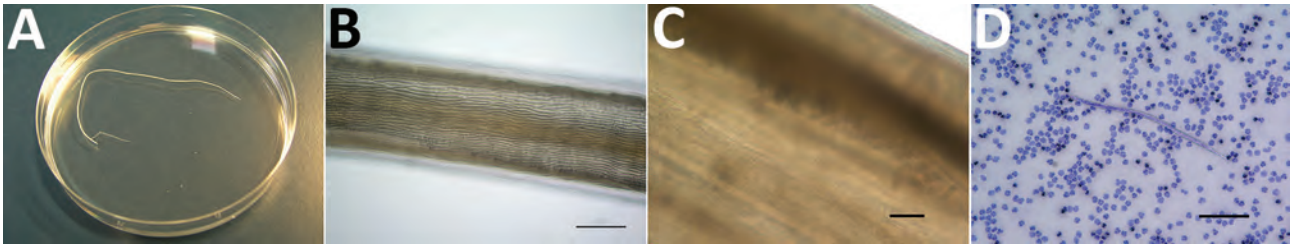

Figure. *Dirofilaria repens* adult worm isolated from the right conjunctiva of a 76-year-old man who returned to Belgium from Senegal, and microfilaria detected by using the Knott test. A) Macroscopic image of the adult. B) Microscopic image of the adult cuticle, showing the typical longitudinal ridges. Scale bar indicates 200 μm. C) Eggs in utero, indicating that the adult is a gravid female worm. Scale bar indicates 50 μm. Panel C has been cropped and contrast was increased to improve visibility of eggs. D) Microfilaria found in the blood of the patient. In a Knott test, microfilariae usually appear stretched out and slightly longer than those observed in a Giemsa-stained blood film. Scale bar indicates 100 μm.

Human dirofilariasis is currently considered an emerging zoonosis (*2*). *D. repens* nematodes have a large geographic distribution that includes Africa, Asia, and Europe and have recently spread into colder regions (*3*). Studies of primates indicate that *D. repens* nematodes need to develop for ≈25–34 weeks before they are fully mature and produce microfilariae (*4*). This finding suggests that the patient we report acquired the infection in Senegal, possibly through close contact with dogs.

Initially, loiasis was suspected as a diagnosis, given the location of the adult worm and presence of microfilaremia. However, the length (10 cm) of the adult worm did not correspond to a *Loa loa* worm, which can reach a maximum length of ≈7 cm. Microscopic examination of the cuticle identified conspicuous longitudinal ridges, which are typical for certain *Dirofilaria* spp. but absent in *L. loa* worms (Figure, panel B). These ridges also ruled out *D. immitis* worms.

When we took the largest diameter of the adult worm (470 μm) into account, we made a diagnosis of *D. repens* nematode infection (*5*). Eggs found in utero (Figure, panel C) confirmed that the worm was a gravid adult female. This diagnosis was supported by morphologic features of the blood microfilariae: terminal extremities that did not contain nuclei (*L. loa* microfilariae have nuclei extending to the tip of the tail) and short cephalic spaces containing 2–4 nuclei (Figure, panel D; online Technical Appendix Figure, https://wwwnc.cdc.gov/EID/article/24/9/18-0462-Techapp1.pdf). We measured 25 larvae, and they had an average length of 376 μm (range 357–395 μm) and an average diameter of 9.7 μm (range 7.5–10.0 μm), all features compatible with *D. repens* microfilariae (*6*,*7*).

We attempted to provide molecular confirmation of the infecting species by using 2 PCRs: 1 reported by Gioia et al. in 2010 (*8*) and 1 reported by Latrofa et al. in 2012 (*9*). Both techniques, which were performed with material from the adult worm, did not confirm identification of infecting species, probably because of prolonged preservation of the worm in formaldehyde.

D. repens worms seldom fully develop and produce microfilariae in humans. To our knowledge, 5 such cases have been reported: 3 with microfilariae in tissues surrounding adult worms and 2 with microfilariae in blood (*10*). There might have been immune impairment in our patient with diabetes, which enabled completion of the worm cycle, a phenomenon also observed in macaques with decreased immunity (*4*).

In conclusion, this case highlights the need for careful parasitologic examination when clinical and laboratory findings (i.e., presence of an eye worm and microfilaremia) lead to a diagnosis that is epidemiologically unexpected. In addition, clinicians should be aware that similar clinical presentations might also be increasingly observed in nontropical settings.

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About the Author

Mr. Potters is a skills laboratory teacher and a medical laboratory technologist at the national reference laboratory for parasitology at the Institute of Tropical Medicine, Antwerp, Belgium. His research interest is tropical parasitology.

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Address for correspondence: Idzi Potters, Department of Clinical Sciences, Institute of Tropical Medicine Antwerp, Kronenburgstraat 43/3, 2000 Antwerp, Belgium; email: ipotters@itg.be

Rubella Virus Genotype 1E in Travelers Returning to Japan from Indonesia, 2017

Daiki Kanbayashi, Takako Kurata, Yuka Nishino, Fumi Orii, Yuki Takii, Masaru Kinoshita, Toshitake Ohara, Kazushi Motomura, Takahiro Yumisashi

Author affiliations: Osaka Institute of Public Health, Osaka, Japan (D. Kanbayashi, T. Kurata, K. Motomura, T. Yumisashi); Osaka Prefectural Government Department of Health and Medical Care, Osaka (Y. Nishino, F. Orii, Y. Takii, M. Kinoshita); Osaka Prefectural Government Ikeda Healthcare Center, Osaka (T. Ohara)

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Although rubella is epidemic in Indonesia, the phylogenetic profile of circulating rubella virus strains has not been clarified. In 2017, rubella virus was detected in 2 travelers who returned from Indonesia to Japan. These strains were classified into genotype 1E lineage 2, which may be an indigenous strain in Indonesia.

Rubella is a mild contagious disease caused by the rubella virus, genus *Rubivirus*, family *Togaviridae* (*1*). Fetal death or congenital rubella syndrome (CRS) can occur when infection arises in pregnant women (*1*). Rubella infections and CRS cases have declined in many countries because of vaccination (*2*); however, an estimated 110,000 CRS cases occurred globally in 2010, with almost half developing in Southeast Asia because routine immunization programs against rubella virus had scarcely been introduced in these countries at that time (*3*,*4*). As of 2016, of the 11 countries in Southeast Asia, 8 (Bangladesh, Bhutan, Maldives, Myanmar, Nepal, Sri Lanka, Thailand, and Timor-Leste) had introduced routine immunization (*5*). However, large epidemics still exist in Southeast Asia, mainly in India and Indonesia, which had not introduced routine immunization as of 2016 (*5*). In addition, CRS cases in Indonesia were the highest worldwide in 2016 (*5*). Contrarily, only 1 sequence of the virus in Indonesia was registered in GenBank, from a patient who returned to the United States in 2011 (Hendersonville. NC.USA/15.11, accession no. JX477651). Although these rubella-endemic countries greatly affect the efforts of neighboring countries to control the virus, genetic information of epidemic strains in Southeast Asia remains unclear.

In October 2017, a 29-year-old man in Japan experienced a slight fever and sore throat. He had traveled to Jakarta, Indonesia, in late September, 14 days before symptom onset. He was not previously vaccinated against rubella virus. On day 4 after onset, rashes appeared on his body. Testing by real-time reverse transcription PCR did not detect the measles virus genome, but it detected rubella virus genome via throat swab sample collected on day 7 after onset (*6*). His illness was diagnosed as rubella; we strongly suspected that he acquired the infection in Indonesia, because the incubation period of rubella virus is ≈14 days and Japan has had no domestic rubella epidemic since 2013.

We amplified the E1 protein-coding region genome of the virus and sequenced the molecular window region (739 nt) (*7*). We classified this rubella strain into genotype 1E and deposited it into GenBank (RVs/Osaka.JPN/41.17[1E], accession no. LC333396). We generated a phylogenetic tree including 61 strains using the maximum-likelihood method; it revealed that rubella virus can be classified into 5 distinct lineages (L0–L4), as previously described (*7*,*8*). 1E-L1 strains are mainly detected in China and Russia. 1E-L2 strains are mainly detected in or imported from Malaysia, China, and Japan. 1E-L3 strains are detected in or

imported from African countries, such as the Democratic Republic of the Congo and Tunisia. 1E-L4 strains are detected in Sudan, Yemen, and Uganda. Both RVs/Osaka.JPN/41.17[1E] and Hendersonville.NC.USA/15.11 belonged to 1E-L2; these sequences were closely related to the recently identified 1E-L2 sequences. We also detected RVs/Yokohama.JPN/3.17[1E] in a traveler who returned to Japan from Indonesia in January 2017 (deposited in GenBank under accession no. LC215401) who had contact with a local rubella patient. Our findings indicate that 1E-L2 strains may circulate as indigenous strains in Indonesia.

To verify rubella elimination, interruptions in transmission of indigenous or imported rubella virus strains must be confirmed through effective surveillance systems (*9*). However, it is difficult to distinguish imported strains from endemic strains and to confirm the control status on the basis of genotype information because the genotypes of global epidemic strains converge to genotypes 1E and 2B (*3*,*7*,*8*,*10*).

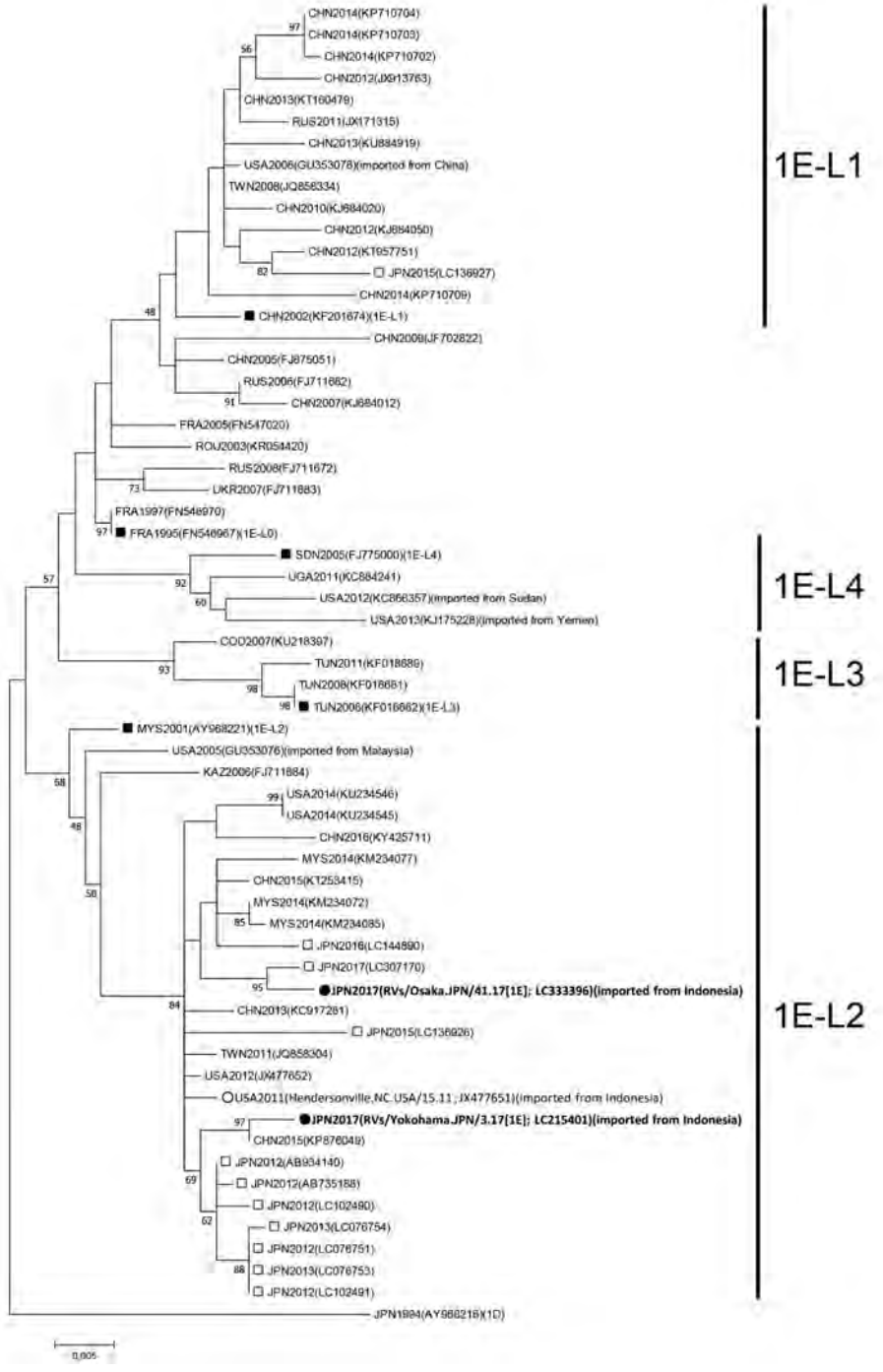

Figure. Maximum-likelihood phylogram of the molecular window region (739 nt) within the E1 gene of rubella virus genotype 1E from a 29-year-old man in Japan who had traveled to Indonesia (black circles). We constructed a phylogenetic tree using 61 strains, including the genotype reference strains and the candidate lineage reference strains, using MEGA version 7.0 (http://www.megasoftware.net) and the Tamura-Nei model. Numbers at nodes indicate the bootstrap support values, given as a percentage of 1,000 replicates (values <45 are omitted). The genotype 1D reference strain (RVi/Saitama.JPN/0.94/[1D]) is included as an outgroup. White circles indicate the genotype 1E strain detected in patients returning to the United States from Indonesia in 2011. Black squares indicate candidate genotype 1E lineage reference strains. White squares indicate the strains detected in Japan from 2012–2017. Each strain identification consists of a 3-letter country name abbreviation and detection year. Accession numbers are shown in parentheses. Scale bar indicates nucleotide substitutions per site. COD, Democratic Republic of the Congo; FRA, France; JPN, Japan; KAZ, Kazakhstan; CHN, China; MYS, Malaysia; ROU, Romania; RUS, Russia; SDN, Sudan; TWN, Taiwan; TUN, Tunisia; UGA, Uganda; UKR, Ukraine; USA, United States of America.

Therefore, several studies were conducted to subdivide the genotypes on the basis of detailed phylogenetic analysis (*7*,*8*). We reported that a large epidemic in Japan in 2013 might have occurred due to the transport of multiple lineages of rubella virus from rubella-endemic countries (*7*). According to the National Epidemiological Surveillance of Infectious Diseases (NESID) of Japan, during 2015–2017, ≈100 cases of rubella, which is a notifiable disease in Japan, were reported annually (*5*), and genotype 1E strains, including a strain closely related to RVs/Osaka.JPN/41.17[1E], were detected. Although these strains might have been transported from countries with endemic rubella, their origin remains unclear because of insufficient genomic information.

Japan has a high risk for subsequent rubella epidemics because the proportion of persons susceptible to rubella virus (≈9.0%) has not changed since 2013. In addition, an epidemic can occur when rubella virus is transported from rubella-endemic countries and the infection occurs in susceptible populations, as happened in Japan in 2013. Of the 11 imported cases of rubella to Japan reported in 2017, 4 were from Indonesia, according to the NESID of Japan. In the case we describe, we identified the rubella-exporting country and clarified the genetic information of the strain, which may contribute to countermeasures for worldwide importation of rubella virus. Rubella control by 2020 is the flagship goal of the World Health Organization South-East Asia region. Indonesia is conducting rubella immunization campaigns targeting ≈70 million children in 2017–2018. Therefore, constructing effective surveillance systems, accumulating genetic information, and promoting immunization in rubella-endemic countries are steps toward the global elimination of rubella.

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About the Author

Mr. Kanbayashi is a researcher at the Osaka Institute of Public Health. His research interests include rubella.

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Address for correspondence: Daiki Kanbayashi, Osaka Institute of Public Health, 1-3-69 Nakamichi, Higashinari-ku, Osaka 537-0025, Japan; email: kanbayashi@iph.osaka.jp

Spondweni Virus in Field-Caught *Culex quinquefasciatus* Mosquitoes, Haiti, 2016

Sarah K. White,[1] John A. Lednicky, Bernard A. Okech, J. Glenn Morris, Jr., James C. Dunford

Author affiliations: University of Florida, Gainesville, Florida, USA (S.K. White, J.A. Lednicky, B.A. Okech, J.G. Morris, Jr.); US Navy and Marine Corps Public Health Center, Portsmouth, Virginia, USA (J.C. Dunford)

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[1]Current affiliation: Brammer Bio, Alachua, Florida, USA.

Spondweni virus (SPONV) and Zika virus cause similar diseases in humans. We detected SPONV outside of Africa from a pool of *Culex* mosquitoes collected in Haiti in 2016. This finding raises questions about the role of SPONV as a human pathogen in Haiti and other Caribbean countries.

Spondweni virus (SPONV) and Zika virus are closely related flaviviruses that were first described in Africa in 1952 and 1947, respectively (*1*). Humans infected by these viruses have similar clinical manifestations; asymptomatic infections are common, and illness is generally self-limiting (*1*). In the 6 documented human SPONV infections, fever occurred in all. Other symptoms included headache, nausea, myalgia, conjunctivitis, and arthralgia; only 1 SPONV-infected person had maculopapular and pruritic rash (*1*). The similar clinical presentations for these virus infections and reportedly high serologic cross-reactivity have resulted in frequent misdiagnosis (*1*).

Because of the 2015–2016 epidemic of Zika fever in the Western Hemisphere and the link between microcephaly and Zika virus infection, Zika virus has been studied more comprehensively than SPONV (*1*). SPONV was first isolated from *Mansonia uniformis* mosquitoes during virus surveillance in 1955 in South Africa (*2*). No new reports of SPONV surfaced despite continued mosquito surveillance until 1958, when it was identified in 4 additional mosquito species, including *Aedes circumluteolus*, a tropical sylvatic mosquito found in Africa (*2*). Little is known about possible vertebrate hosts, although SPONV antibodies have been detected in birds, small mammals, and ruminants (*2*). In a recent study by Haddow et al., strains of *Ae. aegypti*, *Ae. albopictus*, and *Culex quinquefasciatus* mosquitoes were not susceptible to SPONV infection (*3*).

We detected SPONV from a pool of 7 mixed-sex *Cx. quinquefasciatus* mosquitoes collected in July 2016 during ongoing arbovirus surveillance in Gressier, Haiti. During May–August 2016, we caught 1,756 mosquitoes using Biogents Sentinel traps (BioQuip Products, Rancho Dominguez, CA, USA) within a 10-mile radius in Gressier, a semirural setting. Trap locations were selected based on environmental considerations, low risk for traps being disturbed, and known human arbovirus-caused illnesses in the area (*4*). Trap bags were transported to a field laboratory in Haiti, where mosquitoes were frozen at –20°C, then identified by species and sexed by trained technicians using morphologic keys and identification guides (*5*,*6*). After identification, the mosquitoes were pooled by location, collection date, species (*Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus*, and other), and sex. All pools were screened for chikungunya virus, dengue virus (DENV) serotypes 1–4, and Zika virus RNA by real-time reverse transcription PCR (rRT-PCR) (online Technical Appendix Table 1, https://wwwnc.cdc.gov/EID/article/24/9/17-1957-Techapp1.pdf), as we previously have done with human specimens from Haiti (*4*). Mosquito homogenates positive by rRT-PCR were used for sequencing using primer walking and Sanger sequencing methods as previously reported (*4*; online Technical Appendix Table 2). In addition, we confirmed *Aedes* and *Culex* mosquito species by molecular methods (*7*,*8*). In initial screens of a pool of 7 mixed-sex *Cx. quinquefasciatus* mosquitoes (non–blood-fed) collected on July 4, 2016, rRT-PCR results suggested the presence of Zika virus RNA (cycle threshold value 39), but this same pool was negative for chikungunya virus and DENV RNA by rRT-PCR. After unsuccessful attempts to amplify Zika virus–specific amplicons using previously described Zika virus sequencing primers, we used an unbiased sequencing approach after treatment of virions in mosquito homogenate with cyanase (*4*). Because we suspected a closely related virus, we next

Table. Comparison of nucleotide and amino acid identities of representative strains of SPONV and Zika virus, Haiti*

Virus type and nucleotide GenBank accession no. (country of origin, year)	Nucleotide identity, %					
	SPONV, GenBank accession no.			Zika virus, GenBank accession no.		
	MG182017	DQ859064	KX227369	KY989511	KU501215	MF384325
SPONV MG182017 (Haiti, 2016)	100	98.8	96.8	70.7	70.4	70.4
SPONV DQ859064 (South Africa, 1954)		100	97.8	70.9	70.6	70.7
SPONV KX227369 (Nigeria, 1952)			100	71.1	70.8	70.8
Zika virus KY989511 (Uganda, 1947)				100	89.0	89.0
Zika virus KU501215 (Puerto Rico, 2015)					100	99.6
Zika virus MF384325 (Haiti, 2016)						100
Virus type and protein GenBank accession no. (country of origin, year)	**Amino acid identity, %**					
	SPONV, GenBank accession no.			Zika virus, GenBank accession no.		
	AVD68687	ABI54480	AOZ57820	ARM59240	AMC13911	ASF57880
SPONV AVD68687 (Haiti, 2016)	100	98.8	98.3	74.1	74.0	74.1
SPONV ABI54480 (South Africa, 1954)		100	99.1	74.9	74.7	74.8
SPONV AOZ57820 (Nigeria, 1952)			100	74.9	74.8	74.9
Zika virus ARM59240 (Uganda, 1947)				100	96.9	96.9
Zika virus AMC13911 (Puerto Rico, 2015)					100	99.8
Zika virus ASF57880 (Haiti, 2016)						100

*SPONV, Spondweni virus.

tested random hexamers and SPONV-specific primers (online Technical Appendix Table 3), which resulted in formation of virus-specific amplicons (online Technical Appendix). Thereafter, using SPONV primers, we determined a 10,290-nt nearly complete genome and deposited it in GenBank (accession no. MG182017).

The SPONV genome from Haiti shared 10,174 (98.8%) of 10,290 nt identity with a SPONV isolate from mosquitoes in South Africa in 1954 (GenBank accession no. DQ859064) and 9,958 (96.8%) of 10,287 nt identity with the SPONV Chuku strain from blood of a febrile human patient in Nigeria in 1952 (accession no. KX227369) (Table). When compared with the Zika virus reference strain from Uganda (accession no. KY989511), a strain from Puerto Rico (accession no. KU501215), and a strain from Haiti in 2016 (accession no. MF384325), Zika virus and SPONV clearly continue to diverge because the nucleotide and amino acid identities of SPONV are less similar to more recent strains of Zika virus (Table). Few SPONV sequences have been deposited into GenBank, resulting in insufficient information to predict how and when SPONV was introduced in Haiti.

In the Americas and the Caribbean, SPONV is a potential emergent arbovirus and public health threat that manifests clinically with symptoms and signs similar to those of Zika virus infection (*2*,*9*). Misdiagnosis has been documented, and it is possible that SPONV has caused human infection in Haiti but has been misidentified as infection from DENV or other arboviruses (*9*). Little is known about SPONV pathogenesis, host range, and vector competency, especially with vectors present in the Western Hemisphere. Our detection of SPONV in *Cx. quinquefasciatus* mosquitoes raises questions about the role of this species as a vector for this virus and highlights the need for ongoing surveillance for SPONV infection among humans in the Caribbean, combined with studies of potential vector populations.

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About the Author

Dr. White is a senior scientist in Assay Development and Analytic, and Small Scale Development at Brammer Bio. During this study, she was a postdoctoral associate in the Department of Environmental and Global Health at the Emerging Pathogens Institute, University of Florida, under the mentorship of Dr. John Lednicky. Her primary research interests include emerging arboviruses, influenza D virus, and novel viral vector therapeutics.

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Address for correspondence: Sarah K. White, Brammer Bio, 13859 Progress Blvd, Alachua, FL 32615, USA; email: sek0005@tigermail.auburn.edu

Fluconazole-Resistant *Candida parapsilosis* Bloodstream Isolates with Y132F Mutation in ERG11 Gene, South Korea

Yong Jun Choi,[1] Yae-Jean Kim,[1] Dongeun Yong, Jung-Hyun Byun, Taek Soo Kim, Yun Sil Chang, Min Ji Choi, Seung Ah Byeon, Eun Jeong Won, Soo Hyun Kim, Myung Geun Shin, Jong Hee Shin

Author affiliations: Chonnam National University Medical School, Gwangju, South Korea (Y.J. Choi, M.J. Choi, S.A. Byeon, E.J. Won, S.H. Kim, M.G. Shin, J.H. Shin); Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea (Y.-J. Kim, Y.S. Chang); Yonsei University College of Medicine, Seoul (D. Yong, J.-H. Byun); Seoul National University Hospital, Seoul (T.S. Kim)

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We recently observed the emergence of fluconazole-resistant *Candida parapsilosis* bloodstream isolates harboring a Y132F substitution in Erg11p in South Korea. These Y132F isolates had a higher propensity to cause clonal transmission than other fluconazole-resistant isolates and persisted within hospitals for several years, as revealed by microsatellite typing.

Candida parapsilosis is the second most common species isolated from patients with *Candida* bloodstream infections (BSIs) in Latin America and eastern Asia (*1*,*2*). Although uncommon, fluconazole-resistant *C. parapsilosis* isolates harboring the Y132F substitution in Erg11p (referred to as Y132F isolates) have been reported in Brazil, the United States, and Kuwait (*3*–*6*). The precise reason for the emergence of *C. parapsilosis* Y132F isolates has yet to be defined; it may be related to selective drug pressure, and the mutation at position 132 may be a hot spot for resistance mediated by *ERG11*, a gene encoding the azole target (*3*). Alternatively, *C. parapsilosis* Y132F isolate emergence may be associated with exogenous clonal transmission (*4*). We recently observed the emergence and nosocomial spread of Y132F isolates in South Korea. In this study, we report a greater increase in the clonal spread of *C. parapsilosis* Y132F BSI isolates than of non-Y132F fluconazole-resistant isolates within hospitals during the past several years.

We assessed the first 47 *C. parapsilosis* BSI isolates that were fluconazole-resistant (MIC ≥8 mg/L) according to the Clinical and Laboratory Standards Institute (CLSI) species-specific clinical breakpoint (*7*,*8*). All 47 isolates were obtained from multicenter surveillance cultures from 8 university hospitals (A–H) during 2005–2016. For all fluconazole-resistant isolates, we examined genotypic relationships using microsatellite typing. We defined ≥2 isolates with identical genotypes according to microsatellite typing as clonal isolates. We sequenced the *ERG11* gene and 3 transcription factor genes: *TAC1*, which can lead to the upregulation of *CDR*; *MRR1*, which can lead to the upregulation of MDR; and *UPC2*, which can lead to the upregulation of *ERG11* (*5*); we compared the results with those of 20 fluconazole-susceptible (MIC 0.5–2 mg/L) isolates. This study was approved by the Institutional Review Board of Chonnam National University Hospital (IRB CNUH-2014-290).

Of the 47 *C. parapsilosis* fluconazole-resistant isolates, 30 (63.8%) had the Y132F substitution in Erg11p; however, none of the 20 fluconazole-susceptible isolates had the Y132F mutation in *ERG11*. Recently, 31%–57% of fluconazole-resistant *C. parapsilosis* isolates from different parts of the world were reported to be Y132F isolates, but the Y132F mutation was absent in all fluconazole-susceptible isolates (*3*–*6*). These data confirm that a Y132F substitution in Erg11p is the predominant fluconazole resistance mechanism for *C. parapsilosis* worldwide.

Microsatellite typing revealed that 4 clonal Y132F isolates (M1–4) were persistently recovered in 2 hospitals (A and B) over a period of 3–7 years, and the proportion of clonal isolates was much higher in Y132F isolates (86.7%, 26/30) than in non-Y132F fluconazole-resistant isolates (11.8%, 2/17) (Table). In a previous microsatellite study from a US surveillance study by Grossman et al. (*4*), no hospital specificity was detected among 13 non-Y132F fluconazole-resistant isolates; however, 2 notable clusters of isolates from 17 Y132F isolates were found over 8- or 18-month periods. The results obtained in our study and those of Grossman et al. indicate that Y132F isolates may have a higher propensity to cause clonal transmission and persist in particular hospitals than do non-Y132F fluconazole-resistant isolates. The Y132F substitution in Erg11p has also been detected in *C. auris* isolates from Pakistan (10/16 isolates), India (12/17 isolates), and Venezuela (5/5 isolates); these isolates are strongly associated with clonal transmission (*9*). Further studies are needed to determine whether the Y132F mutation in Erg11p has a direct effect on clonal transmission of *C. parapsilosis* or *C. auris* isolates.

Two previous studies conducted in the United States detected the Erg11p Y132F substitution in combination with the Erg11p R398I substitution in almost all *C. parapsilosis* BSI isolates (*4*,*5*). In addition, no Y132F isolates detected in a US surveillance study contained an *MRR1* polymorphism, according to *MRR1* sequence analysis

[1]These authors contributed equally to this article.

Table. Molecular characterization of 47 fluconazole-resistant isolates and 20 fluconazole-susceptible isolates of *Candida parapsilosis*, South Korea*

Microsatellite genotypes†	Hospital	No. isolates	MICs, mg/L‡		Amino acid substitutions§				Isolation year (no. patients)
			FLC	VRC	Erg11p	Mrr1p	Tac1p	Upc2p	
Fluconazole-resistant with Y132F in Erg11p, n = 30 isolates									
M1	A	8	8–32	0.25–0.5	Y132F	K177N			2006 (1), 2009 (1), 2010 (2), 2011 (2), 2012 (1), 2013 (1)
	B	3	16–32	0.5	Y132F	K177N			2012 (2), 2013 (1)
M2	A	10	8–32	0.125–0.5	Y132F	K177N			2012 (1), 2016 (9)
M3	A	3	8–16	0.25	Y132F	K177N, Q1053*			2007 (1), 2011 (1), 2012 (1)
M4	A	2	8–>64	0.5–4	Y132F	K177N			2013 (1), 2016 (1)
M5	A	1	32	0.25	Y132F	K177N			2012 (1)
M6	A	1	8	0.5	Y132F	K177N			2013 (1)
M7	A	1	8	0.25	Y132F	K177N			2016 (1)
M8	C	1	64	2	Y132F				2016 (1)
Other fluconazole-resistant, n = 17 isolates									
M9	D	2	>64	1		G583R			2007 (1), 2009 (1)
M10	E	1	16	0.5	R398I		L877P		2005 (1)
M11	A	1	8	0.25					2006 (1)
M12	E	1	8	0.25	R398I		L877P		2011 (1)
M13	F	1	16	0.06	R398I		L877P		2011 (1)
M14	E	1	8	0.125	R398I		L877P		2012 (1)
M15	G	1	8	0.125	R398I		L877P		2012 (1)
M16	G	1	8	0.06	R398I		L877P		2012 (1)
M17	E	1	8	0.125			N900D		2012 (1)
M18	C	1	8	0.125	R398I	P250S	L877P		2012 (1)
M19	C	1	8	0.25	R398I	S1081P	L877P		2012 (1)
M20	D	1	8	0.125	R398I			D394N	2012 (1)
M21	E	1	32	0.5	R398I	P295R	L877P		2015 (1)
M22	E	1	16	0.125	R398I				2015 (1)
M23	H	1	32	0.125	K128N	W872C			2015 (1)
M24	E	1	16	0.25		G927D			2016 (1)
Fluconazole-susceptible controls									
M3	A	1	1	0.03		K177N, Q1053*			2010 (1)
M25	C	2	0.5	0.03	R398I				2012 (2)
M26	F	2	0.5	0.03–0.06			R208G		2012 (1), 2013 (1)
M27	A	1	2	0.06					2010 (1)
M28	A	1	0.5	0.03		K177N, Q1053*			2011 (1)
M29	A	1	1	0.06			L877P		2011 (1)
M30	A	1	1	0.03			R208G		2012 (1)
M31	A	1	0.5	0.03			R208G		2012 (1)
M32	E	1	2	0.03					2012 (1)
M33	G	1	0.5	0.03			R208G		2012 (1)
M34	G	1	0.5	0.03					2012 (1)
M35	A	1	0.5	0.03			R208G		2013 (1)
M36	A	1	1	0.06	R398I			D394N	2013 (1)
M37	A	1	0.5	0.03	R398I				2013 (1)
M38	D	1	0.5	0.06	R398I				2014 (1)
M39	D	1	0.5	0.06			R208G		2014 (1)
M40	E	1	0.5	0.03			R208G		2015 (1)
M41	A	1	1	0.03	R398I		L877P		2015 (1)

*CLSI, Clinical and Laboratory Standards Institute; FLC, fluconazole; VRC, voriconazole.
†For microsatellite typing, each strain was characterized by a genotype resulting from combination of the sizes of the 4 markers (CP1, CP4, CP6, and B). See the Technical Appendix Figure (https://wwwnc.cdc.gov/EID/article/24/9/18-0625-Techapp.pdf) for results of microsatellite genotyping presented as an UPGMA tree.
‡Antifungal MICs were determined by the CLSI M27–A3 broth microdilution method (*7*). The fluconazole MICs of 30 Y132F isolates determined by Etest were ≥8 mg/L. All 67 isolates tested were susceptible to amphotericin B (MIC 0.25–1 mg/L) and micafungin (MIC 0.25–2 mg/L) according to the CLSI method.
§All were homozygote alleles except for 6 heterozygote alleles (Q1053,* G583R, P250S, P295R, W872C, and G927D) in Mrr1p.

results (*4*). However, a single Y132F substitution in Erg11p was found in all 30 fluconazole-resistant isolates from South Korea hospitals. The same K177N substitution in Mrr1p was found in all Y132F isolates except 1; none of the Y132F isolates showed missense mutations in Tac1p or Upc2p (Table). Taken together, these findings demonstrate low genetic diversity among Y132F isolates from the same country (the United States or South Korea).

In our study, 76.7% (23/30) of patients with Y132F isolates had no antifungal exposure within 30 days before

candidemia detection, and their clonal transmission was not detected by routine hospital surveillance, partly because more than half of the patient hospitalizations did not overlap. These findings indicate that clonal Y132F isolates may be dormant over long periods and can survive and persist outside their host on hospital environmental surfaces, which may be similar to the behavior of *C. auris* (*10*). Although our study was limited by the relatively low number of isolates, our data suggest that *C. parapsilosis* Y132F isolates should be identified in clinical microbiology laboratories to prevent further clonal transmission of BSI caused by Y132F isolates.

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About the Author

Dr. Y.J. Choi is a clinical pathologist at Chonnam National University Hospital, Gwangju, South Korea. His current research interest is molecular epidemiology of fungal infections.

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Address for correspondence: Jong Hee Shin, Chonnam National University Medical School, Department of Laboratory Medicine, 42 Jebong-ro, Dong-gu, Gwangju 61469, South Korea; email: shinjh@chonnam.ac.kr

Borrelia miyamotoi Disease in an Immunocompetent Patient, Western Europe

Dieuwertje Hoornstra,[1] Joris Koetsveld,[1] Hein Sprong, Alexander E. Platonov, Joppe W. Hovius

Author affiliations: Academic Medical Center, Amsterdam, the Netherlands (D. Hoornstra, J. Koetsveld, J.W. Hovius); National Institute for Public Health and the Environment, Bilthoven, the Netherlands (H. Sprong); Central Research Institute of Epidemiology, Moscow, Russia (A.E. Platonov)

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Borrelia miyamotoi disease is a hard tick–borne relapsing fever illness that occurs across the temperate climate zone. Human *B. miyamotoi* disease in immunocompetent patients has been described in Russia, North America, and Japan. We describe a case of *B. miyamotoi* disease in an immunocompetent patient in western Europe.

A 72-year-old woman in the Netherlands sought treatment in her third day of fever (≤38.6°C) and reported myalgia, arthralgia, headache, and a 2.5-kg weight loss. Three weeks earlier she had noticed a tick bite after gardening. Several days later, an erythematous lesion appeared, increasing to palm size within 1.5 weeks and dissolving in a similar period. Full medical history was not

[1]These authors contributed equally to this article.

suggestive of other causes of fever. Her previous medical history included cervical carcinoma and breast cancer, curatively treated.

Physical examination showed a moderately ill patient with a temperature of 36.7°C, heart rate of 59 bpm, blood pressure of 100/72 mmHg, an erythematous skin lesion (1.5 cm in diameter) on the thigh, and mild generalized lymphadenopathy. Initial laboratory tests revealed increased C-reactive protein (22.7 mg/L), leukopenia (2.1 × 10^9 cells/L), elevated monocytes (11%), and thrombocytopenia (144 × 10^9 platelets/L) (reference ranges in online Technical Appendix Table 1, https://wwwnc.cdc.gov/EID/article/24/9/18-0806-Techapp1.pdf). All other test results, including urinalysis, were unremarkable. Molecular tests of blood and skin biopsy and serologic testing for *Borrelia burgdorferi* sensu lato and syphilis were repeatedly negative, except for a C6 EIA IgM/IgG seroconversion (Immunetics, Boston, MA, USA) in convalescent-phase serum samples that was positive but could not be confirmed by either IgM or IgG immunoblot (Mikrogen, Neuried, Germany) (online Technical Appendix Table 2). We did not admit the patient to the hospital, and we did not initiate antimicrobial drug treatment because her symptoms had largely resolved. At a 2-month follow-up visit, the patient had fully recovered, and laboratory test results were normal.

On the basis of the patient's description, we suspect that she was bitten by an *Ixodes ricinus* tick, the most prevalent tick species in western Europe (*1*), which can potentially carry several tickborne pathogens: *Borrelia burgdorferi* s.l., *B. miyamotoi*, *Rickettsia helvetica* and *R. monacensis*, *Anaplasma phagocytophilum*, *Babesia divergens* and *B. microti*, *Neoehrlichia mikurencis*, and tick-borne encephalitis virus (*2*). Specific molecular and serologic diagnostic tests for all of these pathogens were negative, expect for 1 (false-positive) tick-borne encephalitis virus IgM ELISA result in convalescent-phase serum samples (online Technical Appendix Table 2).

B. miyamotoi, a relapsing fever *Borrelia* species uniquely found in *Ixodes* spp. ticks in Eurasia and North America, is the causative agent of *Borrelia miyamotoi* disease (BMD), a tickborne febrile disease (*3*,*4*). Diagnosis of BMD relies on detection of spirochetes by quantitative PCR of blood and experimental serology based on glycerophosphodiester phosphodieasterase (GlpQ) antigen detection (*3*,*5*). GlpQ is present in relapsing fever *Borrelia* but not in *B. burgdorferi* s.l. and therefore can discriminate between the 2 types (*4*). In a well-described cohort of PCR-positive patients in Russia, characteristic clinical symptoms were fever, myalgia, nausea, and headaches; laboratory findings showed thrombocytopenia and diffuse organ damage (*3*).

In this patient, results of pan–relapsing fever *Borrelia* PCR and *B. miyamotoi*–specific PCR (*6*) of blood drawn at the day of clinical visit were negative. However, the fever and symptoms had subsided, which probably impeded these direct diagnostic tests. We tested for anti-GlpQ and anti–variable major proteins (Vmps) IgM and IgG using ELISA

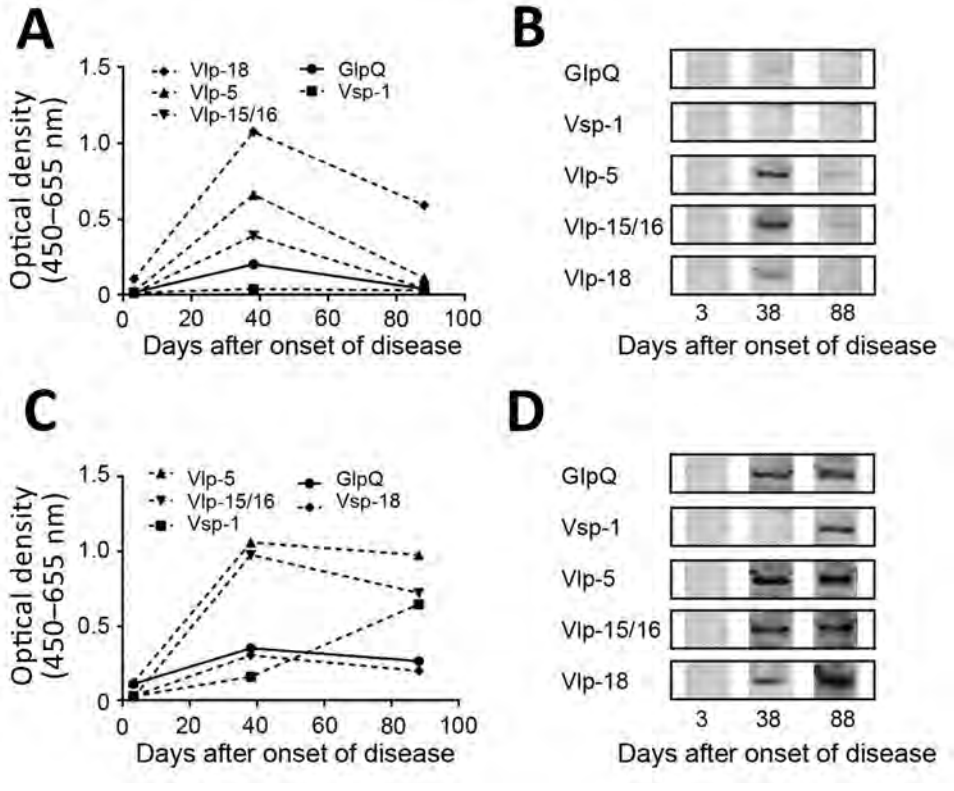

Figure. Results of GlpQ and variable major proteins (Vmps) IgM and IgG ELISA and confirmatory Western blot tests in testing of a 72-year-old woman in the Netherlands who showed evidence of *Borrelia miyamotoi* disease. A) Anti-GlpQ and anti–Vmps IgM ELISA results representative of 3 individual ELISAs. B) Confirmatory IgM Western blot results of samples taken at 3 different time points with recombinant proteins. C) Anti-GlpQ and anti–Vmps IgG ELISA results representative of 3 individual ELISAs. D) Confirmatory IgG Western blot results of samples taken at 3 different time points with recombinant proteins. GlpQ, glycerophosphodiester phosphodieasterase; Vlp, variable large protein; Vsp, variable small protein.

and Western blot in serum samples taken on the day of the hospital visit (3 days after disease onset), after 5 weeks (38 days), and after 3 months (88 days). Results demonstrated a clear seroconversion for predominantly IgG against GlpQ (Figure). We had previously shown that Vmps are highly immunogenic in patients with BMD (*7*) and that the presence of antibodies against GlpQ combined with antibodies against Vmps had 100% specificity for IgM and 98.3% for IgG (*8*). In this case, we could demonstrate antibodies against multiple Vmps over time (Figure). Finally, our findings were further confirmed by preferential IgM and IgG reactivity to lysates of the *B. miyamotoi* strain HT31 (tick isolate, Japan) and Izh-16 (clinical isolate, Russia) compared with reactivity to the *B. afzelii* strain PKo (skin isolate, Germany) and *B. hermsii* HS-1 (tick isolate, United States) control lysates (online Technical Appendix Figure).

These findings, combined with the established presence of *B. miyamotoi* in *I. ricinus* ticks throughout Europe, clinical presentation, and laboratory findings, strongly suggest that *B. miyamotoi* was the causative agent of the patient's symptoms. That the patient recovered even without antimicrobial treatment is consistent with a recent BMD case described in the United States (*9*). Because of the initial skin rash, we did not completely rule out *B. burgdorferi* s.l. co-infection; however, prior evaluation by an independent dermatologist, a negative *B. burgdorferi* s.l. immunoblot despite high C6 reactivity, and a negative PCR on DNA obtained from the skin biopsy argue against co-infection. Regardless, the clinical picture of fever and mild leukopenia and thrombocytopenia is compatible with BMD and not with Lyme borreliosis. Of interest, C6 reactivity in combination with a negative *B. burgdorferi* s.l. immunoblot has been described in BMD patients in the United States (*10*).

This case identifies *B. miyamotoi* as an emerging tickborne pathogen in western Europe. Because of the widespread presence of multiple other tickborne pathogens across Europe, more attention and awareness for other tickborne diseases is warranted.

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About the Authors

Dr. Hoornstra and Dr. Koetsveld are PhD students at the University of Amsterdam at the Department of Infectious Diseases in Professor Joppe W. Hovius's laboratory. They focus on tickborne pathogens, and their work has been key in research on *B. miyamotoi* disease.

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Address for correspondence: Dieuwertie Hoornstra, Academic Medical Center, Center for Experimental and Molecular Medicine, Meibergdreef 9 Amsterdam 1105 AZ, the Netherlands; email: d.hoornstra@amc.uva.nl

Seroprevalence of Chikungunya Virus after Its Emergence in Brazil

Patrick Gérardin,[1] André Ricardo Ribas Freitas,[1] Daouda Sissoko[1]

Author affiliations: Centre Hospitalier Universitaire Réunion, Saint Pierre, France (P. Gérardin); Unité Mixte 134 Processus Infectieux en Milieu Insulare Tropical, Sainte Clotilde, France (P. Gérardin); São Leopoldo Mandic School of Medicine, Campinas, Brazil (A.R.R. Freitas); Biomerieux Africa Cluster, Abidjan, Côte d'Ivoire (D. Sissoko)

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To the Editor: In their well-designed and timely serosurvey, Dias et al. provide evidence of high prevalence of East/Central/South African (ECSA) genotype chikungunya virus (CHIKV) (seropositivity rate 51.0%) in population subsets of 2 urban communities in Bahia state, Brazil (*1*). The authors found a high proportion of asymptomatic CHIKV patients (63.2%; 268/424). In addition, the prevalence of chronic arthralgia among infected persons (26.4%; 112/424) was lower or within the expected range of previously reemerging clades of CHIKV, namely ECSA diverged (Indian Ocean lineage) or Asian lineage, respectively. However, a high proportion of the symptomatic participants in Dias et al. reported chronic symptoms lasting >3 months (71.8%; 112/156). We comment on these findings.

First, Dias et al. report that the selected locations were at the epicenter of the transmission area and, therefore, the data cannot be extrapolated to other cities. To better understand the dynamics of the disease, it would be useful to select locations more representative of other infected areas.

Second, the modest participation at the study locations (66.5%; 831/1250) could be related to using the more painful venipuncture method instead of a fingerstick to draw blood. In comparison, the participation rate was ≈80% in a Réunion Island serosurvey for CHIKV for which the fingerstick method was used (*2*). The participation level suggests the possibility of self-selection bias toward infected patients, who might be more interested in knowing their serologic status. Self-selection bias might explain the high proportion of symptomatic patients who self-reported having a chronic form of chikungunya.

Last, because of their unreliable discriminatory performance, using fever and arthralgia to identify symptomatic patients might have increased the proportion of patients misclassified as asymptomatic (i.e., patients with symptoms other than fever and arthralgia being falsely classified as negative) (*3*). These limitations being specified, the prevalence of chronic arthralgia among symptomatic patients in Dias et al. falls within the expected range of the Asian lineage of CHIKV, the other clade circulating in the Americas (*4*,*5*), which confers external validity to the study.

[1]All authors contributed equally to this article.

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Address for correspondence: Patrick Gérardin, INSERM CIC 1410 Clinical Epidemiology, Centre Hospitalier Universitaire, Groupe Hospitalier Sud Réunion, BP 350, 97448 Saint Pierre CEDEX, Reunion, France; email: patrick.gerardin@chu-reunion.fr

Correction: Vol. 24, No. 8

The author list was incorrect in Death from Transfusion-Transmitted Anaplasmosis, New York, USA, 2017 (R. Goel et al.), and a name was missing from the acknowledgments. Melissa M. Cushing should have been listed as senior author. Ljljana V. Vasovic provided assistance with the article. The article has been corrected online (https://wwwnc.cdc.gov/eid/article/24/8/17-2048_article).

Correction: Vol. 24, No. 9

Several corrections to the text were needed in Phenotypic and Genotypic Characterization of Enterobacteriaceae Producing Oxacillinase-48–Like Carbapenemases, United States (J.D. Lutgring et al.). The article has been corrected online (https://wwwnc.cdc.gov/eid/article/24/4/17-1377_article).

Emerging Infectious Literatures and the Zombie Condition

Joanna Verran,[1] Xavier Aldana Reyes[1]

The book club format has enabled expert and nonexpert exploration of infection and epidemiology as encountered in popular literature. This exploration reveals that fiction focusing on apocalyptic disease often uses the zombie as embodiment of infection, as well as an exemplar of current knowledge on emerging disease.

The Bad Bugs Book Club (https://www2.mmu.ac.uk/engage/what-we-do/bad-bugs-bookclub/) was established in 2009 (*1*). This reading group meets every 2 months to discuss works of literary fiction from any genre that features infectious disease. The aim of these meetings is to engage scientists and nonscientists in discussions about epidemiology and infection and to consider what the texts tell us about our perception of science and its advances.

Book clubs, or reading groups, have increased in popularity since the late 1990s. Estimates in 2003 were of ≈50,000 book clubs in Britain and ≈500,000 in the United States (*2*). Some clubs are specialized groups whose members read restricted genres such as crime fiction, science fiction, or the classics. Fiction and nonfiction texts focusing on microbiology have been incorporated into the book club format, led primarily by academics for student education (*3*–*5*), but no evidence has been found in the literature for such groups for the general public.

Adults have been identified as 1 of 3 key underserved audiences in terms of engagement with science (*6*). The reading group format addresses this need and contrasts with unidirectional science communication activities from scientists (experts) to members of the public (nonexperts) (*7*) in that reading groups provide an opportunity for adults to contribute their knowledge, experience, and perceptions about the reading subject matter on a level platform.

Bad Bugs Book Club meetings take place in an informal environment (a bar) in the evening, typically comprising up to 8 participants, of whom around half have been members since 2009. New members are welcome; meetings are advertised online, as well as through an email group. At each meeting one book is discussed, selected by the group at the previous meeting. Discussions tend to be led by the group leader (J.V.), but all members can lead discussions, particularly for books that they have suggested. The meeting leader prepares questions before the meeting to guide discussion and publishes them online on the book club's website after the meeting, but usually conversation does not require prompting. Meeting reports are also posted online, enabling themes to be identified across books and genres, as well as establishing a rich, freely accessible resource that has informed much of the content of this article.

Our findings, based on the reports accessible from the book club's website, show that fiction content in epidemiologic narratives is often influenced by epidemiologic outbreaks—authors absorbing and recasting what have been called "outbreak narratives" (*8*) within plotlines—as well as by the pervading rhetoric of fear that surrounds pandemics in the media (*9*). We found that the representation of vampires and, particularly, zombies as agents of infection was frequent; these monsters appeared often as epidemiologic avatars (*10*–*12*). This article therefore examines the role of the zombie as a metaphor for infectious disease and the emergence of new literature describing apocalyptic disease as examples of the ways in which fiction can lead to a widespread discussion and understanding of pandemics. We use examples from books discussed in our book club meetings.

The Zombie Research Society defines a zombie as "a relentlessly aggressive human or reanimated human corpse driven by a biologic infection" (http://zombieresearchsociety.com/about-us). This description neatly summarizes the current state of zombies, both narratively (in the stories told about them) and in terms of how they might become useful in our understanding of pandemics, contagion patterns, and prevention. One aim of the book club is to redress the balance between fear of infection and the importance of a working knowledge of microbiology, but zombies also provide a useful means for examining concerns about fast-spreading diseases in the first world, the "shock doctrine" used in the reporting of pandemics (*13*), emerging disease, and, more recently, the impact of antimicrobial resistance. Indeed, Bishop (*14*) proposes that "post-9/11 anxieties about potential terrorist attacks via anthrax, avian influenza, swine flu, and other forms of biologic warfare" may be responsible for this emergence and suggests that apocalyptic contagion narratives might outlive interest in the zombie.

Author affiliation: Manchester Metropolitan University, Manchester, UK

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[1]The authors contributed equally to this article.

The Zombie as Allegory of Infectious Disease Epidemiology

In fiction focusing on infectious disease, the invisible pathogen is an embodiment of the unknown, existing in intimate contact with us, yet beyond the boundaries of our senses. The infection is carried by its host and transmitted to another; its effects become apparent as symptoms develop. The pathogen as a microscopic Gothic presence can be represented metaphorically and macroscopically in the figure of the zombie, much as the ghost, the undead (the vampire), and the "weird creature" have traditionally acted as springboards for the exploration of the beyond and the numinous (*15*–*17*). In the zombie, internal damage to the host becomes externalized, and contagion patterns among populations are demonstrated as the zombie hordes rampage. With no subclinical manifestation, the zombie makes the apocalypse visible, enabling us to physically map the spread of infection. In other words, the zombie becomes an "allegory of infectious disease" and a "metaphor of ubiquitous contagion" (*18*). In their hordelike structure, zombies also operate metonymically, standing in for large swaths of the population (the infected), or viruses (the infection). The mathematics of zombie outbreaks has therefore also been explored as an education tool to represent contagion patterns and containment strategies (*19*,*20*).

In 1996, the influential horror survival video game *Resident Evil* was developed by Capcom (Capcom USA, San Francisco, CA, USA); this release was the first zombie game to rely on infection as the catalyst for the zombie state. Since then, and particularly after 2002, when a spate of infection-zombie texts emerged (such as the *Resident Evil* movie series [2002 onward; directed by Paul W.S. Anderson], *28 Days Later* [directed by Danny Boyle, 2002], and *The Walking Dead* comic series [2003–present] [*21*]), infection has tended to become the primary cause of the zombie condition itself. A blurring of the lines between the traditional zombie and the rabid human has shortened the distance between fantasy and reality (*22*). The zombie dominates the horror fiction landscape because it has adapted well to the real-life scenario of pandemic outbreaks as represented by and in the media. Like microorganisms themselves, zombies respond well to selection pressures.

Other parallels between the infection process and the infectious zombie text are apparent. In 2007's *28 Weeks Later* (directed by Juan Carlos Fresnadillo), a woman and son appear to be immune to infection but carry the Rage virus to susceptible populations. The novel *I Am Legend* (*10*) describes the scientific methods applied to isolate the cause of the undead plague, but ultimately it is the evolution of the agent of zombie infection that enables the survival of the host and the pathogen in the novel (although the monsters are infected with the "vampiris bacillus," their behavior by night is zombie-like). Likewise, in *The Girl with All the Gifts* (*23*), airborne fungal spores ultimately bring about the extinction of the human race: the new world is populated by partially immune, but infected, children. Killing the host limits spread of infection and survival of the pathogen. In these novels, symbiosis is advantageous to both partners in the host–parasite relationship.

Tolerance of infection leads to recovery in the novel *Warm Bodies* (*24*); the immune response is stimulated when the host begins to interact socially with humans. *Breathers: A Zombie's Lament* (*25*) is narrated by a zombie who regains his self-confidence through attendance at "Undead Anonymous" meetings and becomes a champion for zombie rights (with a taste for human flesh). In comparable texts in other media, such as the television series *In the Flesh* (written and produced by Dominic Mitchell, 2013–2014) and *iZombie* (directed by Rob Thomas, 2016–present), zombies are also likable main characters who suffer at the hands of a society that does not understand them. This cross-media development suggests that the zombie condition is evolving heterogeneously, sometimes (especially in melodrama and romance fiction) moving away from the image of the monstrous apocalyptic vector and into a more individually focused host–parasite relationship. The sentient zombie of *Breathers* or *Warm Bodies* can cohabit the same cultural space as the more traditional aggressors of Seth Grahame-Smith's book *Pride and Prejudice and Zombies* (*26*) and Darren Shan's *Zom-B* (*27*) series, and even zombie-like creatures, such as the rabid attackers of David Moody's *Hater* (*28*). What unites all these zombies is a similar approach to the cause of their ontological status, namely, infection as the point of origin.

In contemporary zombie fiction, 3 different contagion outcomes predominate that parallel the pathogenesis of infection: success of the predator, mutualism, or a defeat of the predator. Our innate knowledge of real disease epidemiology is thus illustrated in much zombie literature by the behavior of the humans who are under threat. In the absence of any treatment strategy, options are restricted to quarantine (isolation of the infected, as in Cherie Priest's *Boneshaker* [*29*]), immunization strategies (protection of the uninfected in *Warm Bodies*, Charlie Higson's *The Enemy* [*30*], and Jonathan Maberry's *Rot and Ruin* [*31*]), and control (extermination of the agent in Max Brooks' *World War Z* [*32*]). As zombies become the manifestation of virulent infection, they do not just address our fear of pandemic disease and apocalypse; they also allow us to explore coping strategies.

Pathogens Influencing Zombie Epidemiology

Viruses are the perfect mechanistic microbiological comparison for the zombie, whose sole function is essentially to replicate/transmit the infection. Although some bacterial infections have had an impact on a global scale, viral

pandemics are a greater threat: viruses replicate inefficiently, frequently creating different versions of themselves against which we have reduced or no immunological defense (*33*). The attributes of airborne transmission, high infection rate, and high virulence are the worst possible outcome for humanity because airborne transmission is extremely difficult to control or prevent, a high infection rate ensures high numbers of cases, and high virulence results in high rates of illness and death (as depicted in the 2011 infectious disease–themed movie *Contagion*, directed by Steven Soderbergh). In addition, the incubation period needs to be sufficiently long to enable others to become infected. These patterns have been followed in popular fictional narratives. For example, Ebola virus disease has varying infectivity and virulence: in the 1976 Zaire and Sudan outbreaks, infectivity was relatively low (contact with infected fluids was the route of infection), but fatality rates were high, approaching 90%, whereas in the 2014 outbreak, the fatality rate was 50% (*34*). Ebola epidemiology enabled dramatic scenarios in the book *The Hot Zone* (*35*) and the movie *Outbreak* (directed by Wolfgang Petersen, 1995). The zombie apocalypse draws from such scenarios, yet simultaneously eclipses them all in its scale.

The epidemiology of viral infection we have described may not always be directly applicable to the spread of the zombie condition (e.g., airborne infection happens only when the zombie origin is fungal), but noteworthy patterns do emerge. The novel *World War Z* (as opposed to the 2013 movie of the same name, directed by Marc Forster, which bears little resemblance to the novel) is a good example of how zombie fiction uses real epidemiologic scares to shape the ultimate viral zombie horror narrative. In microbiological terms, the book describes the emergence and spread of a pandemic whose infection and mortality rates are 100%, with an incubation period of a few days, whose symptoms make those infected extremely dangerous to society, and for which there is no treatment. Inactivation of infected persons by destruction of the brain becomes the only solution and prevention strategy. The infection is not airborne; rather, it is transmitted by biting or entry of infected tissue through injured skin and via transplants. Still, its other traits correlate with those of several true infectious agents, such as rabies virus, Creutzfeldt-Jakob prion disease, cytomegalovirus, herpes virus, and HIV (*33*). The zombie incubation period in the novel is also extended ("slow burns") if a major blood vessel is missed during biting. This particular aspect of the virus is itself borrowed from rabies, in which a longer incubation period results from a bite to the leg rather than a bite to the neck. As with many influenza pandemics and severe acute respiratory syndrome, *World War Z*'s pandemic begins in China. In this novel, there are other localized outbreaks, but the pandemic develops via misinformation and obfuscation—as occurred with the spread of severe acute respiratory syndrome from China. Politics plays a major part in the spread of the pandemic.

The zombie, an insentient creature with a tendency to swarm, has been used in several disciplines in recent years to shed light on the dynamics of economics, capitalism, and international politics and to channel fears connected to social alienation, especially as a result of digital and communication technologies and overpopulation (*22*,*36*–*38*). In contrast to the intellectual and allegorical use of the zombie in such disciplines, articles describing the epidemiologic properties and preventive measures in the event of a "zombie outbreak" have been presented in the scientific literature in a more ironic tone, nevertheless taking cues from emerging public interest in zombies. For example, *BMJ* has provided information on epidemiology, treatment, and prevention (*39*). However, the use of zombie epidemiology as an education tool requires careful planning; for instance, the CDC's section on "zombie preparedness" (http://www.cdc.gov/phpr/zombies.htm) has been accused of "trivialization" of the preparedness topic (*40*).

At least 1 of the popular reimaginings of the late postmillennial zombie proposes that zombified humans may have a "new strain of prion disease." In the novel *Zombie Autopsies: Secret Notes from the Apocalypse* (*41*), the private notes of a neurodevelopmental biologist written in a remote laboratory setting "where the world community could focus its efforts on the scientific study of ANSD [Ataxic Neurodegenerative Satiety Deficiency Syndrome]" describe the dissections of 3 zombie subjects before the author succumbs to the disease himself. This narrative is framed as the main section of a highly confidential memorandum from the United Nations outpost. Two working hypotheses on the nature of the pandemic are proposed. The first is that ANSD may be caused by an airborne engineered plague, a symbiosis that would result in 3 contagions operating through a single vector, specifically a combination of influenza and prion and a third unknown infectious agent. The second option is that humans may be faced "with something new… with distinct and adaptive properties. Something that hijacks the host."

Two different fictional worlds, in Charlie Higson's *The Enemy* series (2009–2015) (*30*) and *The Girl with All the Gifts* (*23*), adopt and modify the "zombie fungus" *Ophiocordyceps unilateralis* as the apocalyptic zombie agent. In both cases, airborne fungal spores provide an inescapable source of infection, with the sporulation cycle being critical to the plot. Mira Grant's novel *Feed* (*42*) is the first volume in a series following life in a postapocalyptic America where a third of the population has succumbed to the Kellis-Amberlee virus; zombies are a result of an ecoterrorism act that "released a half-tested 'cure for the common cold' into the atmosphere." The novel's dormant pathogen is based on *Toxoplasma gondii*, a pathogen that would not wipe out

the entire susceptible population. In all these new novels, the aim is to provide a microbiologically accurate backdrop and story in which the population either succumbs to infection or learns to cope with a pathogen—for example, in *Feed*, through out-of-bounds contamination areas, complex decontamination routines, and constant screening.

Books like *Zombie Autopsies* shows how new infectious zombie texts act as virological repositories. The opposite is also true; since *I Am Legend*, several publications have attempted to use the symptoms of zombieism to explain the workings of specific pathogens and scientific principles. Many examples exist; a particularly notable one is *Do Zombies Dream of Undead Sheep? A Neuroscientific View of the Zombie Brain* (*43*), largely an introduction to neurology, in which consciousness deficit hypoactivity disorder (CDHD) is deemed to be the result of infection from external pathogens that hijack human systems, which could be caused by either *Cordyceps*-style fungi (as in *The Girl with All the Gifts*), prion disease, or evolved brain tapeworms (parasites or protozoans). The zombie status is used here to explain viral attacks on the brain, as well as to describe how this organ generally operates. A book like *Do Zombies Dream of Undead Sheep?* shows both the tremendous reach of the zombie in the 21st century and how fiction may, in turn, end up delivering the very science it uses as inspiration.

Conclusion: Zombies and Emerging Infectious Literatures

New infectious zombie texts evince the main shifts in fictional representations of infection narratives; in them, symptoms and epidemiology are often based on real infections. We have termed the wider phenomenon within which the zombie narrative has manifested "emerging infectious literatures," an echo of the term "emerging infectious diseases." Generically, emerging infectious literatures are varied: some show clear horrific leanings, whereas others are more obviously defined as science fiction or thrillers. Influenza is a particularly malleable candidate for such narratives; the varying possible rates of transmission and virulence have been used to frame different postapocalyptic scenarios since the publication of Stephen King's *The Stand* (*44*). For example, in *Immunity* (*45*), 4% of the population is lost in a matter of months, and screening is deployed to detect the infected, coupled with immunization of selected persons. In *Station Eleven* (*46*), a much more virulent strain almost wipes out humanity; survivors are few and required to construct new, small, civilizations. Yet another book, *Not Forgetting the Whale* (*47*), focuses on how an isolated Cornish community manages to avoid succumbing to an outbreak affecting the urban environment. Novels about the future impact of antimicrobial resistance are as yet few and far between, but no less interesting: *A Fierce Radiance* (*48*) describes the industrial production of antimicrobial drugs during World War II, the prioritization of combat troops to receive treatment, and the impact of antimicrobial drugs on public health, and *The Deep Zone* (*49*) is concerned with the discovery of new antimicrobial drugs in unusual environments (caves), couched in industrial and political espionage. More recently, short stories (for example, *Infectious Futures*, published by NESTA [http://www.nesta.org.uk/search?search_api_views_fulltext=Infectious%20futures]), comic books (*50*), and other public information efforts are attempting to raise awareness and change behavior. Perhaps Zika virus and Middle East respiratory syndrome will provide inspiration for the next epidemiologic antiheroes. Zombies will likely remain a returning concern for epidemiologically inclined writers.

To return to our initial premise, the book club format successfully allows discussion between experts and nonexperts about the overlaps between pandemic fact and fiction. Through these discussions, participants can focus on key messages about disease and infection that underpin the fiction narrative. Meeting reports and reading guides posted on the Bad Bugs Book Club website over the past 9 years provide evidence of the success of multiway discussion, and are a rich resource for others wishing to engage in similar activities. Book club discussions have enabled identification of different themes emerging from such texts, the most notable of which has, for us, been the zombie infection narrative. In the case of the novels discussed throughout this article, our meetings helped us come to grips with the contemporary significance, porosity, and ubiquity of the zombie as contemporary monster. The zombie has enabled the exploration of our behaviors when confronted with infection and served as an indication of how fiction reflects current knowledge about pandemics. In the same way that the changing virulence of pathogens has occurred throughout history, the zombie trope is flexible, something that has enabled its survival in 21st century literature. Emerging infectious diseases and their management likewise provide a rich lode for continuous exploration of the microbiological present and potential future in fiction.

About the Authors

Dr. Verran is a professor of microbiology at Manchester Metropolitan University, Manchester, UK. Her laboratory-based research focuses on the interactions occurring between microorganisms and inert surfaces, but she also investigates how art and literature can facilitate discussion and enhance understanding of infectious disease epidemiology among students and public audiences.

Dr. Aldana Reyes is a senior lecturer in English literature and film at Manchester Metropolitan University and a founding member of the Manchester Centre for Gothic Studies.

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Address for correspondence: Joanna Verran, Manchester Metropolitan University School of Healthcare Science, Chester Street, Manchester M1 5GD, UK; email: J.Verran@mmu.ac.uk

Paul Klee (1879–1940), *Tropische Dämmerung (Tropical Twilight)*, 1921. Oil on white primer on paper on cardboard; 13.5 in × 9.1 in/33.5 cm × 23 cm. Fondation Beyeler, Riehen/Basel, Switzerland; Beyeler Collection; Photo: Robert Bayer.

Tropical Sunset Blues

Byron Breedlove and Paul M. Arguin

"Color is the place where our brain and the universe meet," according to painter, printmaker, draftsman, and teacher Paul Klee. This month's cover image, *Tropische Dämmerung (Tropical Twilight)*, is a shimmering example of Klee's early work during his very productive decade at the German art school Bauhaus. In this painting, Klee uses colors, shapes, and forms that defy expectations for a tranquil twilight in the tropics, in some ways suggestive of the peculiar, imaginary plant kingdom envisioned half a century later in Leo Lionni's book *Parallel Botany*. Brisk, pale brushstrokes make up the broad leaves and twitching tendrils of vegetation set against a menacing crimson sunset. A crosshatched fence bisects the canvas and is repeated again in the lower left. Hieroglyphic shapes, particularly a starlike symbol, contrast with his organic forms, creating additional tension to the scene. The viewer scans, ruminates, and ponders how Klee's iconography, arrangement, and color form a seamless and foreboding snapshot of the tropics at that moment when colors fade into darkness.

Klee was born in Münchenbuchsee, Switzerland, in 1879. According to a Tate Museum biography, "Klee came from a generation that would shape the modern world.

Author affiliation: Centers for Disease Control and Prevention, Atlanta, Georgia, USA

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Albert Einstein . . . was born in March 1879, Georges Braque and Pablo Picasso in 1880 and 1881 respectively." Klee is remembered as a prodigious artist, caring teacher, talented violinist, and thoughtful writer. His father, Hans Wilhelm Klee, a German music teacher, and his mother, Ida Marie Klee, a Swiss singer, no doubt nurtured his interest and instruction in music, but Klee shifted his attention to visual arts while a teenager and, starting in 1898, he studied drawing and painting in Munich, Germany. Early in his career, Klee struggled to find his path as an artist, noting after a trip to Italy "that a long struggle lies in store for me in this field of color."

When he returned to Munich in 1911, Klee became involved with Der Blaue Reiter (The Blue Rider), an organization of diverse artists founded by Wassily Kandinsky and Franz Marc. Those associations and travels to Paris in 1912 exposed the young artist to emerging, new theories about color and art forms and lead to his discovery of work by Pablo Picasso and Georges Braque. It was during a brief excursion to Tunisia in 1914, however, that Klee experienced an epiphany about color, attributed to the quality of light there, leading him to boldly proclaim, "Colour has taken possession of me; no longer do I have to chase after it, I know that it has hold of me forever... Colour and I are one. I am a painter." Klee taught at the Bauhaus from 1921 to 1931, and in 1923, Kandinsky and Klee formed Die Blaue Vier (The Blue Four).

His influences and his output reveal a gracious fluidity in his vast trove of artwork, thought to comprise 9,000–10,000 works. Identifying Klee by a single category or school of art—whether cubist, abstract, surrealist, expressionist, or perhaps Dadaist—is simply not possible. Klee worked simultaneously on multiple projects in various media, and those could also be quite dissimilar in style and approach. (Klee was also ambidextrous: he used his left hand to paint, his right hand to write.) Alexxa Gotthardt, staff writer and editor for *Arsty*, explains that "Klee's body of work isn't easily bucketed into a single category, thanks in large part to the system of throbbing forms, mystical hieroglyphs, and otherworldly creatures that he developed to populate his compositions."

Whether Klee's rendering of a tropical twilight came from a place he visited or imagined does not matter. But Klee asserted in his 1920 *Creative Confession* that "Art does not reproduce the visible, rather, it makes visible." We see the tropical twilight through his eyes as both the natural beauty and the hidden dangers together.

It is at this juncture between day and night when crepuscular fauna are active and the stealthy nocturnal denizens of the tropics begin to stir. Before you can see them, the sting of that first sandfly bite is often the signal that you have lingered too long watching the sunset and it is time to head inside for the evening. Tiny female *Anopheles* mosquitoes also become active at this time, seeking a blood meal for sustenance while perpetuating the devastating cycle of malaria infections. Alphonse Laveran, the scientist who discovered the malaria parasite *Plasmodium falciparum,* died in 1922, the year after Klee finished this arresting painting that evokes the world's tropical areas. By 2050, according to the report *State of the Tropics,* more than half of the world's population and 60% of children will live in the tropics. Within the tropics, malaria, the World Health Organization's 17 neglected tropical diseases, and numerous other zoonotic infections such as leptospirosis and human trypanosomiasis are leading causes of death and disability in humans.

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Address for correspondence: Byron Breedlove, EID Journal, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop H16-2, Atlanta, GA 30329-4027, USA; email: wbb1@cdc.gov

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Article Title

National Surveillance for *Clostridioides difficile* Infection, Sweden, 2009–2016

CME Questions

1. You are advising a large Swedish hospital regarding anticipated needs for *Clostridioides difficile* infection (CDI). According to the analysis of data from the Swedish national surveillance program by Rizzardi and colleagues, which of the following statements about CDI incidence rates and resistance from 2012 to 2016 is correct?

A. National CDI incidence increased by 22% from 2012 to 2016
B. The proportion of multidrug-resistant (MDR) isolates increased by 18% from 2012 to 2016
C. Among European countries, Sweden still has a comparatively high CDI incidence
D. From 2012 to 2016, geographical variation in incidence among counties increased

2. According to the analysis of data from the Swedish national surveillance program by Rizzardi and colleagues, which of the following statements about distribution of CDI types, including known outbreaks from 2012 to 2016, is correct?

A. High incidence of CDI in Sweden is best explained by nationwide outbreaks
B. RT017 was the most common polymerase chain reaction (PCR) ribotype throughout the whole period from 2012 to 2016
C. Ribotypes frequently associated with MDR isolates were most common in 2014 and 2015
D. A clonal outbreak not always apparent in clinical practice or in infrequent surveillance programs could explain geographical clustering of MDR isolates

3. According to the analysis of data from the Swedish national surveillance program by Rizzardi and colleagues, which of the following statements about the impact of diagnostic methods on CDI incidence and other possible reasons for changes in incidence and resistance is correct?

A. A decrease in CDI incidence and resistance is caused exclusively by decreased antibiotic use
B. The disappearance of geographical clusters of specific *C. difficile* PCR ribotypes indicates reduced nosocomial spread
C. Use of stand-alone nucleic acid amplification testing (NAAT) explains higher CDI incidence
D. Minimal inhibitory concentration distributions of isolates collected in Sweden between 2009 and 2016 were very similar to those from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for all tested antibiotics

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Manuscript Submission. To submit a manuscript, access Manuscript Central from the Emerging Infectious Diseases web page (www.cdc.gov/eid). Include a cover letter indicating the proposed category of the article (e.g., Research, Dispatch), verifying the word and reference counts, and confirming that the final manuscript has been seen and approved by all authors. Complete provided Authors Checklist.

Manuscript Preparation. For word processing, use MS Word. Set the document to show continuous line numbers. List the following information in this order: title page, article summary line, keywords, abstract, text, acknowledgments, biographical sketch, references, tables, and figure legends. Appendix materials and figures should be in separate files.

Title Page. Give complete information about each author (i.e., full name, graduate degree(s), affiliation, and the name of the institution in which the work was done). Clearly identify the corresponding author and provide that author's mailing address (include phone number, fax number, and email address). Include separate word counts for abstract and text.

Keywords. Use terms as listed in the National Library of Medicine Medical Subject Headings index (www.ncbi.nlm.nih.gov/mesh).

Text. Double-space everything, including the title page, abstract, references, tables, and figure legends. Indent paragraphs; leave no extra space between paragraphs. After a period, leave only one space before beginning the next sentence. Use 12-point Times New Roman font and format with ragged right margins (left align). Italicize (rather than underline) scientific names when needed.

Biographical Sketch. Include a short biographical sketch of the first author—both authors if only two. Include affiliations and the author's primary research interests.

References. Follow Uniform Requirements (www.icmje.org/index.html). Do not use endnotes for references. Place reference numbers in parentheses, not superscripts. Number citations in order of appearance (including in text, figures, and tables). Cite personal communications, unpublished data, and manuscripts in preparation or submitted for publication in parentheses in text. Consult List of Journals Indexed in Index Medicus for accepted journal abbreviations; if a journal is not listed, spell out the journal title. List the first six authors followed by "et al." Do not cite references in the abstract.

Tables. Provide tables within the manuscript file, not as separate files. Use the MS Word table tool, no columns, tabs, spaces, or other programs. Footnote any use of boldface. Tables should be no wider than 17 cm. Condense or divide larger tables. Extensive tables may be made available online only.

Figures. Submit editable figures as separate files (e.g., Microsoft Excel, PowerPoint). Photographs should be submitted as high-resolution (600 dpi) .tif or .jpg files. Do not embed figures in the manuscript file. Use Arial 10 pt. or 12 pt. font for lettering so that figures, symbols, lettering, and numbering can remain legible when reduced to print size. Place figure keys within the figure. Figure legends should be placed at the end of the manuscript file.

Videos. Submit as AVI, MOV, MPG, MPEG, or WMV. Videos should not exceed 5 minutes and should include an audio description and complete captioning. If audio is not available, provide a description of the action in the video as a separate Word file. Published or copyrighted material (e.g., music) is discouraged and must be accompanied by written release. If video is part of a manuscript, files must be uploaded with manuscript submission. When uploading, choose "Video" file. Include a brief video legend in the manuscript file.

Types of Articles

Perspectives. Articles should not exceed 3,500 words and 50 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles should provide insightful analysis and commentary about new and reemerging infectious diseases and related issues. Perspectives may address factors known to influence the emergence of diseases, including microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures.

Synopses. Articles should not exceed 3,500 words in the main body of the text or include more than 50 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (not to exceed 150 words), a 1-line summary of the conclusions, and a brief biographical sketch of first author or of both authors if only 2 authors. This section comprises case series papers and concise reviews of infectious diseases or closely related topics. Preference is given to reviews of new and emerging diseases; however, timely updates of other diseases or topics are also welcome. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

Research. Articles should not exceed 3,500 words and 50 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Report laboratory and epidemiologic results within a public health perspective. Explain the value of the research in public health terms and place the findings in a larger perspective (i.e., "Here is what we found, and here is what the findings mean").

Policy and Historical Reviews. Articles should not exceed 3,500 words and 50 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles in this section include public health policy or historical reports that are based on research and analysis of emerging disease issues.

Dispatches. Articles should be no more than 1,200 words and need not be divided into sections. If subheadings are used, they should be general, e.g., "The Study" and "Conclusions." Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed 2); tables (not to exceed 2); and biographical sketch. Dispatches are updates on infectious disease trends and research that include descriptions of new methods for detecting, characterizing, or subtyping new or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome.

Research Letters Reporting Cases, Outbreaks, or Original Research. EID publishes letters that report cases, outbreaks, or original research as Research Letters. Authors should provide a short abstract (50-word maximum), references (not to exceed 10), and a short biographical sketch. These letters should not exceed 800 words in the main body of the text and may include either 1 figure or 1 table. Do not divide Research Letters into sections.

Letters Commenting on Articles. Letters commenting on articles should contain a maximum of 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication.

Commentaries. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references (not to exceed 15) but no abstract, figures, or tables. Include biographical sketch.

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

Books, Other Media. Reviews (250–500 words) of new books or other media on emerging disease issues are welcome. Title, author(s), publisher, number of pages, and other pertinent details should be included.

Conference Summaries. Summaries of emerging infectious disease conference activities (500–1,000 words) are published online only. They should be submitted no later than 6 months after the conference and focus on content rather than process. Provide illustrations, references, and links to full reports of conference activities.

Online Reports. Reports on consensus group meetings, workshops, and other activities in which suggestions for diagnostic, treatment, or reporting methods related to infectious disease topics are formulated may be published online only. These should not exceed 3,500 words and should be authored by the group. We do not publish official guidelines or policy recommendations.

Photo Quiz. The photo quiz (1,200 words) highlights a person who made notable contributions to public health and medicine. Provide a photo of the subject, a brief clue to the person's identity, and five possible answers, followed by an essay describing the person's life and his or her significance to public health, science, and infectious disease.

Etymologia. Etymologia (100 words, 5 references). We welcome thoroughly researched derivations of emerging disease terms. Historical and other context could be included.

Announcements. We welcome brief announcements of timely events of interest to our readers. Announcements may be posted online only, depending on the event date. Email to eideditor@cdc.gov.

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