day 11, tiny colonies grew poorly on sheep blood agar (Appendix Figure, https://wwwnc.cdc.gov/EID/article/27/12/21-0649-App1.pdf). We did not observe growth on chocolate, MacConkey, or Columbia colistin-nalidixic acid agars. Microscopic examination of a Gram-stained smear revealed gram-negative rods with bulbar swellings (Appendix Figure). We used matrix-assisted laser desorption/ionization time-of-flight mass spectrometry to confirm the colonies as *S. moniliformis* (score 2.35); we did not conduct susceptibility testing.

We diagnosed subacute polyarticular septic arthritis, which has a recommended treatment of penicillin G (200,000 units 2×/d for 5–7 days); the alternative option is a 4-week course of ceftriaxone. We stopped steroid treatment and prescribed ceftriaxone because the patient had a severe penicillin allergy. She responded very well to intravenous treatment, and her joint pain and swelling improved remarkably. Two months before symptom onset, she had cleaned a research laboratory housing rats and homes that had mousetraps. She was not aware of any bites or scratches. We obtained informed consent for her participation in this research.

S. moniliformis is the etiologic agent of rat-bite fever, which usually causes fever, rash, and arthralgia. However, this patient and others had polyarticular involvement without fever or rash (*5*,*6*). Previous case reports have described *S. moniliformis* as favoring synovial and serosal surfaces (*7*,*8*).

S. moniliformis is difficult to identify because of its fastidious nature and slow growth on culture; as a result, it is sometimes misdiagnosed as inflammatory arthritis. An informed diagnosis requires raised clinical awareness and attention to patient social history. Arthrocentesis should be conducted in any case of suspected septic arthritis. As shown in this case, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry is a useful tool for diagnosing *S. moniliformis* infection.

About the Author
Dr. Uddin is a resident in internal medicine at the University of Pittsburgh, Pittsburgh, Pennsylvania, USA. Her research interests include infectious disease.

References
1. Strangeways WI. Rats and carriers of *Streptobacillus moniliformis*. J Pathol Bacteriol. 1933;37:45–51. https://doi.org/10.1002/path.1700370106
2. Centers for Disease Control and Prevention. Fatal rat-bite fever—Florida and Washington, 2003. MMWR Morb Mortal Wkly Rep. 2005;53:1198–202.
3. Place EH, Sutton LE. Erythema arthriticum epidemicum (Haverhill fever). Arch Intern Med (Chic). 1934;54:659–84. https://doi.org/10.1001/archinte.1934.00160170002001
4. Stehle P, Dubuis O, So A, Dudler J. Rat bite fever without fever. Ann Rheum Dis. 2003;62:894–6. https://doi.org/10.1136/ard.62.9.894
5. Wang TK, Wong SS. *Streptobacillus moniliformis* septic arthritis: a clinical entity distinct from rat-bite fever? BMC Infect Dis. 2007;7:56. https://doi.org/10.1186/1471-2334-7-56
6. Dendle C, Woolley IJ, Korman TM. Rat-bite fever septic arthritis: illustrative case and literature review. Eur J Clin Microbiol Infect Dis. 2006;25:791–7. https://doi.org/10.1007/s10096-006-0224-x
7. Rumley RL, Patrone NA, White L. Rat-bite fever as a cause of septic arthritis: a diagnostic dilemma. Ann Rheum Dis. 1987;46:793–5. https://doi.org/10.1136/ard.46.10.793
8. Adams SH, Mahapatra R. Rat bite fever with osteomyelitis and discitis: case report and literature review. BMC Infect Dis. 2021;21:479–86. https://doi.org/10.1186/s12879-021-06172-x

Address for correspondence: Mohamed Yassin, Division of Infectious Diseases, University of Pittsburgh, Pittsburgh, PA 15219, USA; email: yassinm@upmc.edu

Coxiella burnetii in 3 Species of Turtles in the Upper Midwest, United States

William E. Sander, Richard King, William Graser, Joshua M. Kapfer, Aubrey I. Engel, Laura Adamovicz, Matthew C. Allender

Author affiliations: University of Illinois Urbana–Champaign, Urbana, Illinois, USA (W. E. Sander, A.I. Engel, L. Adamovicz, M.C. Allender); Northern Illinois University, DeKalb, Illinois, USA (R. King); Kane County Forest Preserve, Geneva, Illinois, USA (W. Graser); University of Wisconsin–Whitewater, Whitewater, Wisconsin, USA (J.M. Kapfer)

Coxiella burnetii, the causative bacterium of the zoonotic disease Q fever, has been documented in many different species. We describe documented turtles that were PCR positive for *C. burnetii* from multiple locations in Illinois and Wisconsin, USA. Assessing the conservation implications, reservoir potential, and zoonotic risk requires further research.

Two studies have identified *Coxiella burnetii* in poikilotherms (vertebrates that cannot regulate body temperature physiologically); both studies originated in India. Two tortoises had antibodies to *C. burnetii* by capillary agglutination testing of their serum samples in Uttar Pradesh (*1*). Additional reptiles, including snakes and skinks, had serum samples positive for *C. burnetii* in a separate study in Karnataka (*2*). Although both studies are useful in clarifying how this bacterium might interface with reptiles, there is no other evidence to support the role played by this large class of vertebrates (*3*). Furthermore, serologic assays applied to species that they were not designed for are difficult to interpret (Appendix, https://wwwnc.cdc.gov/EID/article/27/12/21-1278-App1.pdf).

Serologic testing, typically using indirect immunofluorescence assay, is the primary method used to diagnose *C. burnetii* infection, which causes Q fever in humans and coxiellosis in domestic ruminants (*4*). Additional serologic testing includes complement fixation and ELISA (*5*). Serologic assay benefits include commercial availability and insights into acute, treated, and chronic patients, depending on titers (*6*). Several PCR-based assays have been developed for detection of *C. burnetii* in samples from nontraditional mammals, birds, and arthropods (*7*). PCR provides a simple and reliable method for detection of the bacterium even retrospectively from tissues (*6*). Therefore, we tested turtles from multiple locations in Illinois and Wisconsin, USA, for *C. burnetii*.

This study was approved by the institutional animal care and use committees of the University of Illinois (20258), Northern Illinois University (LA16–0016), and University of Wisconsin–Whitewater (K145011020Q). The Wildlife Epidemiology Laboratory, based at the University of Illinois College of Veterinary Medicine, continually conducts long-term, prospective health assessments of several turtle species across Illinois and neighboring states in natural habitats. Reptiles can be an excellent proxy for the health of environments, and many turtle species have small home ranges with diverse diets reflecting local conditions (*8*).

As part of these annual surveys, turtle species collected have various morphometric data, blood samples, or oral and cloacal swab specimens obtained before being released. Several diagnostic tests are performed with these samples, such as PCR screening for several pathogens, including *C. burnetii*. Other pathogenic organisms include *Ambystoma tigrinum* virus, Bohle iridovirus, Terrapene herpesvirus 1, Terrapene herpesvirus 2, epizootic hematopoietic necrosis virus, *Emydomyces testavorans*, frog virus 3, Emydid herpesvirus 1, Emydoidea herpesvirus 1 (in Blanding's turtles), *Mycoplasma agassizii*, *M. testudineum*, *Salmonella* spp., and Testudinid herpesvirus 2 (*9*).

We extracted DNA from frozen, combined oral/cloacal swab specimens from each turtle by using the DNA Blood Mini Kit (QIAGEN, https://www.qiagen.com). We assessed spectrophotometrically DNA concentration and purity by using NanoDrop 1000 (Thermo Fisher Scientific Inc., https://www.thermofisher.com). We performed quantitative PCR by using a QuantStudio3 Real Time PCR System (Applied Biosystems, https://www.thermofisher.com) and a TaqMan primer–probe assay targeting the *C. burnetti icd* gene as described (*10*).

We assayed all samples, standards, and non-template controls in triplicate and quantified positive samples by using a 7-point standard curve (10^1–10^7 target copies). Samples were considered positive if all 3 replicates had a lower cycle threshold value than the lowest detected standard dilution. We used a highly sensitive and specific quantitative PCR for *C. burnetti*.

During 2019, samples from 5/605 turtles encountered across 8 counties showed positive results for quantitative PCRs, indicating presence of *C. burnetii* (Figure). We collected positive samples from 3 Blanding's turtles (*Emydoidea blandingii*), 1 painted turtle (*Chrysemys picta*), and 1 ornate box turtle (*Terrapene ornata*). These positive turtles were found in Kane and Lee Counties in Illinois and Sauk County in Wisconsin. We did not perform serologic analysis for these animals. One Blanding's turtle had a microchip and transmitter, was sampled again during 2020, and showed a negative PCR result. All of these turtles were found within a 1-hour drive to the Illinois–Wisconsin state border within protected preserves. However, the 3 locations in which the 5 turtles varied in proximity to farms, livestock, industry, residential areas, and major highways; we found no geographic associations. All other screening tests showed negative results for pathogenic organisms for these 5 animals.

C. burnetii is a ubiquitous bacterium that has been found in many different species, often

without pathogenicity (*4*). A variety of species of turtles are sampled annually in Illinois and surrounding areas through the Wildlife Epidemiology Laboratory. Over time, the testing for various organisms has expanded, especially as additional tests are validated. Screening for the

bacterium that causes Q fever has been conducted for many species but infrequently in poikilotherms. These results show that the bacteria can be detected in these species and should be further researched to understand additional sources of this reportable disease, including potential management or regulatory decisions.

Continued investigation and screening in poikilotherms for zoonotic pathogens should be prioritized to understand the potential risk from additional hosts. The pet trade is a potential avenue of risk for exposure between humans and turtles. As these pathogens of concern are better characterized, the implications of different and varied hosts will drive the need for continued One Health research and dialogue between environmental, animal, and human health professionals.

About the Author

Dr. Sander is an assistant professor of preventive medicine and public health and director of the DVM/MPH joint degree program in the Department of Veterinary Clinical Medicine, University of Illinois College of Veterinary Medicine, Urbana, IL. His primary research interest is One Health approaches, including the interface of infectious disease and toxicities between humans and animals both domestically and internationally.

Figure. Location (gray areas) of turtles PCR positive for *Coxiella burnetii*, by county, Wisconsin (top) and Illinois (bottom), USA.

References

1. Yadav MP, Sethi MS. Poikilotherms as reservoirs of Q-fever (*Coxiella burnetii*) in Uttar Pradesh. J Wildl Dis. 1979;15:15–7. https://doi.org/10.7589/0090-3558-15.1.15
2. Stephen S, Rao KN. Coxiellosis in reptiles of South Kanara district, Karnataka. Indian J Med Res. 1979;70:937–41.
3. Marschang RE. Clinical virology. In: Mader DR, Divers JJ. Current therapy in reptile medicine and surgery. New York: Elsevier Inc.; 2013. p. 32–52.
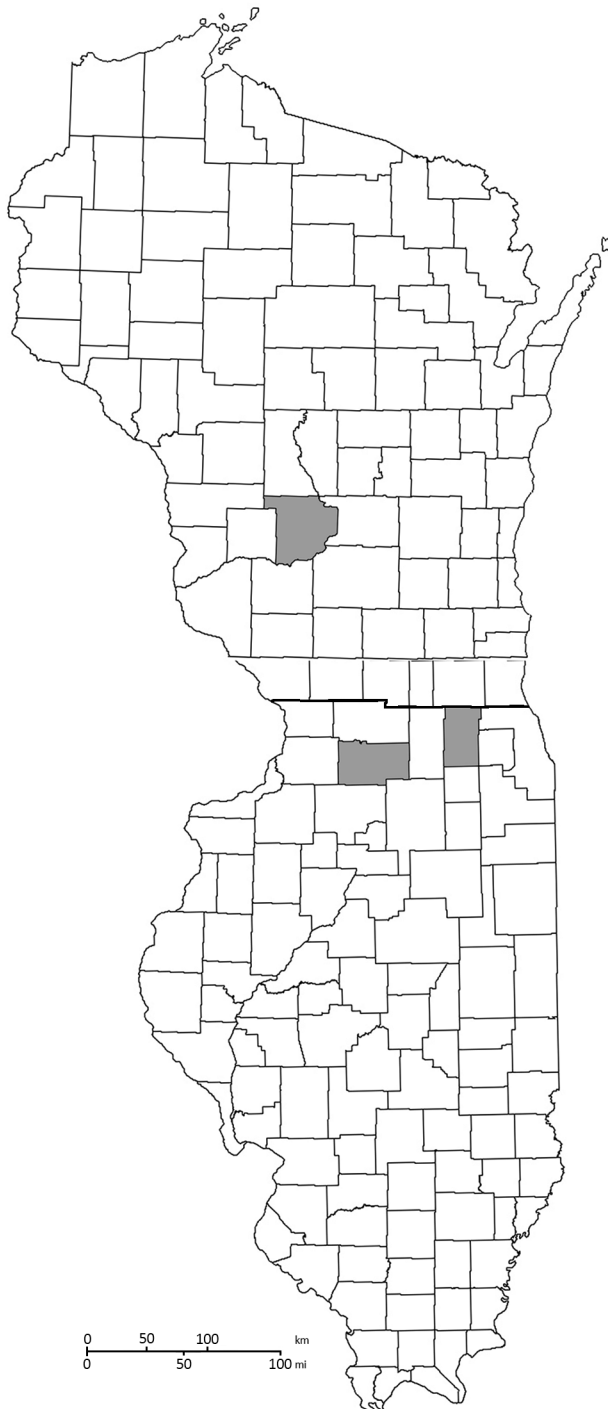4. Tissot-Dupont H, Raoult D. Q fever. Infect Dis Clin North Am. 2008;22:505–14, ix. https://doi.org/10.1016/j.idc.2008.03.002

5. Eldin C, Mélenotte C, Mediannikov O, Ghigo E, Million M, Edouard S, et al. From Q fever to *Coxiella burnetii* infection: a paradigm change. Clin Microbiol Rev. 2017;30:115–90. https://doi.org/10.1128/CMR.00045-16

6. Fournier P-E, Marrie TJ, Raoult D. Diagnosis of Q fever. J Clin Microbiol. 1998;36:1823–34. https://doi.org/10.1128/JCM.36.7.1823-1834.1998

7. Sahu R, Rawool DB, Vinod VK, Malik SV, Barbuddhe SB. Current approaches for the detection of *Coxiella burneti*i infection in humans and animals. J Microbiol Methods. 2020;179:106087. https://doi.org/10.1016/j.mimet.2020.106087

8. Way Rose BM, Allender MC. Health assessment of wild eastern box turtles (*Terrapene carolina carolina*) in east Tennessee. J Herpetological Med Surg. 2011;21:107. https://doi.org/10.5818/1529-9651-21.4.107

9. Archer GA, Phillips CA, Adamovicz L, Band M, Byrd J, Allender MC. Detection of copathogens in free-ranging eastern box turtles (*Terrapene carolina carolina*) in Illinois and Tennessee. J Zoo Wildl Med. 2017;48:1127–34. https://doi.org/10.1638/2017-0148R.1

10. Klee SR, Tyczka J, Ellerbrok H, Franz T, Linke S, Baljer G, et al. Highly sensitive real-time PCR for specific detection and quantification of *Coxiella burnetii*. BMC Microbiol. 2006;6:2. https://doi.org/10.1186/1471-2180-6-2

Reassortant Influenza A(H1N1)pdm09 Virus in Elderly Woman, Denmark, January 2021

Jakob N. Nissen, Sophie J. George, Charlotte K. Hjulsager, Jesper S. Krog, Xiaohui C. Nielsen, Tina V. Madsen, Klara M. Andersen, Tyra G. Krause, Lasse S. Vestergaard, Lars E. Larsen, Ramona Trebbien

Author affiliations: Statens Serum Institut, Copenhagen, Denmark (J.N. Nissen, C.K. Hjulsager, J.S. Krog, K.M. Andersen, T.G. Kause, L.S. Vestergaard, R. Trebbien); University of Copenhagen, Copenhagen (S.J. George, L.E. Larsen); Zealand University Hospital, Koege, Denmark (X.C. Nielsen, T.V. Madsen)

A case of human infection with influenza A(H1N1)pdm09 virus containing a nonstructural gene highly similar to Eurasian avian-like H1Nx swine influenza virus was detected in Denmark in January 2021. We describe the clinical case and report testing results of the genetic and antigenic characterizations of the virus.

Human infection with swine influenza A virus (IAV) had not previously been detected in Denmark, but sporadic cases have been reported from other countries (*1*). We report the identification of a case of zoonotic swine influenza infection in Denmark during a low-activity influenza season.

The variant IAV was detected by the National Influenza Center at Statens Serum Institut (Copenhagen, Denmark), as part of routine surveillance. A sputum sample was collected on January 21, 2021, in Zealand, Denmark, from a female patient in her 70s with various concurrent conditions, including a chronic respiratory disease, who was admitted to hospital after 2 days of moderate influenza-like symptoms: fever (39°C), coughing, sore throat, and difficulty breathing. The patient sample was positive for IAV in analyses at the local hospital microbiology laboratory; remaining sample material was submitted to the National Influenza Center, which confirmed it positive for influenza A(H1N1)pdm09 (Appendix, https://wwwnc.cdc.gov/EID/article/27/12/21-1361-App1.pdf).

We performed whole genome sequencing on the virus (*2*), and named it A/Denmark/1/2021 (vH1N1), and submitted to GISAID (https://www.gisaid.org; accession no. EPI_ISL_909652). BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and phylogenetic analyses revealed that all segments except the nonstructural gene belonged to influenza A(H1N1)pdm09 clade 1A3.3.2 (*3*), which is most similar (97%–98% nt identity) to viruses collected from swine in France and Germany in 2014 and 2015 (Table; Figure). The nonstructural gene was most similar (95%) to Eurasian avian-like H1Nx swine viruses of clade 1C. No segments had a near-exact match to sequences in GenBank or GISAID, and all were distinct from the seasonal vaccine strain, A/Guangdong-Maonan/SWL1536/2019 (Table).

Because of the suspected swine origin of the case virus, we used whole-genome sequencing to retrospectively analyze 68 IAVs with a hemagglutinin (HA) gene belonging to clade 1A.3.3.2 sampled from swine herds in Denmark during 2020–2021. Nine of the samples, collected April 2020–January 2021 from ≥7 different herds in different parts of Denmark, including Zealand, contained the same