

EMERGING INFECTIOUS DISEASES[®]



Microbial World

October 2025



Mija-tesse Ververs (1962–), *The Pathogen Quilt*, 2021 (detail). Fabric, beads, and iron wire. 43 in x 45 in/110 cm x 114 cm. Personal collection. Digital image courtesy of Mija-tesse Ververs.

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On the Cover

Mija-tesse Ververs (1962–), *The Pathogen Quilt*, 2021. Fabric, beads, and iron wire. 43 in × 45 in/110 cm × 114 cm. Personal collection. Digital image courtesy of Mija-tesse Ververs.

About the Cover p. 2068

Synopses

Retrospective Analysis of Historical *Listeria monocytogenes* Clinical Isolates, New York, USA, 2000–2021

H. Samut et al. 1883

Organ Donor Transmission of *Rickettsia typhi* to Kidney Transplant Recipients, Texas, USA, 2024

J.C. Jones et al. 1893

Research

Medscape
EDUCATION
ACTIVITY

Recent Systemic Antifungal Exposure and Nonsusceptible *Candida* in Hospitalized Patients, South Africa, 2012–2017

Previous antifungal use drives *Candida* resistance in older patients but does not explain resistance in young infants.

C. Rabault et al. 1901

Medscape
EDUCATION
ACTIVITY

Reptile Exposure in Human Salmonellosis Cases and *Salmonella* Serotypes Isolated from Reptiles, Ontario, Canada, 2015–2022

Awareness of serotypes associated with reptile and amphibian contact can inform public health investigation by local authorities.

K. Paphitis et al. 1912

Comparative Epidemiology of *Salmonella enterica* Serovars Paratyphi A and *Salmonella* Typhi Causing Enteric Fever, Bangladesh, 2018–2020

S.I.A. Rahman et al. 1922

Prolonged Monkeypox Virus Infections, California, USA, May 2022–August 2024

S.H. Schildhauer et al. 1935

Differences in COVID-19 Fatality Rates among Ethnic Groups, Hawaii, USA, 2020–2022

G. Devendra et al. 1942

Effect of Seasonal Influenza Vaccines on Avian Influenza A(H5N1) Clade 2.3.4.4b Virus Infection in Ferrets

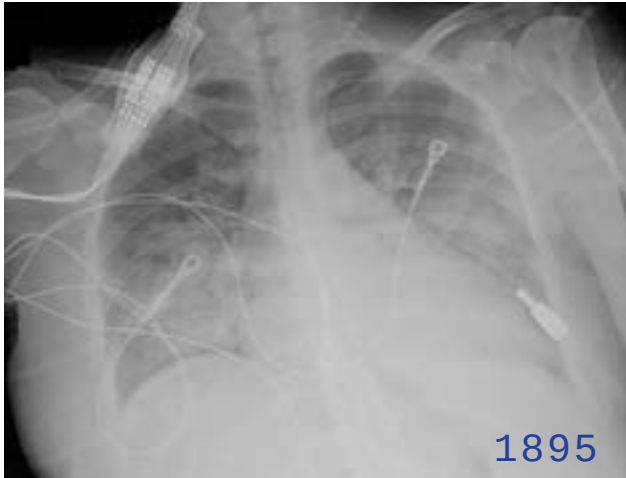
X. Sun et al. 1950

Spotted Fever Group Rickettsioses among Hospitalized Patients and Circulation of *Rickettsia* in Ticks, Kazakhstan, 2019

Y.V. Bumburidi et al. 1961

Multidrug-Resistant pESI-Harboring *Salmonella enterica* Serovar Muenchen Sequence Type 82 in Poultry and Humans, Israel, 2020–2023

J. Perry et al. 1969



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October 2025

Genomic Investigation of Increased Gonococcal Infections, Minnesota, USA, 2024

D. Evans et al. 2003

Fatal Pneumocephalus Caused by Hypervirulent *Klebsiella pneumoniae*, Germany

N. Gläser et al. 2008

Zoonotic *Baylisascaris procyonis* in Raccoons, Mississippi, USA, 2023–2024

B.L. Huerta-Beltrán et al. 2013

Dispatches

Bat-Associated Hemotropic Mycoplasmas in Immunosuppressed Children, Spain, 2024

F. Esperón et al. 1980

Detection of Monkeypox Virus Clade 1b DNA in Wastewater Solids at Wastewater Treatment Plants, United States

A.B. Boehm et al. 1984

Seoul Virus Infection Acquired at Private Pet Rat Breeding Facility, Germany, 2024

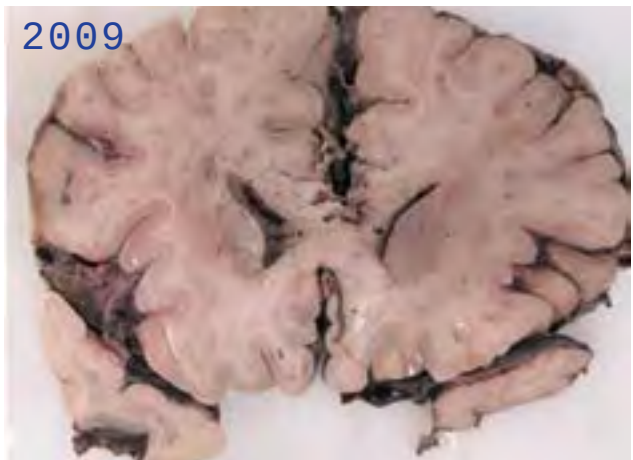
F. Baalman et al. 1988

Antimicrobial-Resistant Clonal Complex 11 *Neisseria meningitidis*-Associated Urethritis Cluster, Thailand

J.C. Cartee et al. 1992

Emergence and Polyclonal Dissemination of *bla*_{NDM-7}-Carrying InX3 Plasmid in *Enterobacter cloacae* Complex, France, 2021–2023

I. Rezzoug et al. 1998



Genetic Cluster of Extended-Spectrum β -lactamase-Producing *Klebsiella pneumoniae* in Humans and Food, Switzerland, 2018–2019

L. Aguilar-Bultet et al. 2017

Emergence of *Bordetella holmesii*-Associated Pertussis-Like Illness, Northern India, 2019–2023

N. Shekhar et al. 2022

Escherichia coli Sequence Type 131-H22 in Parrots from Illegal Pet Trade, Brazil, 2024

V.G.P. Rocha et al. 2025

Zoonotic Soil-Transmitted Helminth Infections among Humans, Gabon

H. Zhao et al. 2029

Investigation of Possible Intraoperative Transmission of *Brucella melitensis*, Slovenia

I. Potparić et al. 2034

Research Letters

Cutaneous Coccidioidomycosis Mimicking Rosacea in Immunosuppressed Patient, Arizona, USA, 2024

S. Obagi et al. 2038



2038



Jorge Lobo's Disease in Child with Tick Exposure, Brazil

F.G. Gonçalves et al. 2040

Neonatal Gonococcal Conjunctivitis Caused by Fluoroquinolone-Resistant *Neisseria gonorrhoeae*

H. Mizushima et al. 2043

Disseminated Blastomycosis Mimicking Tuberculosis, China

C. Guo et al. 2046

Crimean-Congo Hemorrhagic Fever Virus Circulation in Wild European Rabbits, Portugal, 2018–2023

C. Baptista et al. 2048



2055

EMERGING INFECTIOUS DISEASES

October 2025

Increased Rates of *Purpureocillium lilacinum* Mold among Laboratory Culture Results, United States

D.J. Smith et al. 2051

***Angiostrongylus cantonensis* Nematodes in Definitive and Intermediate Hosts, Madagascar, 2023**

L.A. Maminirina et al. 2054

Detection of *Burkholderia thailandensis* in Soil Samples, Suriname

J. Savelkoel et al. 2057

Detection of Immunity Gap before Measles Outbreak, Ho Chi Minh City, Vietnam, 2024

T. Ong et al. 2059



2044

EIN Letter

Emerging Sexual Transmission of *Trichophyton mentagrophytes* Genotype VII Infections, United States

P. Anand et al. 2064

Comment Letter

Invasive Urogenital *Neisseria meningitidis* Serogroup Y Multilocus Sequence Type 1466

C.R.R. George et al. 2067

About the Cover

A Personal Journey Through the Microbial World in Quilting

L. Mitchell 2068

Etymologia

Malassezia

F.P. Sellera et al. 2063

2026

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CDC is pleased to announce the launch of the **CDC Yellow Book 2026**. The CDC Yellow Book is a resource containing the U.S. government's travel medicine recommendations and has been trusted by the travel medicine community for over 50 years. Healthcare professionals can use the print and digital versions to find the most up-to-date travel medicine information to better serve their patients' healthcare needs.

The CDC Yellow Book is available online now at www.cdc.gov/yellowbook and in print starting in June 2025 through Oxford University Press and other major online booksellers.

Retrospective Analysis of Historical *Listeria monocytogenes* Clinical Isolates, New York, USA, 2000–2021¹

Hilal Samut, Damaris V. Mendez-Vallellanes, Hannah Hoyt, Samantha E. Wirth, Lisa Mingle, Brian D. Sauders, Gregory A. Deiulio, Alyssa W. Dickey, Maria L. Ishida, William J. Wolfgang, Martin Wiedmann, Renato H. Orsi

We used whole-genome sequencing data to investigate historical *Listeria monocytogenes* clinical (n = 1,046) and nonclinical (n = 1,325) isolates from New York, USA. Applying a threshold of ≤ 20 single-nucleotide polymorphism differences for single-linkage clustering, 321 clinical isolates clustered into 85 clusters ranging from 2–33 isolates per cluster. Fourteen clusters included isolates with outbreak codes (4 clusters with New York codes and 10 with multistate codes). Three New York outbreak codes were assigned to isolates that were genetically highly related and from cases < 2 months apart. Fifteen clusters included isolates that were obtained > 10 years apart; 33 clusters included isolates from the same or contiguous counties. Seventeen clusters included food and environmental isolates highly related to clinical isolates. These findings suggest that some listeriosis clusters can be local and span a long period, demonstrating the importance of investigating small, localized listeriosis cases with closely related isolates, even over long timeframes.

Listeria monocytogenes contamination affects various food commodities, including dairy products (e.g., cheeses, ice cream), ready-to-eat meats (e.g., deli meats), fruits, and vegetables. The ability to survive under adverse conditions enables *L. monocytogenes* to persist in food processing environments over

extended periods (1), which can lead to recurrent contamination of finished, ready-to-eat food products.

L. monocytogenes outbreak investigations pose challenges because of long incubation time, small outbreak size, and long timespan between cases (2). Identifying the source becomes even more difficult when genetically related environmental and food isolates are geographically widespread or associated with multiple food commodities or environmental sources (3). Those challenges complicate understanding of *L. monocytogenes* transmission patterns, often resulting in the detection of primarily large or localized outbreaks (2) and the characterization of most listeriosis cases as sporadic.

With the advent of high-throughput DNA sequencing, the Centers for Disease Control and Prevention (CDC) PulseNet and the US Food and Drug Administration GenomeTrackr programs started the routine use of whole-genome sequencing (WGS) as a subtyping tool in *L. monocytogenes* outbreak surveillance in September 2013. This tool has enabled public health agencies to connect cases over extended periods, refine case definitions, connect sporadic cases to sources, and link cases to common sources using WGS data from food and environmental isolates obtained through surveillance efforts (2,4). In this study, we sequenced historical *L. monocytogenes* clinical isolates obtained in New York, USA, during 2000–2021 to retrospectively analyze listeriosis cases with the aim of assessing temporal and geographic distribution of clustered isolates and patterns associated with

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DOI: <https://doi.org/10.3201/eid3110.241876>

¹Preliminary results from this study were presented at the International Association for Food Protection Conference; July 14–17, 2024; Long Beach, California, USA.

clusters investigated by the New York State Department of Health (NYSDOH) and CDC.

Materials and Methods

We have provided a complete description of methods in the Appendix (<https://wwwnc.cdc.gov/EID/article/31/10/24-1876-App1.pdf>). In brief, we performed WGS on 957 *L. monocytogenes* clinical isolates collected by NYSDOH (2000–2021) and 89 by the New York City Department of Health and Mental Hygiene (2000–2004) using the Illumina NextSeq 500 platform (Illumina, <https://www.illumina.com>). We sequenced 802 other nonclinical isolates (i.e., food and environmental) (2000–2021) using Illumina MiSeq and obtained WGS data for 523 nonclinical isolates (2000–2020) from previous studies (5,6). We submitted all data to PulseNet and the National Center for Biotechnology Information Pathogen Detection database, where single-nucleotide polymorphism (SNP) clusters and pairwise SNP distances were generated (7).

We obtained metadata (e.g., collection date, county, source) from Food Microbe Tracker, NYSDOH, New York City Department of Health and Mental Hygiene, and New York State Department of Agriculture and Markets. We filtered duplicate sequences of the same isolate and excluded 40 clinical isolates from mother–child pairs or the same patient. Clinical isolates were clustered into New York clusters using single-linkage at ≤ 20 SNPs (8–10); nonclinical isolates within ≤ 50 SNPs of a cluster were added (11). We classified clusters by county proximity (i.e., same, contiguous, or noncontiguous).

We performed core-genome multilocus sequence typing (cgMLST) in PulseNet 2.0 with automated quality control, SPAdes assemblies (<https://ablab.github.io/spades>), and allele calling against 1,748 loci. We performed statistical analyses in R version 4.2.1 (The R Project for Statistical Computing, <https://www.r-project.org>) using generalized least square to assess pairwise SNP distances within clusters and generalized linear model with negative binomial distribution for predicting maximum SNP distances between clusters. We reported the estimated mean differences in pairwise SNP distances (β_{pairwise}) and maximum SNP distance between clusters (β_{cluster}).

Results

Cluster Size

In total, 1,046 clinical isolates were obtained in New York during 2000–2021. Although 725 (69%) of the isolates showed >20 SNP differences from another

New York clinical isolate, 321 (31%) showed ≤ 20 SNP differences from ≥ 1 other New York clinical isolate; those closely-related isolates were grouped into clusters such that a cluster included ≥ 2 clinical isolates differing by ≤ 20 SNPs, for a total of 85 clusters (median of 2 isolates per cluster of 2–isolates) (Figure 1).

We divided clusters into 3 arbitrary categories: small (2 or 3 clinical isolates), medium (4–10 clinical isolates), and large (>10 clinical isolates). Of the 85 clusters, 67 (79%) clusters were small, 14 (16%) were medium, and 4 (5%) were large, suggesting that most potential outbreaks involve 2–3 identified cases only.

Timespan of Clusters

The average timespan (i.e., time interval between the collection dates of the first and last collected clinical isolates in a cluster) among 85 clusters was 4.59 years (median 2.42 years, interquartile range [IQR] 0.33–6.75 years) (Figure 1); the range was 0 months (observed in 4 small clusters) to 21 years (covering the entire study period). Moreover, 15 clusters (18%) exhibited timespans >10 years, underscoring the diversity in temporal dynamics of the clinical isolates. Although 39 (46%) clusters lasted relatively short durations of ≤ 2 years, only 89 (28%) of the clinical isolates were represented in those clusters. Conversely, most clusters' clinical isolates (72%, 232) were grouped in clusters (54%, 46) that lasted >2 years. Of the 46 clusters spanning >2 years, 30 (65%) were small clusters, and 5 small clusters extended beyond 10 years (Appendix Table 1), suggesting the presence of long-lasting small outbreaks in New York during the study period.

Clusters including isolates exclusively obtained before 2014 ($n = 41$), when pulsed-field gel electrophoresis (PFGE) was the only subtyping method for *L. monocytogenes* clinical isolates in New York, were significantly associated with their isolates not having NYSDOH-assigned cluster codes ($p < 0.001$ by Pearson χ^2 test with Yates' continuity correction) (Appendix Table 1). Moreover, although 13 of the 20 New York clusters including isolates exclusively obtained in or after 2014 (when WGS was first used alongside with PFGE as a subtyping method for *L. monocytogenes* clinical isolates in New York) had all their isolates assigned a NYSDOH cluster code, only 1 out of 65 clusters with ≥ 1 isolate obtained before 2014 had all its isolates assigned a NYSDOH cluster code.

County Origin of Isolates

The 1,046 clinical isolates were obtained from 59 of 62 New York counties (no isolates from Hamilton, Herkimer, and Lewis Counties); clusters included isolates

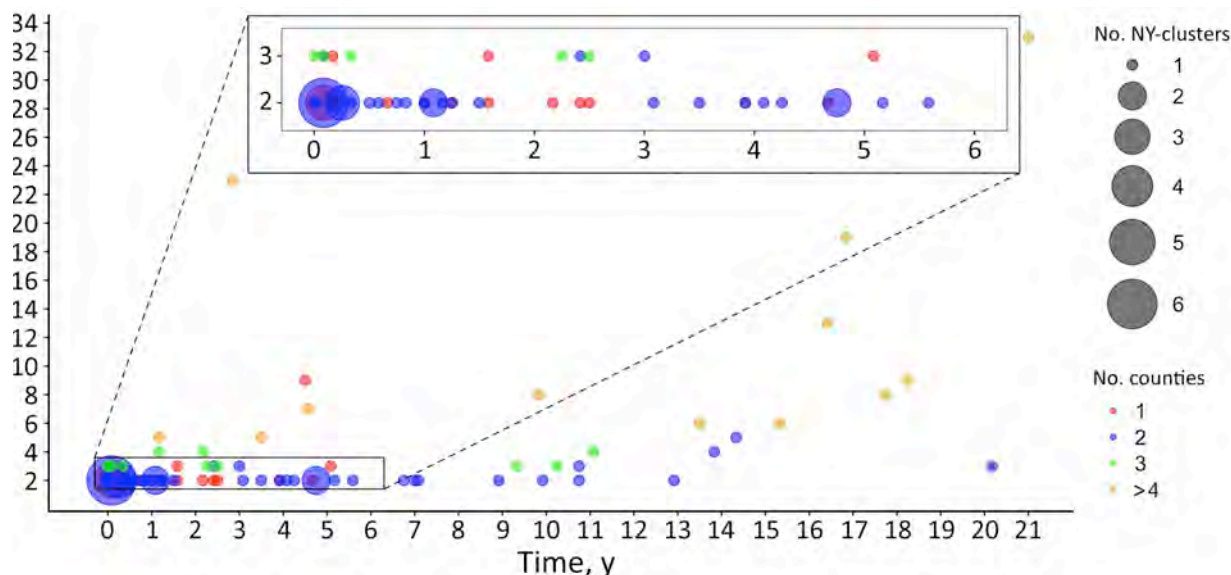


Figure 1. Distribution of timespan, number of clinical isolates, and number of counties among clusters in retrospective analysis of historical *Listeria monocytogenes* clinical isolates, New York, USA, 2000–2021. Clinical isolates that show a single-nucleotide polymorphism (SNP) distance of ≤ 20 SNPs to ≥ 1 of the other clinical isolates were grouped into a cluster; therefore, each cluster contains ≥ 2 clinical isolates. Each circle represents between 1 and 6 clusters, and the size of the circle is proportional to the number of clusters represented. Circles are color-coded according to the number of counties in each cluster. Timespan represents the interval between the first and the last collected clinical isolates in a cluster, measured in years with a month (1/12 of a year) as the minimum measurable unit. If the first and last collected clinical isolates in a cluster were obtained within the same month of the same year, the timespan for the cluster is zero.

from 49 counties (Figure 2, panels A, B). Excluding the 5 New York City (NYC) boroughs (Figure 2, panel A), Nassau ($n = 41$) had the highest number of isolates, followed by Suffolk ($n = 38$), Westchester ($n = 24$), Erie ($n = 22$), Onondaga ($n = 14$), and Monroe ($n = 12$) Counties (Appendix Table 2). During January 2000–September 2021, the highest incidence rates were recorded in Yates and Genesee Counties; rates were 86.9/1,000,000 persons over the study time span in Yates County and 81.8 isolates/1,000,000 persons over the study time span in Genesee County (data not shown).

Fifty-two clusters (61%) consisted of isolates collected from noncontiguous counties; 38 were small clusters (Appendix Table 1). Excluding NYC, most isolates were obtained in the Metropolitan area, which includes Long Island and the Hudson Valley. The Central region had the lowest number of *L. monocytogenes* isolates in this study (Appendix Table 3). Excluding NYC isolates, we observed no significant association between cluster size and the regions where clinical isolates were recorded ($p = 0.83$ by Pearson χ^2 test) (Appendix Table 3).

A total of 33 (39%) clusters included isolates from the same or contiguous counties (Appendix Table 1). Among the 18 clusters from the same county, 17 were small clusters. One medium cluster of 9 clinical isolates (NY-cluster 18) with all isolates from the same

county had a timespan of 4.5 years (Appendix Table 1), and the isolates were all highly related (SNP distances ranging from 0 to 6); although the 3 isolates collected during June–October 2014 were assigned 1 outbreak code, the other 6 isolates, collected during June 2016–December 2018, were assigned a different outbreak code (Appendix Table 4).

SNP Differences in Clinical Isolates

On the basis of the highest SNP distance within each cluster, we applied arbitrary SNP thresholds: highly related (0–10 SNP differences) and closely related (11–20 SNP differences). More than half (61%, 52/85) of New York clusters had highly related isolates (Appendix Table 1), consisting of 46 small, 5 medium, and 1 large cluster. Although the maximum SNP distances in a cluster were not statistically different between clusters classified as same and contiguous counties ($\beta_{\text{cluster}} -0.40$ [95% CI -0.88 to 0.09]; $p = 0.10$) compared with noncontiguous counties ($\beta_{\text{cluster}} -0.10$ [95% CI -0.58 to 0.41]; $p = 0.69$), 89% (16/18) of clusters from the same county presented high genetic relatedness (maximum SNP distance ≤ 10 SNPs) (Figure 3). Conversely, only 60% (9/15) of clusters from contiguous counties and 52% (27/52) of clusters from noncontiguous counties exhibited high relatedness, suggesting that localized clusters tend to be associated with more

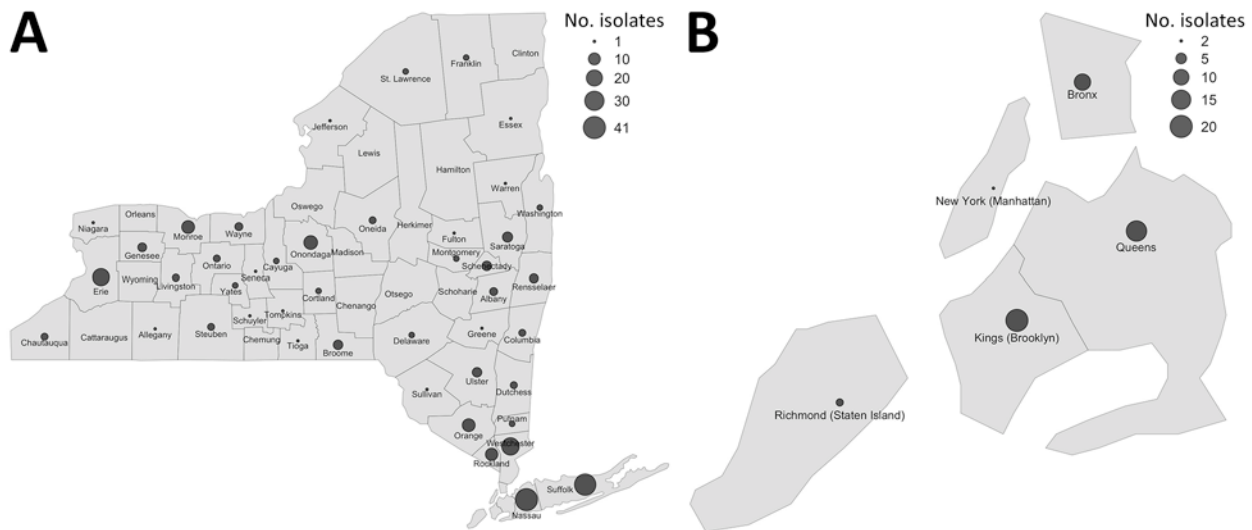


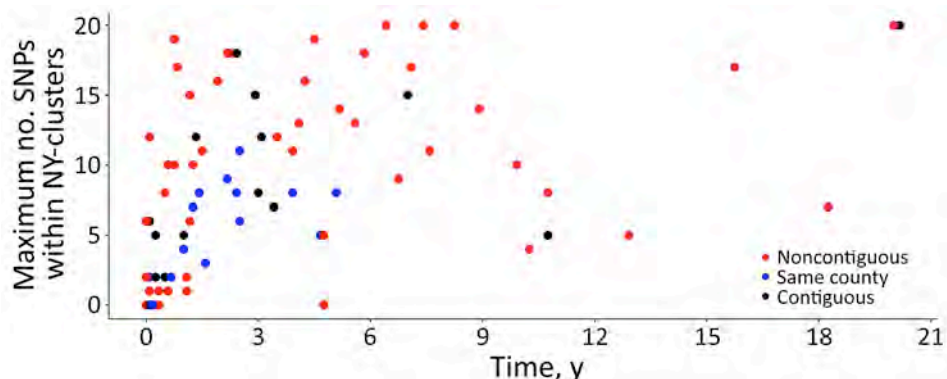
Figure 2. Distribution of clinical isolates within clusters (i.e., the isolates with pairwise SNP distance ≤ 20) in retrospective analysis of historical *Listeria monocytogenes* clinical isolates, New York, USA, 2000–2021. A) Distribution of isolates by New York county during 2000–2021 (except for the New York City [NYC] area); B) distribute of isolates in NYC during 2000–2004. The boroughs of NYC (Manhattan, Brooklyn, Queens, the Bronx, and Staten Island) were separated from New York counties outside NYC because of the limited number of isolates available for these regions.

genetically related isolates. In addition, the within-cluster pairwise SNP distance between isolates from same and contiguous counties (Figure 4, panels A, B) were significantly lower ($\beta_{\text{pairwise}} -3.68$ [95% CI -4.72 to -2.64]; $p = 0.03$) than the pairwise SNP distance between isolates from noncontiguous counties ($\beta_{\text{pairwise}} -1.05$ [95% CI -2.00 to -0.09]; $p < 0.001$) (Figure 4, panel C). Both the maximum SNP distance ($\beta_{\text{cluster}} 0.005$ [95% CI 0.001 – 0.009]; $p = 0.003$) and the within-cluster pairwise SNP distances between isolates ($\beta_{\text{pairwise}} 0.015$ [95% CI 0.01 – 0.02]; $p < 0.001$) were significantly positively associated with the timespan between isolates.

In addition to SNP-based distances, we assessed the relationship between pairwise SNP distances and cgMLST allele distances across all isolate pairs for

isolates in a given cluster. We observed a strong positive correlation between SNP and allele distances (Pearson correlation coefficient 0.96) (Appendix Figure 1), indicating that cgMLST allele distances closely mirror SNP distances; SNP distances ranged from 0 to 67 and cgMLST allele distances ranged from 0 to 32. We observed a strong linear relationship between SNP and allele distances across the range of 0 to 67 SNPs. Specifically, the slope of the regression line was ≈ 0.5 , indicating 1 allele difference for every 2 SNP differences. Therefore, the SNP threshold of 20 SNP differences used here was equivalent to a difference of 10 cgMLST alleles. In general, isolates considered to be highly related (i.e., ≤ 10 SNPs) showed ≤ 5 allele differences between each other.

Figure 3. Maximum SNP distances observed in clusters in retrospective analysis of historical *Listeria monocytogenes* clinical isolates, New York, USA, 2000–2021. Timespan corresponds to the interval in years between an isolate pair where the maximum number of SNPs was observed among closely related isolates (i.e., SNPs ≤ 20). If the clinical isolates in a NY-cluster were collected within the same month of the same year, the timespan for the NY-cluster is 0. SNP, single-nucleotide polymorphism.



Isolates with New York–Specific Outbreak Codes

In a listeriosis multistate outbreak investigation, CDC PulseNet assigns outbreak codes to clusters with ≥ 3 clinical isolates collected within 120 days, with ≥ 2 clinical isolates differing by ≤ 5 cgMLST alleles. Seven isolates with outbreak codes that were previously assigned to only 1 clinical isolate in our dataset were assigned to New York clusters, suggesting that more cases might have been part of those outbreaks but were not previously identified. Clusters included isolates with 22 different outbreak codes, including 5 New York–specific and 17 multistate codes (Appendix Table 4). Five New York outbreak codes were linked to 1 medium and 3 small clusters; 2 codes were assigned to isolates in the same medium cluster (NY-cluster 18). The multistate outbreak codes were linked to 5 small, 3 medium, and 2 large clusters; the 2 large clusters were linked with 2 multistate outbreak codes (NY-cluster 84) and 7 multistate codes (cluster 85) (i.e., isolates obtained at similar times were assigned the same code, whereas genetically related isolates obtained at different times were assigned different outbreak codes) (Appendix Table 4).

All 4 clusters linked with New York outbreak codes included highly related isolates with SNP differences ranging from 0 to 6 SNPs (median 2 SNPs) (Appendix Table 1). Of those clusters with New York outbreak codes, 3 were also short-lasting (0–1 month); the other cluster with a New York outbreak code spanned 4.5 years (NY-cluster 18). Of the 4 clusters with New York outbreak codes, 2 included isolates from the same county (including NY-cluster 18), whereas the other 2 clusters included isolates from noncontiguous counties. Hence, no clusters with New York–specific outbreak codes included isolates from different counties or with cases >1 month apart (Appendix Table 1).

Relationship with Nonclinical Isolates

A total of 307 nonclinical isolates (78 food, 4 animal, and 225 environmental) differed by ≤ 50 SNP differences from ≥ 1 New York clinical isolate in 37 clusters (Appendix Table 5). The most common types of food isolates within clusters were deli meats ($n = 18$) and fish-related products ($n = 15$) (Figure 5). Fourteen clusters had ≥ 2 clinical and nonclinical isolates

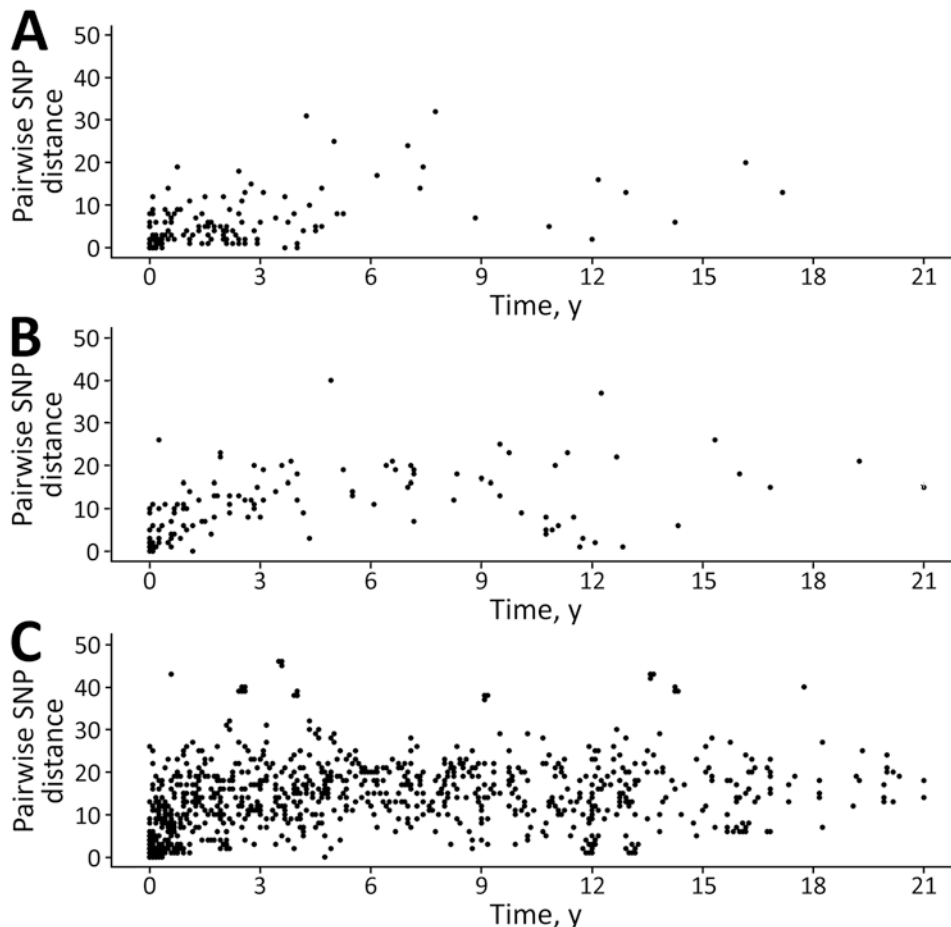


Figure 4. Pairwise SNP distances observed among 321 clinical isolates in clusters in retrospective analysis of historical *Listeria monocytogenes* clinical isolates, New York, USA, 2000–2021. Each data point represents a pair of isolates from the same county (A), contiguous counties (B), or noncontiguous counties (C). The timespan corresponds to the interval between each isolate pair within clusters in years with a month (1/12 of a year) as the minimum measurable unit. If the clinical isolates in a cluster were collected within the same month of the same year, the timespan for the cluster is 0. SNP, single-nucleotide polymorphism.

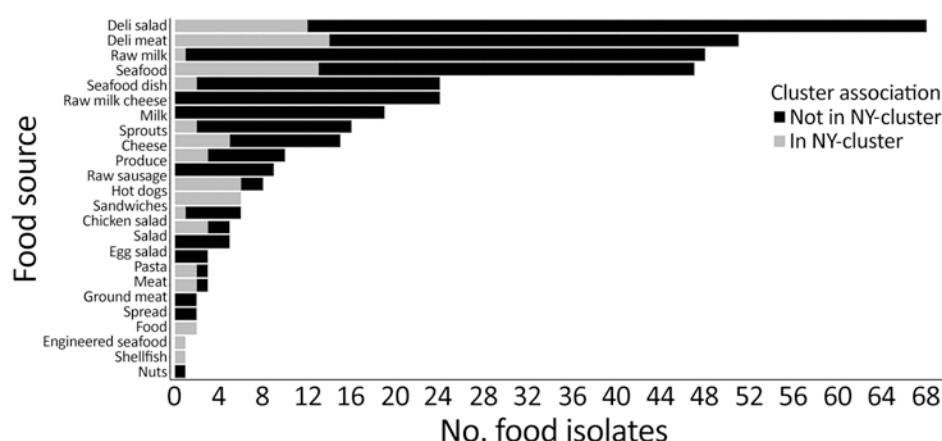


Figure 5. Number of food isolates obtained by food category in retrospective analysis of historical *Listeria monocytogenes* clinical isolates, New York, USA, 2000–2021. Some categories reflect limited metadata detail: food indicates unspecified food products, spread includes unspecified spreadable foods (e.g., cheese or other dips), and seafood dish includes prepared seafood dishes, whereas seafood indicates raw or unspecified seafood items.

obtained from the same county (Appendix Table 6). Seventeen clusters included nonclinical isolates highly related (SNP distances ≤ 10) to ≥ 1 clinical isolate (Appendix Table 6); 13 were small or medium.

Discussion

Before WGS became the major subtyping method applied to public health, US multistate listeriosis outbreaks were considered rare (<10 outbreaks/year), short-lasting (usually <1 year in length), and medium to large (most outbreaks had >10 cases) (2,12). Since WGS was implemented in the United States in 2013 (4), more outbreaks (>10 per year) have been identified, many of which last >1 year and involve fewer cases (most with <10 cases) (2). We used a collection of >1,000 clinical *L. monocytogenes* isolates obtained in New York during 2000–2021 to assess temporal and geographic distribution of clusters of closely related isolates and the patterns associated with clusters that included isolates with New York cluster codes and outbreak codes. Our results show that New York clusters were generally small (≤ 3 cases per cluster) and long-lasting (median timespan >2 years) and commonly involved isolates from the same or contiguous counties ($\approx 40\%$). Furthermore, NYSDOH-assigned cluster codes were significantly associated with clusters with isolates exclusively obtained after 2014, when WGS began to be used alongside PFGE as a subtyping method for *L. monocytogenes* clinical isolates, suggesting that WGS has greatly improved the identification of *L. monocytogenes* clusters. In addition, New York outbreak codes were linked to clusters, including highly related (≤ 10 SNP differences) clinical isolates that occurred within 2 months of each other or originated from the same county. Of note, 37 clusters, including 23 (62%) clusters with 2 or 3 cases only, had ≥ 1 environmental or food isolate related (within ≤ 50

SNPs) to ≥ 1 clinical isolate, suggesting that a possible source could be linked to those clusters.

Our findings indicate that a substantial number of listeriosis cases represent small, long-lasting clusters. Surveillance systems capable of detecting such clusters could enhance prevention efforts. Most clusters in our study were small (2–3 clinical isolates) and spanned >2 years. Similarly, a previous study analyzed *L. monocytogenes* population structure of clinical isolates in New York during 2000–2021 and found 38 epidemiologically linked clusters (including ≥ 3 clinical isolates) showing multiyear persistence of 20 identified clusters (13). Likewise, studies in the Netherlands (14,15), Germany (16), United Kingdom (17), and Italy (18,19) using WGS data on historical *L. monocytogenes* clinical isolates showed small clusters (i.e., 2–3 cases) scattered over time. The observation that many small clusters were scattered over long periods suggests that investigations of *L. monocytogenes* clusters involving as few as 2 cases, when the isolates are separated by ≤ 20 SNPs, even if the cases occurred >1 year apart, might be valuable and could contribute to improved prevention.

The observation of many clusters scattered over long periods might suggest that these clusters might be caused by reoccurring, emerging, or persisting (REP) *L. monocytogenes* strains. CDC defines these strains as reoccurring (causing repeated cases over an extended period but not causing continuing cases), emerging (strains start to cause an increasing number of cases), or persisting (strains that consistently cause illnesses over extended periods) (20). In addition to strains of *Salmonella enterica* (21) and *Escherichia coli* O157:H7 (22), *L. monocytogenes* also includes REP strains (<https://www.cdc.gov/foodborne-outbreaks/php/rep-surveillance/index.html>). Although none of the 85 clusters identified included confirmed *L. monocytogenes* REP strains, our

findings indicate that several *L. monocytogenes* strains in New York might represent local or previously unidentified REP-like strains. For example, the largest cluster identified in this study included ≥ 1 isolate reported in each year during 2000–2021, except for the years 2003, 2014, 2015 and 2019; this cluster could, thus, be linked to a persisting *L. monocytogenes* strain (Appendix Figure 2). All large and most medium clusters showed evidence for persistence similar to that reported as characteristic for REP strains; more specifically, most medium and all large clusters included clinical isolates obtained >2 years apart. Identifying REP strains can be key for outbreak source attribution. According to CDC, REP strain designation considers the number of cases and rising case numbers associated with the strain, and how frequent or large the outbreaks associated with this strain are. REP strains might show multidrug resistance, increased virulence, or increased transmissibility than non-REP strains (20). Because we did not characterize strains comprehensively, we refer to some of the clusters persisting >2 years as REP-like strains. The many REP-like strains identified in this and previous studies (13–19) is not necessarily surprising, given *L. monocytogenes*'s ability to persist for many years in food-associated environments (1,23–31) and to recontaminate finished products after many years (26,32–35).

To further understand genetic relatedness within clusters, we compared pairwise SNP and cgMLST allele distances. The strong correlation between SNP and cgMLST allele distances (Pearson correlation coefficient 0.96) supported the concordance between the 2 high-resolution typing methods (36). However, the broader numerical range of SNP distances (0–67) compared with cgMLST allele distances (0–32) indicates that cgMLST might reveal less genetic variation; that difference is likely because of SNP differences that fall in genes not included in the cgMLST scheme or that fall in intergenic regions. Hence, for the 1,748-gene cgMLST scheme, a threshold of ≤ 7 allele difference has been suggested for clustering related isolates (37), which closely aligns with the ≤ 10 allele difference we observed for isolates ≤ 20 SNPs apart. Although cgMLST offers standardization and ease of comparison for routine surveillance (e.g., through PulseNet), our results highlight the importance of SNP analysis offering finer discrimination and more precise cluster boundaries, supporting its use as a complementary method for validating or refining cgMLST-based clusters (36,38).

Although linking nonclinical isolates to clinical clusters might provide valuable clues for investigating clusters and identifying sources, traceback and

epidemiologic evidence remain essential to link clusters to specific sources. That process can be particularly challenging for small or temporally dispersed clusters, where obtaining conclusive evidence is often difficult. In our analysis, a ≤ 50 SNP threshold to link nonclinical isolates with clusters was intended as an exploratory approach. That threshold is not intended to confirm that isolates are part of an outbreak linked to a common source but rather to identify potentially related isolates that can guide hypotheses and prioritize targets for epidemiologic investigations. Several small and medium clusters included food and environmental isolates highly related to clinical isolates from the same county, even when collected years apart. This finding could suggest local common sources and persistent contamination in local small processors, farms, retail establishments, or private (e.g., residential) and public (e.g., schools, hospitals, restaurants) kitchens (1). Similarly, long-lasting listeriosis outbreaks linked to local settings have been previously reported, including a hospital-linked outbreak in Finland consisting of 6 listeriosis cases during 2015–2019 (39) and a food-service-linked outbreak in the United States consisting of 15 listeriosis cases during 2014–2017 (40). Those results support the importance of comprehensive data collection and analysis of environmental and food isolates, which can support Sample-Initiated Retrospective Outbreak Investigations and help identify potential sources and initiate inspections to determine the specific source of outbreaks (4).

Our findings indicate that WGS of historical *L. monocytogenes* isolates could contribute to identifying long-lasting outbreaks previously recognized as sporadic cases or as smaller, independent, acute outbreaks. Only 4 clusters were linked to New York outbreak codes and just 10 clusters were linked to multistate outbreak codes, despite 25 clusters containing highly related clinical isolates (≤ 10 SNPs) from the same or contiguous counties. Although clusters containing closely related clinical isolates (≤ 20 SNPs) obtained during 2000–2013 might have been overlooked because of PFGE's lower resolution (2,4), 20 clusters included isolates that were exclusively obtained after 2013. Clusters without a linked outbreak code were usually small (2–3 clinical isolates) and had clinical isolates that were closely but not highly related or that were obtained >1 year apart. Since the advent of WGS as a subtyping tool in public health, long-lasting listeriosis outbreaks have been more commonly recognized (e.g., outbreaks linked to queso fresco and cotija cheese in 2014–2024 [41], enoki mushrooms in 2016–2019 [42], leafy greens in 2018–2023 [43], packaged

salads in 2015–2022 [44], and deli meats and cheeses in 2016–2019 [45]), suggesting that at least some outbreaks initially considered to be short-lasting might actually represent larger, long-lasting outbreaks. This possibility is supported by clusters 18, 84, and 85, which included clinical isolates that were linked to multiple short-spanning outbreaks (i.e., the isolates in those clusters were linked to multiple PulseNet-assigned outbreak codes). Moreover, cases years apart that included closely related isolates tended not to be investigated as part of an outbreak. For example, 15 clusters included highly related (≤ 10 SNPs) clinical isolates that were obtained >2 years apart and were not assigned outbreak codes; only 1 of the 15 clusters included >3 isolates. This possibility is particularly likely for clusters with closely related isolates from cases that occurred when different subtyping methods (i.e., PFGE and WGS) were being used. Although both PFGE and WGS were used during 2014–2017, cases before 2014 were analyzed using PFGE, which is less sensitive for detecting outbreaks (2), whereas cases after 2017 were only analyzed by WGS.

In conclusion, using a data-driven approach, we demonstrated that outbreak investigations often prioritize temporally tight clusters of highly related isolates, whereas small clusters that are spread across time but are from the same or contiguous counties are frequently overlooked. Our results emphasize the importance of comparing new and historical *L. monocytogenes* clinical isolates using WGS because even small listeriosis outbreaks could span a long period of time because of the bacteria's long-term persistence in food-associated environments. Moreover, small and medium clusters including food and environmental isolates from the same counties as the clinical isolates could also indicate that the source might be a small local food-associated environment (e.g., local ice cream manufacturer, packing house, restaurant, hospital). Our study also highlights the importance of performing routine environmental and food surveillance to enable public health and regulatory agencies to identify sources prone to persistence and take necessary control measures to mitigate further risk.

Acknowledgments

We thank Erika Mudrak for statistical analysis guidance.

For New York State Department of Agriculture and Markets, whole-genome sequencing was supported by the US Food and Drug Administration (FDA) of the US Department of Health and Human Services (HHS) as part of a financial assistance award (no. 5U19FD007122) 100% funded by FDA/HHS. The contents are those of

the authors and do not necessarily represent the official views of, nor an endorsement, by FDA/HHS or the US government. For New York State Department of Health, this work was supported by New York State, the Centers for Disease Control and Prevention Epidemiology and Laboratory Capacity Grant (cooperative agreement no. NU50CK000423), and the FDA's LFFM Grant (cooperative agreement no. 1U19FD007089). For Cornell University, this work was supported by ELC Foodborne Illness Centers of Excellence (grant no. 5NU50CK000516-05-00 awarded by Health Research Inc.).

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Organ Donor Transmission of *Rickettsia typhi* to Kidney Transplant Recipients, Texas, USA, 2024

Jeffrey C. Jones, Omar G. García, Julian A. Villalba, Rosa Hinojosa, Marissa L. Taylor, Pallavi Annambhotla, Matthias H. Kapturczak, Bonny Mayes, Sandor E. Karpathy, Arlyn N. Gleaton, Linda Moon, Joseph Singleton, Sridhar V. Basavaraju, Christopher D. Paddock

Murine typhus, a fleaborne disease caused by the bacterium *Rickettsia typhi*, is found throughout temperate and tropical regions of the world. Transmission of *R. typhi* to humans involves several species of fleas, and most infections result from direct inoculation of *R. typhi*-infected flea feces into abrasions in the skin. We describe the transmission of *R. typhi* from an organ donor in Texas, USA, to 2 kidney transplant recipients. The donor and 1 recipient died from the infection. The occurrence of *R. typhi* transmission via transplantation is a harbinger for the reemergence of murine typhus in some of the most densely populated metropolitan areas of the United States. Our findings reinforce the need to improve healthcare provider and public awareness of this life-threatening but treatable infection.

Murine or fleaborne typhus, an infection caused by the endotheliotropic bacterium *Rickettsia typhi*, occurs throughout temperate and tropical regions of the world. Murine typhus typically manifests as an undifferentiated febrile illness with severe headache, myalgia, arthralgia, and malaise (1–4) but can progress to severe, multisystem disease characterized by septic shock, multisystem disease characterized by septic shock, pneumonia, hepatitis, acute kidney injury, myocarditis, meningoencephalitis, hemophagocytic lymphohistiocytosis, or death (3–9). The classical transmission cycle of *R. typhi* involves commensal rodents in the genus *Rattus* and Oriental rat fleas (*Xenopsylla cheopis*) (10). During the past several decades, investigators have identified a second transmission cycle in the United States involving

cat fleas (*Ctenocephalides felis*), opossums, and feral cats (11–13). Most human infections are believed to result from inoculation of *R. typhi*-infected flea feces into abrasions in the skin and less often from inhalation or inoculation of mucous membranes with infectious feces (10). Murine typhus was documented once previously in a transplant recipient, albeit 5 years posttransplantation (14). In this article, we describe 2 transplant recipients who acquired murine typhus from organs received from a common donor. This donor resided in a region of the United States where an extraordinary resurgence of this disease has occurred after more than half a century of relative quiescence (15–17). In addition, we discuss how this event could signal broader epidemiologic implications in the 21st Century.

Case Reports

Transplant Recipients

In October 2024, a febrile illness developed in 2 kidney recipients from a common donor several days after transplantation (Figure 1). Recipient 1, a 47-year-old man with end-stage renal disease secondary to diabetes type 2, received the right kidney and remained hospitalized because of delayed graft function. On the fourth day after transplantation, a fever to 39.6°C developed in recipient 1 (Figure 1). Laboratory tests revealed anemia, hyponatremia, and elevated

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DOI: <https://doi.org/10.3201/eid3110.250961>

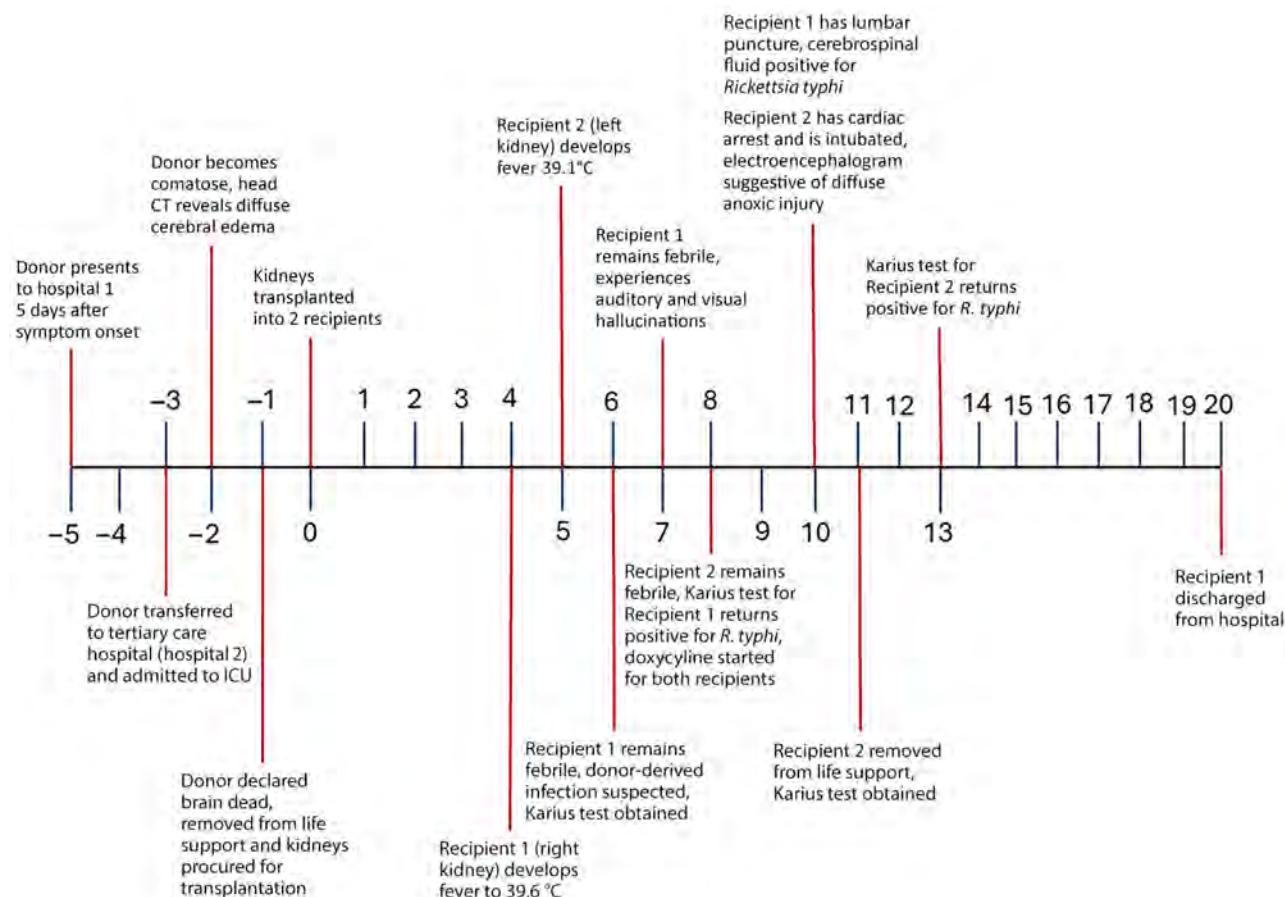


Figure 1. Timeline describing the transmission of *Rickettsia typhi* from an organ donor to 2 kidney transplant recipients, Texas, 2024. Day 1 of donor hospitalization is represented as –5, the day of death as –1, and the day of transplantation as 0, after which posttransplant days are listed, ending with the discharge of recipient 1 from the hospital on posttransplant day 20. CT, computed tomography; ICU, intensive care unit; Karius, <https://kariusdx.com>.

creatinine (Appendix Table 1, <http://wwwnc.cdc.gov/EID/article/31/10/25-0961-App1.xlsx>). Physical examination revealed no specific findings. We obtained blood and urine cultures and treated the patient empirically with cefepime. Micafungin was added after isolation of a *Candida* sp. from donor urine. Results of computed tomography (CT) without contrast of the chest, abdomen, and pelvis were unremarkable. The recipient's fever persisted, and on day 6, we collected plasma for microbial cell-free (mcfDNA) metagenomic sequencing by using the Karius test (Karius, <https://kariusdx.com>). The next day, the patient experienced auditory and visual hallucinations, and a severe frontal headache developed. The Karius test results were returned on day 8 and reported mcfDNA of *R. typhi* in plasma at 65,818 molecules/ μ L (Appendix Table 1). The transplant team provided this unexpected result to the organ procurement organization (OPO), who subsequently notified the organ procurement and transplantation network (OPTN). We

administered doxycycline intravenously to recipient 1. The next day, thrombocytopenia and hypoalbuminemia developed in the patient (Appendix Table 1). A head CT without contrast on day 10 was unremarkable. We performed a lumbar puncture the same day, and routine cultures of cerebrospinal fluid (CSF) were negative. We discharged the patient on the 20th day after kidney transplant.

Recipient 2, a 46-year-old woman with end-stage renal disease secondary to diabetes type 2 and hypertension, received the left kidney and remained hospitalized for delayed graft function. On day 5 after transplantation, a fever to 39.3 °C developed in recipient 2 (Figure 1). Laboratory tests revealed anemia, hyponatremia, and elevated levels of aspartate aminotransferase, alkaline phosphatase, and creatinine (Appendix Table 1). Physical examination revealed no specific findings. We obtained blood and urine cultures, and the patient was treated empirically with cefepime. Her fever persisted, and on day 8 a severe

diffuse headache developed. We began intravenous doxycycline for recipient 2 on the same day that the Karius test result for *R. typhi* returned for recipient 1. Recipient 2 remained febrile, and on day 9, she exhibited echolalia and was transferred to an intensive care unit. The next day she experienced cardiac arrest and was intubated. An electroencephalogram showed diffuse suppression suggestive of diffuse anoxic injury. Magnetic resonance imaging revealed extensive global brain injury. A Karius test was obtained on the same day and detected a nonquantifiable level of *R. typhi* DNA (Appendix Table 1). Her family made the decision to withdraw life support, and recipient 2 died 11 days after transplant.

Organ Donor

Five days before her death, a 34-year-old woman was seen at an emergency department in south Texas with complaints of 5 days of fever, lower extremity weakness, cough, dyspnea, headache, and syncope. She had a history of uncontrolled thyroid endocrinopathy and noncompliance with thyroid medications. Physical examination revealed a jaundiced, toxic-appearing woman with a temperature of 36.1°C, heart rate of 114 beats/min, and respiratory rate of 20/min. No rash was found. Laboratory abnormalities included leukocytosis; thrombocytopenia; anemia; hyperbilirubinemia; multiple hepatic enzyme abnormalities; elevated free triiodothyronine, free thyroxine, and total thyroxine; and low thyroid-stimulating hormone (Appendix Table 1). A chest radiograph revealed no abnormalities (Figure 2, panel A). Doppler ultrasound of her thyroid gland revealed enlargement and marked hypervascularity. She was diagnosed with thyrotoxicosis and was started on methimazole, propranolol, cholestyramine, and hydrocortisone. Uncontrolled tachycardia (133 beats/min) and worsening dyspnea developed on her third hospital day. A chest radiograph revealed extensive, bilateral, alveolar opacities most prominent in the middle and lower lungs (Figure 2, panel B). She required mechanical

ventilation and was transferred to the intensive care unit of a tertiary care center. A chest radiograph revealed extensive alveolar opacities in her middle and lower lungs. Within 24 hours, she became comatose, and head CT revealed diffuse cerebral edema with obliteration of the basal cisterns. A nuclear medicine perfusion scan showed absent cerebral circulation, and she was declared brain dead on the fifth hospital day and withdrawn from life support. After donor eligibility screening and standard tests performed by the OPO, her kidneys were procured for transplantation.

Methods

Clinical and Epidemiologic Review

In the United States, all unexpected, suspected transplant-transmitted diseases must be reported by OPOs or transplant centers to the OPTN for investigation by the ad hoc disease transmission advisory committee. Cases involving pathogens of special interest (https://optn.transplant.hrsa.gov/media/yyhnrkar/special_pathogens_list.pdf) are reported to the Centers for Disease Control and Prevention (CDC) for investigation. CDC was notified by the OPTN disease transmission advisory committee when mcfDNA of *R. typhi* was detected in the plasma of recipient 1. State and local public health officials and the OPO identified residual tissue and body fluid specimens from the organ donor and recipients for confirmatory testing at CDC. Donor and recipient medical records were reviewed by local health officials and by the clinical team. After confirmation of murine typhus in the donor, local public health officials conducted an environmental assessment of the donor's residence in south Texas. This activity was reviewed by CDC and conducted consistent with applicable federal law and CDC policy.

Laboratory Methods

We extracted DNA from residual blood, plasma, serum, CSF, or tissue specimens from the donor and recipient 1. We tested the extracted DNA by using

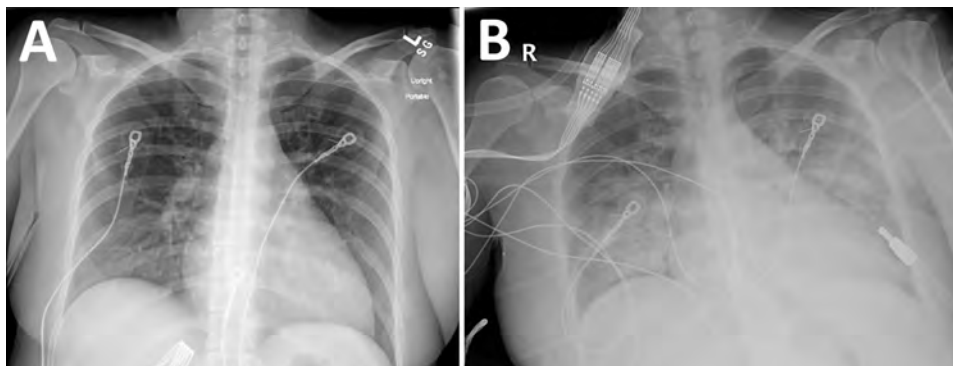


Figure 2. Chest radiographs from an organ donor with murine typhus, from a study describing the transmission of *Rickettsia typhi* from an organ donor to 2 kidney transplant recipients, Texas, 2024. A) Chest radiograph obtained at hospital admission, 5 days after symptom onset, with no specific abnormalities noted. B) Chest radiograph obtained on hospital day 3, revealing extensive alveolar opacities predominantly in the middle and lower lung fields.

a real-time PCR that amplified a 111-bp segment of the 23S rRNA gene of all *Rickettsia* sp. (18) and by using real-time PCRs that amplify 146-bp or 197-bp segments of intergenic regions from the genome of *R. typhi*. The primers and probes and for each target are provided (Appendix Table 2). Threshold cycle (Ct) values <40 were considered positive. A residual serum specimen from the donor was tested for *R. typhi* IgG by an indirect immunofluorescence assay (19). We considered a titer ≥ 128 positive. We evaluated formalin-fixed, paraffin-embedded biopsies of the right and left kidney allografts obtained before transplantation by an immunohistochemical (IHC) stain to detect antigens of typhus group *Rickettsia* sp. (20).

Results

We amplified DNA of *R. typhi* from residual donor samples collected at the time of organ procurement that had mean Ct values of 30.52 (plasma), 30.12 (serum), 32.35 (whole blood), 32.29 (lymph node), and 28.16 (spleen). Antigens of *R. typhi* were detected by IHC in donor lymph node and spleen, and *R. typhi* IgG at a titer of 512 were detected by immunofluorescence assay in donor serum samples obtained on the day of her death. We amplified *R. typhi* DNA at a Ct of 38.86 from residual CSF collected from recipient 1 on posttransplant day 11 (Appendix Table 1). Histopathologic review of the pretransplant allograft biopsies from each transplanted kidney revealed multifocal and predominantly mononuclear inflammatory infiltrates in interstitial peritubular stroma, focal endarteritis, acute tubular injury, and pigmented casts in tubular lumina (Figure 3, panel A). Some glomeruli showed features of focal segmental glomerulosclerosis, including mesangial hypercellularity and tuft adhesions to the Bowman's capsules (Figure 3, panel B). Short chains comprising small, rod-shaped bacteria were identified by the Warthin–Starry silver impregnation staining method (21) in a few glomeruli (Figure 3, panel C). Antigens of *R. typhi* were detected by IHC stain within endothelial cells of small interstitial blood vessels and in the mesangium of several glomeruli (Figures 3, panels D–F).

An epidemiologic assessment of the donor's residence revealed tall grass and abundant loose garbage, wooden planks, and other materials scattered around the yard. An interview conducted with the donor's partner indicated that she had frequent exposures to ≈ 10 flea-infested stray cats that lived on the property.

Discussion

This identification of murine typhus acquired by organ transplantation provides further evidence

for the resurgence of this disease in the United States, particularly south-central Texas (3,4,17,19,22) and southern California (9,23). During 1931–1946, $\approx 42,000$ cases of murine typhus were reported in the United States, predominantly from Alabama, Florida, Georgia, Louisiana, Mississippi, South Carolina, and Texas (15). Estimates of unrecorded cases outnumbered recorded cases by ≈ 4 to 1 (10). From the late 1940s through the early 1950s, coordinated campaigns by local, state, and national public health agencies across the southeastern United States effectively controlled the disease by intensive rodent elimination programs and widespread application of DDT around rodent harborages. National case counts of murine typhus plummeted during the late 1940s, and from the late 1950s through the late 1980s, <100 cases of murine typhus were recorded annually in the United States (15,16). Murine typhus was removed from the list of nationally notifiable diseases in 1987 (16). However, states such as California and Texas, where the disease remained a reportable condition, have recorded huge increases in annual case counts during the past decade (9,16,17,23). For example, the cumulative number of cases in Texas during 2014–2024 was 4.6 times greater than during 2003–2013 (<https://www.dshs.texas.gov/notifiable-conditions/zoonosis-control/zoonosis-control-diseases-and-conditions/flea-borne-typhus>), a trend reminiscent of the explosive rise of murine typhus in Texas almost a century ago (Figure 4).

Murine typhus can progress to severe or life-threatening disease, as evidenced by contemporary surveillance summaries, from which $\approx 60\%$ – 83% of patients are hospitalized (7,22,24); among that cohort, 5%–30% are admitted to an intensive care unit (3,4,19,22,24,25), often at admission to a hospital (4,8,26–29). In this report, the donor died 9 days after the onset of otherwise undifferentiated febrile illness attributed to thyrotoxicosis. The diagnosis of murine typhus is commonly missed in patients seeking care (1,4,29,30), even for those who are critically ill (5–9). This fact reflects the clinical dilemma posed by murine typhus, even in locations such as south Texas (4,22,25,31,32), where the disease has remained endemic for >100 years (33–35). In this article, an otherwise unexpected diagnosis of murine typhus in each kidney recipient was confirmed by metagenomic sequencing of mcfDNA, similar to multiple recent reports from California and Texas that have achieved diagnoses by using this technology (6,9,29–31).

Early clinical descriptions of murine typhus comment on the frequency of a short, hacking cough in

54% to >90% of patients (1,2,36) that for many “was likely to be unnoticed until attention was directed toward it” (36). In this report, the donor had multiple complaints that included cough and dyspnea. Either or both of those clinical findings are documented in 35%–59% of contemporary United States patient series (1–4,22–23, 25), but they are infrequently identified as notable clinical findings. Radiographic evidence of more advanced pulmonary disease, including pneumonitis or pulmonary infiltrates, is described infrequently in most patient series; however,

a recent analysis of 621 patients from 9 worldwide cohort studies identified chest radiograph abnormalities in 104 (16.7%) patients (37). *R. typhi* can remain infectious in desiccated flea feces at ambient temperatures for 9–15 months (38). That observation, evaluated in conjunction with the frequency of early respiratory findings (1,2,36,39), suggests that infections acquired directly from inhalation of desiccated flea feces could be more common than considered previously.

Each patient in this series demonstrated central nervous system involvement, also consistent

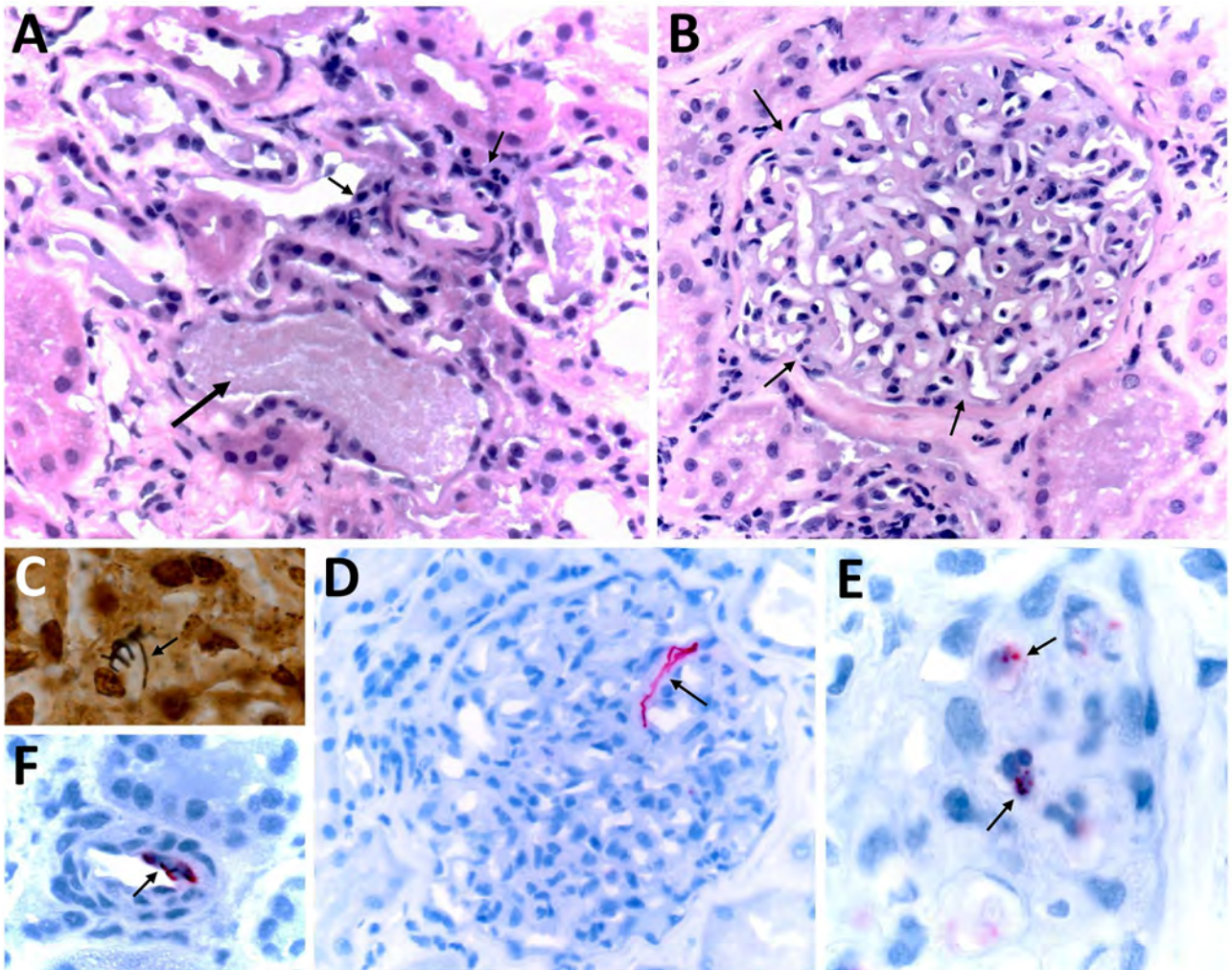


Figure 3. Histopathological and immunohistochemical features of formalin-fixed, paraffin-embedded biopsy specimens collected from the right and left kidney allografts procured from a donor who died of murine typhus, from a study describing the transmission of *Rickettsia typhi* from an organ donor to 2 kidney transplant recipients, Texas, 2024. A) When stained with hematoxylin and eosin, both allografts showed multifocal, interstitial, and predominantly mononuclear peritubular infiltrates, associated with focal endarteritis (thin arrows), features of acute tubular injury, including epithelial attenuation with loss of apical cytoplasm, and pigmented casts (large arrow). B) Glomeruli displayed moderate mesangial hypercellularity and tuft adhesions to the Bowman's capsules (arrows) and intracapillary and mesangial phagocytic foam-cells. C) Short chains comprising small, rod-shaped bacteria were revealed in glomerular capillaries by the Warthin–Starry silver impregnation staining technique. D–F) An immunohistochemical stain for typhus-group rickettsiae revealed intact bacteria within endothelial cells of inflamed small vessels and vascular spaces of mesangial capillaries (arrows) and phagocytized bacterial antigens in the cytoplasm of glomerular foam-cells (arrows). Original magnifications $\times 400$ (A, B, and E) and $\times 1,000$ (C, D, and F).

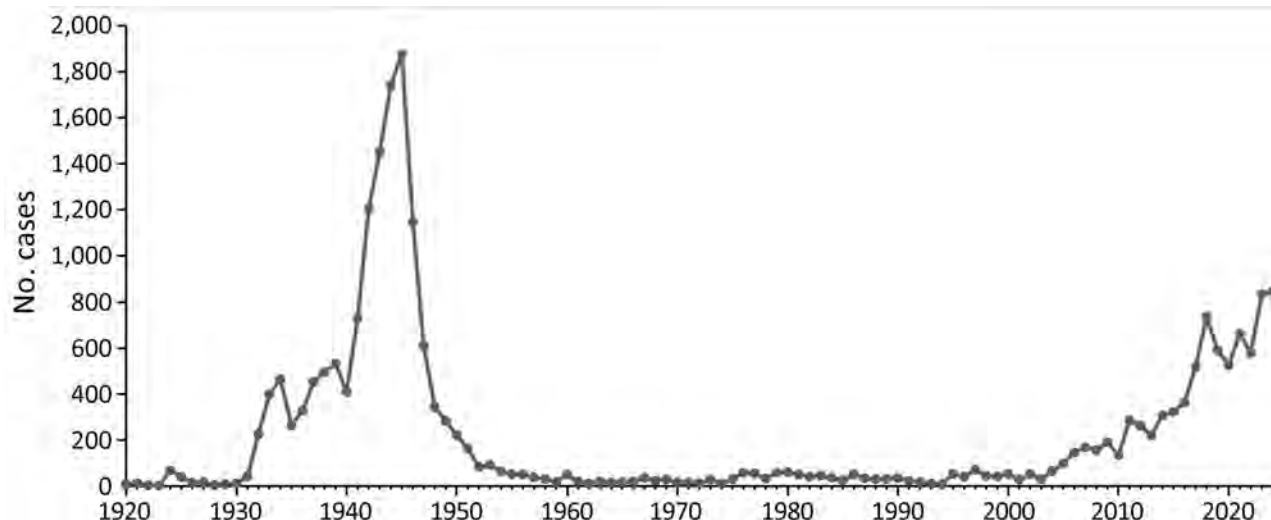


Figure 4. Annual case counts of murine typhus in Texas, USA, during 1920–2024.

with historical and contemporaneous patient series that identify photophobia in 12%–17% of patients, eye pain in 11%–14% of patients, nuchal rigidity in 6%–22% of patients, and stupor and delirium in 16%–17% of patients (1,2,4,23). Two of the patients in this series died from infection with *R. typhi*. During the preantimicrobial era, the case-fatality rate of murine typhus in the southeastern United States was estimated at 3.8%–4.3% (36,39). Contemporary case-fatality rates of murine typhus in the United States are $\leq 1\%$ (33,40), although those estimates are confounded by an unknown number of otherwise undiagnosed survivors and, perhaps more important, an unknown number of undiagnosed deaths. The recent identification of 3 fatal cases of murine typhus in Los Angeles County, California, in a single year exemplifies the lethal potential of this disease (9). In that context, the historical perception of murine typhus as a relatively mild rickettsiosis that typically runs an otherwise benign and self-limited clinical course (41) requires reexamination.

Arthropodborne pathogens are increasingly recognized as infectious agents capable of transmission via organ transplantation (42–44). Not all OPOs collect standardized data for known or potential recent arthropod and animal exposures; nonetheless, routine acquisition of those data could provide early clues to OPOs, transplant centers, and clinicians to consider vectorborne diseases in ill recipients during the early posttransplant period, particularly among recipients who develop otherwise unexplained multisystem disease. Such information could thereby guide and expedite empiric therapy and posttransplantation evaluation for specific vectorborne pathogens.

The public health triumph that almost eliminated murine typhus in the United States during the second half of the 20th Century paradoxically eroded general medical awareness of the disease. For example, the initial diagnosis for 44% of 180 patients was murine typhus in a series from Louisiana (1) in 1945, near the historical zenith of annual case counts in the United States (10,15). Several decades later, only 11% of 345 cases described from Texas during 1980–1987 received an initial diagnosis of murine typhus (25). More recently, murine typhus diagnosis was not considered initially for any of 10 acutely ill patients referred to a tertiary care center in Texas during 2017–2020 (29), or for any of 23 children from Texas when first evaluated as outpatients by primary care physicians and who later required hospitalization (3).

The environmental and social factors contributing to the resurgence of murine typhus in California (9,23) and Texas (3,4,19,22) are not identical to the conditions responsible for the explosive emergence of murine typhus in the United States during the 1930s and 1940s (45). The contemporary ecology and epidemiology of murine typhus in the United States now includes multiple newly recognized species of urban wildlife, including opossums and feral cats (11–13,16,17,46), and a second, highly ubiquitous vector flea species (47,48). Increasing numbers of homeless persons in the United States introduce a cohort of persons at increased risk for exposure to the suite of vertebrate reservoirs and vectors that perpetuate the transmission of *R. typhi* in urban settings (9,23). Those conditions are not restricted to California and Texas and could therefore contribute to additional foci of re-emergence in other states affected historically by this

disease (8,15,16,45,46). Efforts are needed to improve healthcare provider and public awareness of this life-threatening and treatable infection that was largely forgotten but never gone (49,50).

Acknowledgments

We thank Nancy Clark, Kacie Cummings, Ronald Tyler, Amara Bashadi, Johanna Salzer, Nicolette Bestul, Yan Zeng, and Carmen Ramos.

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Recent Systemic Antifungal Exposure and Nonsusceptible *Candida* in Hospitalized Patients, South Africa, 2012–2017

Charlotte Rabault, Liliwe Shuping, Ruth Mpembe, Vanessa Quan, Fanny Lanternier, Olivier Lortholary, Olivier Paccoud, Nelesh P. Govender, for GERMS-SA



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Release date: September 17, 2025; Expiration date: September 17, 2026

Learning Objectives

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

- Assess patterns of antifungal sensitivity among different *Candida* species
- Evaluate the epidemiology and outcomes of *Candida* bloodstream infections
- Identify the rate of antifungal resistance in the current case series of candidemia

CME Editor

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Candida bloodstream infections, and their increasing antifungal resistance, are a global concern. In this cross-sectional study, we analyzed 2,443 culture-confirmed candidemia cases reported in South Africa during 2012–2017 to assess the effect of previous antifungal exposure on nonsusceptible *Candida* infection. We classified cases by species resistance profile and patient's antifungal use within 14 days before infection. We found that 48% of cases were caused by nonsusceptible species, and 20% of patients had prior

antifungal exposure, mainly to fluconazole. In patients >90 days of age, prior antifungal use was significantly associated with nonsusceptible *Candida* bloodstream infection (adjusted OR 2.02, 95% CI 1.43–2.87; $p < 0.001$), with species-specific effects. No such association was found in neonates and young infants, for whom hospital transmission appeared more influential. Our findings underscore the need for targeted antifungal stewardship and enhanced infection prevention to mitigate antifungal resistance in South Africa.

Candida bloodstream infections (BSIs) (i.e., candidemia) are among the most common invasive fungal infections globally, with an estimated 626,000 cases annually (1). Infections are associated with a substantial crude mortality rate of 35% (27%–60%) worldwide (1) and up to 44% in adults (2) and 38% in children (3) within South Africa. Since 2000, the epidemiology of *Candida* BSIs has shifted (4). *C. albicans*, which is largely fluconazole susceptible, has historically accounted for most infections, but it is now increasingly replaced by non-*albicans* *Candida* species (NAC), such as *Nakaseomyces glabratus* (formerly *C. glabrata*), that exhibit reduced susceptibility to ≥ 1 antifungal classes, either intrinsically or through acquisition of resistance mechanisms (5,6).

South Africa is facing a large epidemic of BSI caused by antifungal-resistant (AFR) *Candida*. *C. parapsilosis* was documented as a leading cause of candidemia since 2009; more than two thirds of tested isolates exhibited resistance to azoles (7). *C. auris*, a multidrug-resistant species, has spread rapidly to become the second most common cause of candidemia since 2020 (8,9). The exact drivers of antifungal resistance in *Candida* BSIs remain inadequately understood. Prior exposure to antifungal agents has been described as a potent driver of NAC selection in settings with low resistance prevalence (10,11), in specific populations such as in intensive care units (ICU) (12), in hematologic and oncologic wards (13,14), or through ecologic studies (15). Such exposure has also been described as contributing to acquired resistance, mainly after prolonged therapy (16,17), but might not fully explain dissemination of those last strains (18,19), which has primarily been observed during outbreaks in local or regional healthcare settings

(20–22). Most of those studies have focused on adult populations, with limited data from neonatal and pediatric populations, which account for most candidemia cases in South Africa (8).

Treatment options are currently limited to 4 systemic antifungal classes: azoles, echinocandins, polyenes, and flucytosine (23). In low- and middle-income countries (LMIC), limited access to echinocandins, newer-generation azoles, and lipid amphotericin B formulations leads to a heavy reliance on fluconazole, despite the prevailing resistance patterns (24). Consequently, the effect of AFR is greater in those settings (25), leading to a higher risk for treatment failure (26) and use of toxic drugs such as conventional amphotericin B (27). Understanding factors leading to AFR is crucial to implement prevention or mitigation strategies (28). In this study, we aimed to determine whether recent exposure to systemic antifungal drugs was associated with the occurrence of nonsusceptible *Candida* BSI among hospitalized patients at sentinel sites in South Africa.

Materials and Methods

Study Design and Surveillance Methods

In this cross-sectional study nested within the nationwide laboratory-based surveillance network in South Africa (GERMS-SA), we included all patients with culture-confirmed candidemia caused by any of the 6 most common *Candida* species (*C. albicans*, *N. glabratus*, *Pichia kudriavzevii* [formerly *C. krusei*], *C. auris*, *C. parapsilosis*, or *C. tropicalis*) identified in 31 hospital enhanced surveillance sites (ESS) in South Africa during January 1, 2012–December 31, 2017. GERMS-SA receives annual approvals from relevant university

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DOI: <https://doi.org/10.3201/eid3110.250359>

and provincial ethics committees in South Africa. We obtained additional ethics clearance from the Human Research Ethics Committee (Medical) of the University of Witwatersrand for this substudy (no. M240625).

The GERMS-SA surveillance methodology has been thoroughly described elsewhere (7,8). In brief, diagnostic laboratories at surveillance sites were requested to report all episodes of candidemia to the National Institute for Communicable Diseases (NICD; Johannesburg, South Africa). We defined candidemia as illness in a person from whom any *Candida* species was identified from a blood culture specimen. We defined an episode as a 30-day period starting from the date of the first positive *Candida* species culture. Isolation of a new and different *Candida* species within that 30-day period, or any subsequent positive blood cultures after the 30-day period, defined a recurrent episode and a new case. We considered isolation of multiple *Candida* species within the same blood culture set to be a mixed episode. Nurse surveillance officers at ESS prospectively collected additional clinical data using standardized case report forms. The number of ESS and coverage of surveillance expanded over the study period, from 9 ESS in 2 provinces in 2012–2013 to 16 ESS in 8 provinces in 2014–2015, and finally to 16 ESS as well as non-ESS from the public and private health sectors in 2016–2017. Case definitions and methodology remained consistent throughout the 3 study periods.

Diagnostic Laboratory Practices

We confirmed species identification using the Vitek-2 system (bioMérieux, <https://www.biomerieux.com>) or matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonics, <https://www.bruker.com>). We determined MICs of all tested agents except amphotericin B (fluconazole, voriconazole, itraconazole, posaconazole, micafungin, anidulafungin, caspofungin) by using commercial microbroth dilution panels containing Alamar blue (Thermo Fisher Scientific, <https://www.thermofisher.com>), read visually after 24 hours of incubation. We used *C. parapsilosis* ATCC 22019 and *P. kudriavzevii* ATCC 6258 as quality control isolates. We determined MICs of amphotericin B by Etest (bioMérieux) on RPMI-1640 plates containing 2% glucose (DMP, <https://www.nhls.ac.za>), as recommended by the manufacturer. For isolates with acquired resistance on microbroth dilution testing, we rechecked MICs by Etest. For species other than *C. auris*, we interpreted MICs using the available Clinical and Laboratory Standards Institute clinical breakpoints (29). For *C. auris*, we interpreted MICs using the US Centers for Disease Control and Prevention tentative clinical breakpoints (30).

Definitions

We categorized episodes of candidemia into 2 groups on the basis of their identification and susceptibility profiles. We defined nonsusceptible *Candida* isolates as those exhibiting intrinsic nonsusceptibility (*C. auris*, *P. kudriavzevii*, and *N. glabratus*) or acquired resistance to ≥ 1 antifungal agent, classified as intermediate (I), susceptible-dose-dependent (SDD), or resistant (R). We chose that classification in the context of antifungal access in our setting, where azoles, particularly fluconazole, are the main first-line treatment for candidemia in the public sector. For mixed episodes, we considered only the most resistant isolate. We recorded prior systemic antifungal exposure to ≥ 1 antifungal agent (binary variable) within 14 days before the index blood culture collection date.

Study Population and Participant Selection

We included only episodes with ≥ 1 antifungal agent viable isolate processed at the NICD reference laboratory for which we had species-level identification and antifungal susceptibility test results. We excluded recurrent episodes and patients missing data on the main exposure variable from the analysis.

Data Management and Analysis

We used a probabilistic linking method (dtalink command on Stata [31]) for patient deduplication. We described patient characteristics using Fisher exact or χ^2 tests for categorical variables and Student *t*-test or the Wilcoxon–Mann–Whitney test for continuous variables. To evaluate the effect of each confounder on the main association, we used a classical Mantel Haenszel method. We studied the association between prior antifungal exposure and nonsusceptible *Candida* BSI using a multivariable logistic regression adjusted for major confounders. Independence of individual participant outcomes could not be assumed because of observed and unobserved outbreaks leading to enhanced horizontal transmission within sites. Therefore, we integrated expected variation in candidemia risk factors and infection prevention and control measures at each ESS into the analysis and included a hospital site-level random effect regression analysis for multivariable analysis. To account for risk factors and antifungal prescription practices specific to neonates and young infants, as a prespecified effect modifier, we stratified our analysis by age group: neonates and young infants ≤ 90 days of age and patients >90 days of age. We excluded HIV status from the final models, despite its potential as a confounding factor, because the amount of missing data was substantial ($>40\%$). We considered 2-sided *p* values of <0.05 significant.

We performed all statistical analyses using Stata version 18 (StataCorp LLC, <https://www.stata.com>).

Results

Participants

After deduplication, we noted that 8,647 cases were detected during the 6-year surveillance period, of which 4,337 were nonrecurrent episodes and had both a confirmed identification for 1 of the 6 most prevalent *Candida* species and antifungal susceptibility test results. Of those, 2,443 patients were admitted to an ESS and had a completed case investigation form (Figure). We noted differences in age category, sex, year of diagnosis, healthcare sector, province of

diagnosis, and *Candida* species between excluded and included cases (Appendix Table 1, <https://wwwnc.cdc.gov/EID/article/31/10/25-0359-App1.pdf>).

The population studied included 1,099 (45%) patients ≤ 90 days of age, comprising 831 neonates (≤ 28 days) and 254 young infants (29–90 days of age), and 1,342 (55%) patients > 90 days of age (Appendix Table 2). Overall, 94% (2,290/2,443) of patients were treated in a public-sector facility, and 71% (1,703/2,412) were admitted to an ICU. Most cases were recorded in Gauteng Province (1,397/2,443 cases [57%]) and during the 2016–2017 period (1,241/2,443 cases [51%]). Cases in neonates and young infants were reported more often than in patients > 90 days of age during 2014–2015 (353/1,099 [32%] vs. 242/1,342 [18%];

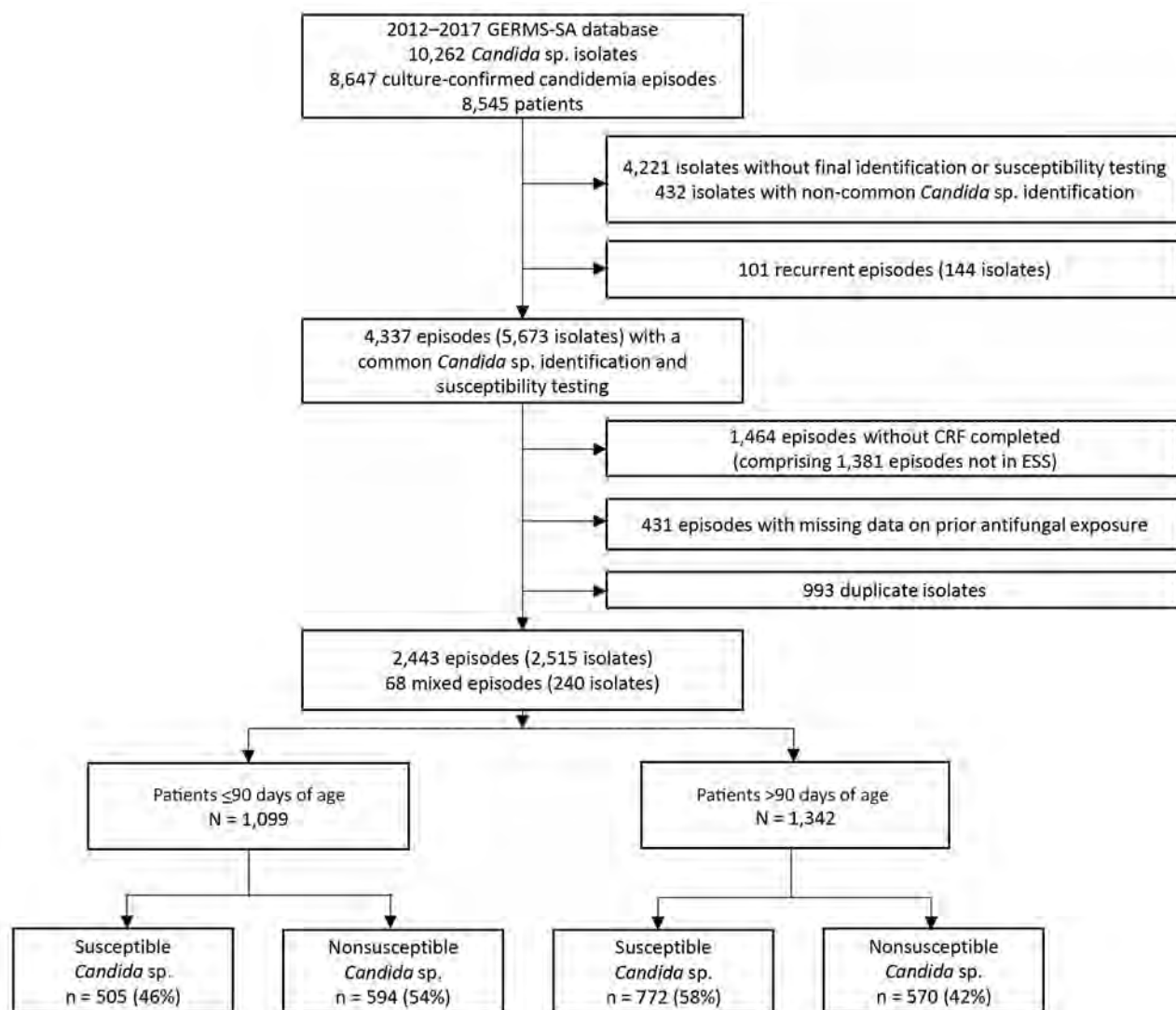


Figure. Flowchart of the selection process for 2,443 cases of candidemia from a 6-year surveillance period for secondary data analysis of recent systemic antifungal exposure and nonsusceptible *Candida* in hospitalized patients, South Africa, 2012–2017. Common *Candida* species were *C. albicans*, *C. parapsilosis*, *Nakaseomyces glabratus* (formerly *C. glabrata*), *C. auris*, *C. tropicalis*, and *Pichia kudriavzevii* (formerly *C. krusei*). CRF, case report form; ESS, enhanced surveillance site.

$p < 0.001$), in Gauteng Province (681/1,099 [62%] vs. 715/1,342 [53%]; $p < 0.001$), and in the public sector (1,094/1,099 [99%] vs. 1,194/1,342 [89%]; $p < 0.001$). Neonates and young infants were also more often hospitalized in an ICU (960/1,089 [88%] vs. 742/1,321 [56%]; $p < 0.001$) and had a longer median hospital stay before infection onset (median [IQR] 14 [9–22] vs. 11 [4–26] days; $p < 0.001$) but less frequently had a central venous catheter in situ (551/1,078 [51%] vs. 742/1,321 [56%]; $p = 0.001$) or total parenteral nutrition administered (211/1,033 [20%] vs. 958/1,308 [24%]; $p = 0.05$).

Prior Antifungal Exposure and Nonsusceptible *Candida* sp.

Overall, recent exposure to a systemic antifungal drug was recorded in 482/2,443 episodes (20%), in a larger proportion of neonates and young infants (272/1,099 [25%]) than older patients (210/1,342 [16%]; $p < 0.001$). The most prescribed agent was fluconazole (340/482 [71%]), in similar proportions in the 2 age groups (196/272 [72%] in neonates and infants vs. 144/210 [69%] in older patients; $p = 0.41$), followed by amphotericin B deoxycholate (AmBD) (106/482 [22%]), in a higher proportion of neonates and young infants than patients >90 days of age (75/272 [28%] vs. 31/210 [15%]; $p = 0.001$). Echinocandins were prescribed for 48 patients, 5/272 (92%) for neonates and young infants versus 43/210 (20%) for patients >90 days of age ($p < 0.001$). Exposure to multiple agents was recorded in 12% of patients ($n = 60$), mainly as a fluconazole and AmBD combination (Table 1; Appendix Table 3).

Of the 2,443 culture-confirmed candidemia cases, 1,165 were classified as nonsusceptible (48%), including 542 (47%) intrinsically nonsusceptible (*N. glabratus* 27%, *C. auris* 7%, *P. kudriavzevii* 13%) and 623 (53%) with acquired resistance (fluconazole-resistant *C. parapsilosis* 51%, fluconazole-resistant *C. albicans* 1%) (Appendix Table 6). Nonsusceptible *Candida* cases were found in a higher proportion of neonates and young infants than patients >90 days of age (594/1,099 [54%] vs. 772/1,342 [43%]; $p < 0.001$).

Effect of Prior Exposure to Antifungals on Nonsusceptible *Candida* BSI

The unadjusted odds ratio (OR) of nonsusceptible *Candida* candidemia among previously exposed neonates and young infants was 1.39 (95% CI 1.05–1.84) times higher than those nonexposed (Appendix Table 4). After adjusting for hospital site, age, sex, time period, province, type of delivery, and birthweight, OR decreased to 1.06 (95% CI 0.75–1.49) (Table 2). The final model revealed a strong cluster effect; differences between hospitals accounted for 14% of the variability

in the occurrence of nonsusceptible *Candida* BSI (intra-cluster correlation coefficient = 0.14; $p < 0.001$).

The unadjusted OR of nonsusceptible *Candida* BSI among previously exposed older patients was 2.23 (95% CI 1.66–3.01) times higher than those nonexposed (Appendix Table 5). After adjusting for hospital site, age, sex, time-period, province, ICU admission and healthcare sector, OR remained at 2.02 (95% CI 1.43–2.87) times higher in patients with a prior exposure compared with those without prior exposure (Table 2). We observed no meaningful cluster effect in the final model (intracluster correlation coefficient <1%; $p = 0.5$).

Among older patients, we observed an increased risk for nonsusceptible *Candida* BSI for those with prior exposure to azoles (unadjusted OR 1.89 [95% CI 1.35–2.64]) and echinocandins (unadjusted OR 6.75 [95% CI 3.10–14.69]) but not after prior exposure to AmBD (OR 1.87 [0.91–3.84]) (Appendix Table 8). Risks remained similar for each class after exclusion of cases with multiagent exposure (data not shown). We compared cases with and without prior exposure to azole; cases with recorded prior exposure to azole had a lower percentage of *C. albicans* (42/151 [28%]) than those without prior exposure (518/1,101 [47%]; $p < 0.001$). We also noted an increase in *C. parapsilosis* in cases with prior azole exposure (54/151 [36%]) than cases without prior exposure (245/1,101 [22%]; $p < 0.001$) (Table 3). We observed similar change in distribution after echinocandins exposure compared with no prior exposure for *C. albicans* (4/41 [10%] vs. 518/1,101 [47%]; $p < 0.001$) and *C. parapsilosis* (24/41 [59%] vs. 245/1,101 [22%]; $p < 0.001$). The proportion of *C. auris* cases was statistically higher after azole prior exposure (16/151 [11%] vs. 44/1,101 [4%]; $p = 0.002$) than that of cases without prior exposure. Among *C. parapsilosis* cases, the proportion of fluconazole-nonsusceptible isolates significantly increased after exposure to echinocandins (22/24 [92%] vs. 121/245 [49%]; $p < 0.001$), but not after azole exposure (32/54 [59%] vs. 121/245 [49%]; $p = 0.19$), compared with cases without exposure recorded.

Discussion

In a high AFR prevalence setting, we found that prior systemic antifungal exposure was independently associated with nonsusceptible *Candida* BSI in patients >90 days of age, but not among neonates and young infants (≤ 90 days of age). Conversely, we observed a significant cluster effect exclusively among neonates and young infants, suggesting that the occurrence of nonsusceptible *Candida* BSIs was not independent in this population. Furthermore, in the older age group, the effect of prior antifungal exposure was drug- and species-specific; we

observed a relatively higher proportion of *C. parapsilosis* and *C. auris* cases after azole and echinocandin preexposure, including a higher proportion of fluconazole-resistant *C. parapsilosis*, compared with cases without recorded prior antifungal exposure.

Previous studies showing an association between prior antifungal exposure and nonsusceptible *Candida*

BSI in adults used heterogeneous definitions and often focused on specific antifungal-species combinations. A surveillance-based study in France that included 2,441 candidemia episodes showed that prior exposure to fluconazole within the previous 30 days was associated with increased odds (OR 2.17 [95% CI 1.51–3.13]) of candidemia caused by species with

Table 1. Baseline characteristics by prior exposure to antifungal drugs in patients >90 days of age with culture-confirmed candidemia at sentinel hospitals, South Africa, 2012–2017*

Characteristic	No prior exposure, n = 1,132	Prior exposure, n = 210	All patients, N = 1,342	p value
Median age, y (IQR)	35 (8–55)	33 (3–54)	34 (8–55)	0.16†
Age group			1,342	
Older infants, 90 d–1 y	112 (10)	31 (15)	143	0.071
Children and adolescents, >1 y–17 y	198 (17)	40 (19)	238	
Adults, 18 y–64 y	663 (59)	105 (50)	768	
Elderly, ≥65 y	159 (14)	34 (16)	193	
Sex			1,341	
F	531 (47)	91 (43)	622	0.335
M	600 (53)	119 (57)	719	
Year of diagnosis			1,342	
2012–2013	288 (25)	63 (30)	351	<0.001
2014–2015	215 (19)	27 (13)	242	
2016	307 (27)	37 (18)	344	
2017	322 (28)	83 (40)	405	
Province§			1,342	
Gauteng	554 (49)	74 (35)	628	<0.001
Other	578 (51)	136 (65)	714	
Healthcare sector			1,342	
Private	99 (9)	49 (23)	148	<0.001
Public	1,033 (91)	161 (77)	1,194	
Prior hospitalization (≤90 d)¶			1,328	
No	251 (22)	35 (17)	286	
Yes	869 (76)	173 (83)	1,042	
ICU admission			1,321	
No	511 (46)	68 (33)	579	<0.001
Yes	601 (54)	141 (67)	742	
Hospital stay before infection onset, d			1,309	
Median (IQR)	10 (3–22)	23 (12–41)	11 (4–26)	<0.001†
≤2	270 (25)	7 (3)	277	<0.001‡
3–7	191 (17)	23 (11)	214	
8–14	227 (21)	37 (18)	264	
15–21	129 (12)	31 (15)	160	
≥22	285 (26)	109 (53)	394	
Systemic antimicrobial drug use			1,081	
No	342 (30)	31 (15)	373	<0.001
Yes	781 (70)	177 (85)	958	
Mechanical ventilation			1,316	
No	749 (68)	126 (61)	875	0.049
Yes	359 (32)	82 (39)	441	
CVC in situ			1,314	
No	505 (46)	51 (25)	556	<0.001
Yes	603 (54)	155 (75)	758	
TPN			1,308	
No	876 (79)	120 (59)	996	<0.001
Yes	230 (21)	82 (41)	312	
HIV status			771	
Seronegative	386 (59)	78 (68)	464	0.069
Seropositive	270 (41)	37 (32)	307	

*Values are no. (%) except as indicated. p value determined by Pearson χ^2 test except as indicated. Numbers for each category indicate no. patients for whom that information was available. Prior antifungal exposure was ≤14 d before blood culture collection. CVC, central venous catheter; ICU, intensive care unit; TPN, total parenteral nutrition.

†p value determined by Wilcoxon rank-sum test.

‡p value determined by Fisher exact test.

§Other province includes Limpopo, Mpumalanga, KwaZulu-Natal, Free State, North West, Northern Cape, Western Cape, Eastern Cape Province.

¶Prior hospital admission was recorded within the previous 90 d before current admission.

Table 2. Random-effect multivariable logistic regression analysis of the effect of prior exposure to antifungal drugs on nonsusceptible *Candida* bloodstream infections among patients with culture-confirmed candidemia, South Africa, 2012–2017*

Variable	<90 d of age†		>90 days of age‡	
	Summary aOR (95% CI) for nonsusceptible <i>Candida</i> spp.	Wald p value	Summary aOR (95% CI) for nonsusceptible <i>Candida</i> spp.	Wald p value
Prior antifungal exposure				
No	Referent		Referent	
Yes	1.06 (0.75–1.49)	0.73	2.02 (1.43–2.87)	<0.001
Age group				
Neonates, ≤28 d	Referent		NA	
Young infants, 29 d–90 d	0.79 (0.53–1.16)	0.23	NA	
Older infants, >90 d–1 y	NA		Referent	
Children–adolescents, >1 y–17 y	NA		0.95 (0.58–1.56)	0.84
Adults, 18 y–64 y	NA		1.92 (1.26–2.91)	0.002
Elderly, ≥65 y	NA		2.84 (1.70–4.74)	<0.001
Sex				
F	Referent		Referent	
M	1.11 (0.84–1.47)	0.36	1.03 (0.81–1.32)	0.47
Year		0.02		
2012–2013	Referent		Referent	
2014–2015	1.97 (1.15–3.38)	0.01	1.06 (0.71–1.58)	0.77
2016	1.47 (0.92–2.32)	0.1	1.65 (1.18–2.32)	0.004
2017	1.82 (1.16–2.84)	0.009	1.49 (1.07–2.08)	0.02
Province				
Other	Referent		Referent	
Gauteng	2.93 (1.21–7.14)	0.01	1.64 (1.25–2.14)	<0.001
ICU admission				
No	NA		Referent	
Yes	NA		1.95 (1.51–2.54)	<0.001
Healthcare sector				
Private	NA		Referent	
Public	NA		0.36 (0.21–0.60)	<0.001
Hospital stay before infection onset, d				
≤2	Referent		Referent	
3–7	1.47 (0.78–2.80)	0.24	0.71 (0.47–1.05)	0.09
8–14	1.86 (1.06–3.28)	0.03	0.77 (0.53–1.13)	0.19
15–21	2.41 (1.33–4.39)	0.004	0.79 (0.51–1.23)	0.29
≥22	2.53 (1.38–4.64)	0.003	0.98 (0.69–1.40)	0.90
Type of delivery				
Cesarean section	Referent		NA	
Vaginal delivery	0.75 (0.56–1.00)	0.05	NA	
Birthweight		0.02		
NBW, ≥2,500 g	Referent		NA	
LBW, <2,500 g	1.32 (0.86–2.02)	0.21	NA	
VLBW, <1,500 g	1.49 (0.98–2.26)	0.06	NA	
ELBW, <1,000 g	1.82 (1.11–2.97)	0.018	NA	

*aOR, adjusted odds ratio; ELBW, extremely low birthweight; ICU, intensive care unit; LBW, low birthweight; NA, not applicable; NBW, normal birthweight; VLBW, very low birthweight.

†23 clusters, mean observations per group = 42.3 (range 1–296); intracluster correlation coefficient = 0.14; $p < 0.001$.

‡31 clusters, mean observations per group = 42.5 (range 1–166); intracluster correlation coefficient <0.0001; $p = 0.5$.

intrinsically reduced fluconazole susceptibility (mainly *N. glabratus* and *P. kudriavzevii*), whereas caspofungin preexposure was responsible for a relative increase in cases of *C. parapsilosis*, *N. glabratus*, and *P. kudriavzevii* compared with *C. albicans* (11). A study focusing on prior fluconazole exposure found an increased risk ratio of 4.47 (95% CI 2.12–9.43) of NAC BSI (10). Another study found that prior exposure to any type of antifungal agent was associated with an increased risk for fluconazole nonsusceptible *Candida* isolate (32). However, most of those studies included <400 episodes; few had >13% of *C. parapsilosis* compared with other species and almost no acquired resistance. Conversely, Shorr et al. (33) did not find an effect

of prior exposure to fluconazole but used a broader definition of prior exposure (within the last 90 days) which might have diluted the effect. Blanchard et al. (34) found a significant positive association between prior exposure to echinocandins and reduced susceptibility to echinocandins in *Candida* spp. (OR 5.25; 95% CI 1.68–16.35). In this study, we used a broad definition of AFR taking into account both intrinsic and acquired resistance; acquired resistance increased recently, particularly in South Africa (6,35). Indeed, acquired resistance accounted for more than half of nonsusceptible episodes overall, largely because of fluconazole-resistant *C. parapsilosis* (95%). Although we also sought to assess echinocandin

resistance, most of the nonsusceptibility patterns encountered in our study concerned azole resistance, and all echinocandin nonsusceptibility was detected among fluconazole-intrinsic resistance species. This is particularly worrisome when considering that fluconazole is one of the only accessible antifungal agents, along with amphotericin B deoxycholate, in the public sector, which covers ≈80% of the South Africa population. The low prevalence of echinocandin resistance observed can be linked to the relatively recent access to this antifungal class in the country; at the time of our study, it was available almost exclusively in the private sector. Similar to previous studies, we found a specific selection of *C. parapsilosis* after exposure to echinocandins, which can be explained by the intrinsically higher MICs of *C. parapsilosis* to echinocandins caused by a polymorphism in the *FKS* gene (11,36). The increased risk for *C. parapsilosis* after prior azole exposure was unexpected, particularly given the lack of observed differences between fluconazole-susceptible and -nonsusceptible isolates. Consistent with data from Vallabhaneni et al. (18), in which 59% of nonsusceptible *N. glabratus* cases had no known prior echinocandin exposure, the substantial proportion of nonsusceptible *Candida* BSI episodes without reported prior antifungal exposure suggests that additional factors may be contributing to antifungal resistance.

We found no association between prior antifungal exposure and nonsusceptible *Candida* BSI among neonates and young infants. However, we observed a cluster effect that was not detected among patients >90 days of age. This finding suggests horizontal transmission during observed or unobserved outbreaks as the main mechanism of acquisition of nonsusceptible *Candida* strains in that population. Indeed, during the study period, the NICD investigated several large outbreaks of resistant *Candida* BSI in neonatal units (37). Studies focusing on the effect of antifungal prophylaxis on deaths in low-birthweight

neonates did not identify an increased risk for non-susceptible *Candida* strains (38).

Furthermore, we hypothesized that the differential impact of prior antifungal exposure could be attributed to a distinct baseline epidemiology with a higher proportion of *C. parapsilosis* and *P. kudriavzevii* in neonates and young infants. Therefore, the influence of prior exposure might be less pronounced compared with horizontal transmission. Most *P. kudriavzevii* isolates were linked to 2 outbreaks in a single neonatal unit in Gauteng Province during 2012–2015, in which overcrowding and suboptimal infection prevention and control practices were suggested as contributing factors despite the absence of a common environmental source (8). Since 2020, reports documenting the spread of *C. parapsilosis* clones in ICUs in the absence of prior antifungal exposure have increased (20); they were linked to invasive devices and found on healthcare workers’ hands (39). Furthermore, a specific clone of *C. parapsilosis* with acquired resistance to fluconazole, related to the *ERG11p* Y132F mutation, has been described globally (21,40) and in Gauteng Province (41); it is associated with persistent strains in the environment (21,42). Finally, the predominant use of polyenes as first-line treatment over azoles in the neonate population might also help explain the absence of observed resistance selection (3,43).

A limitation of this study is that it was not specifically designed to examine the association between prior antifungal exposure and nonsusceptible *Candida* BSI. We collected data on prior antifungal exposure using a binary question, completed by the type of antifungals but without details on indication (prophylaxis, preemptive, or empiric treatment), dosage, or length of exposure. Therefore, we cannot exclude the possibility that some of the recorded prior antifungal exposures were actually empiric or preemptive treatments, introducing a potential reverse causality

Table 3. Comparison of distribution of *Candida* isolates in patients ≥90 d of age (n = 1,301) with and without prior antifungal exposure, South Africa, 2012–2017*

Isolate	No prior exposure, n = 1,101	Prior exposure					
		To azoles, n = 151	p value	To echinocandins, n = 41	p value	To amphotericin B, n = 28	p value
<i>C. albicans</i>	518 (47)	42 (28)	<0.001	4 (10)	<0.001	6 (21)	ND
<i>Nakaseomyces glabratus</i>	202 (18)	20 (13)	0.09	1 (2)	ND	1 (3)	ND
<i>C. auris</i>	44 (4)	16 (10)	<0.001	10 (24)	ND	9 (32)	ND
<i>C. parapsilosis</i>	245 (22)	54 (36)	<0.001	24 (59)	<0.001	8 (29)	ND
FLU-R	121 (11)	32 (21)	0.19	22 (54)	<0.001	2 (7)	ND
FLU-S	124 (11)	22 (15)	ND	2 (5)	ND	6 (21)	ND
<i>C. tropicalis</i>	57 (5)	8 (5)	0.97	2 (5)	ND	2 (7)	ND
<i>Pichia kudriavzevii</i>	34 (3)	11 (7)	0.005	0 (0)	ND	2 (7)	ND

*Values are no. (%) except as indicated. Cases with multiple exposures or mixed episodes are excluded. p values derived by Fisher exact test when validation conditions were met. FLU-R, fluconazole nonsusceptible; FLU-S, fluconazole susceptible; ND, not determined because of the lack of verification of the assumptions required for the statistical tests.

bias. Our analysis was limited to antifungal exposure within the 14 days before the index culture, because of the structure of the case report forms. Further analyses exploring the effect of earlier exposures are warranted, particularly because a previous study (44) reported a higher risk for *N. glabratus* and *P. kudriavzevii* infections after >7 days of prior antifungal treatment compared with shorter treatment durations. Selection bias might be another limitation; we excluded 69% of cases diagnosed at sentinel hospitals because of missing data. Consequently, compared with the overall epidemiology of candidemia in the country, our sample included higher proportions of patients <18 years of age; cases caused by *C. albicans*, *C. tropicalis*, and *P. kudriavzevii*; cases from provinces other than Gauteng; and cases hospitalized in the public sector. The lack of a cluster effect observed in the older population may be attributed to the level (i.e., hospital facility) selected for the random effect; specifically, horizontal transmission appears to occur more frequently within wards than at the facility level. We excluded recurrent episodes from our analysis to avoid autocorrelation bias. However, we acknowledge that those episodes represent high-risk clinical scenarios for antifungal resistance. Dedicated analyses focusing on recurrent episodes are warranted and would contribute valuable insights into the role of prior antifungal exposure in the development of resistance. Finally, data regarding underlying conditions such as diabetes mellitus or immunocompromised status were poorly collected, resulting in residual confounding. We did not observe a change in point estimate for the main exposure effect in a smaller dataset comprising HIV status data (Appendix Table 9). Although those limitations may affect the generalizability of our findings, the large number of patients in our study provides important insights into potential mechanisms underlying AFR emergence and spread in South Africa.

Prior exposure to systemic antifungals appears to be a significant driver of nonsusceptible *Candida* BSI in patients >90 days of age. However, such exposure might not fully account for the epidemiologic patterns observed among neonates, where horizontal transmission may predominate. Neonates and young infants appear to be at particularly high risk of AFR in South Africa; specific studies should focus on that population. Finally, the implementation of antifungal stewardship programs must be accompanied by effective infection prevention and control policies to comprehensively address the large and complex issue of AFR in South Africa.

Members of GERMS-SA (2012–2017): John Black, Vanessa Pearce (Eastern Cape Province); Masego Moncho, Motlatji Maloba (Free State Province); Caroline Maluleka, Charl Verwey, Charles Feldman, Colin Menezes, David Moore, Gary Reubenson, Jeannette Wadula, Merika Tsitsi, Maphoshane Nchabeleng, Nicolette du Plessis, Nontombi Mbelle, Nontuthuko Maningi, Prudence Ive, Theunis Avenant, Trusha Nana, Vindana Chibabhai (Gauteng Province); Adhil Maharj, Fathima Naby, Halima Dawood, Khine Swe Swe Han, Koleka Mlisana, Lisha Sookan, Nomonde Dlamini, Praksha Ramjathan, Prasha Mahabeer, Romola Naidoo, Sumayya Haffeejee, Surendra Sirkar (KwaZulu-Natal Province); Ken Hamese, Ngoaka Sibiyi, Ruth Lekalakala (Limpopo Province); Greta Hoyland, Sindi Ntuli (Mpumalanga Province); Pieter Jooste (Northern Cape Province); Ebrahim Variava, Ignatius Khantsi (North West Province); Adrian Brink, Elizabeth Prentice, Kessendri Reddy, Andrew Whitelaw (Western Cape Province); Ebrahim Hoosien, Inge Zietsman, Terry Marshall, Xoliswa Poswa (AMPATH); Chetna Govind, Juanita Smit, Keshree Pillay, Sharona Seetharam, Victoria Howell (LANCET); Catherine Samuel, Marthinus Senekal (PathCare); Andries Dreyer, Khatija Ahmed, Louis Marcus, Warren Lowman (Vermaak and Vennote); Anne von Gottberg, Anthony Smith, Azwifarwi Mathunjwa, Cecilia Miller, Charlotte Sriruttan, Cheryl Cohen, Desiree du Plessis, Erika van Schalkwyk, Farzana Ismail, Frans Radebe, Gillian Hunt, Husna Ismail, Jacqueline Weyer, Jackie Kleynhans, Jenny Rossouw, John Frean, Joy Ebonwu, Judith Mwansa-Kambafwile, Juno Thomas, Kerrigan McCarthy, Liliwe Shuping, Linda de Gouveia, Linda Erasmus, Lynn Morris, Lucille Blumberg, Marshagne Smith, Martha Makgoba, Mignon du Plessis, Mimmy Ngomane, Myra Moremi, Nazir Ismail, Nelesh Govender, Neo Legare, Nicola Page, Nombulelo Hoho, Ntsieni Ramalwa, Olga Perovic, Portia Mutevedzi, Ranmini Kularatne, Rudzani Mathebula, Ruth Mpembe, Sibongile Walaza, Sunnieboy Njikhlo, Susan Meiring, Tiisetso Lebaka, Vanessa Quan, Wendy Ngubane, Amanda Shilubane, Gloria Thokozile Zulu, Ivy Rukasha, Lerato Qoza, Mabatho Mhlana, Manqoba Rodney Shandu, Mbali Dube, Nozuko Blasich, Phelly Matlapeng, Serisha Naicker, Sydney Mogokotleng, Tsidiso Maphanga, Daniel Desanto, Ernest Tsotetsi, Greg Greene (National Institute for Communicable Diseases).

C.R. received a research grant provided by the Société de Pathologie Infectieuse en Langue Française and by the Assistance Public des Hôpitaux de Paris. N.P.G. was supported by the National Institute for Health and Care Research (grant nos. NIHR134342 and NIHR303140) with UK international development funding from the UK government to support global health research. The views expressed are

those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care.

The authors followed STROBE guidelines in writing this article.

Author contributions: conceptualization: C.R., O.P., N.P.G.; methodology: C.R., L.S., R.M., F.L., O.L., O.P., N.P.G.; data curation: V.Q.; original draft: C.R.; review and editing: L.S., R.M., V.Q., F.L., O.L., O.P., N.P.G.

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Reptile Exposure in Human Salmonellosis Cases and *Salmonella* Serotypes Isolated from Reptiles, Ontario, Canada, 2015–2022

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Release date: September 23, 2025; Expiration date: September 23, 2026

Learning Objectives

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

- Distinguish the subspecies of *Salmonella enterica* responsible for the majority of human infections
- Assess why pet reptiles and amphibians may be frequent hosts for *Salmonella* species
- Analyze the epidemiology of recent contact with a reptile and risk for salmonellosis in the current study
- Compare different reptiles and amphibians regarding their relative risks for salmonellosis after exposure.

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Reptile-associated outbreaks of human *Salmonella* infections are increasing in Canada, coinciding with a rise in the popularity of reptiles as pets. We conducted a retrospective analysis of surveillance data for human *Salmonella* case-patients in Ontario during 2015–2022. We compared serotypes and reptile types for those reporting domestic reptile or amphibian exposure with veterinary *Salmonella* isolates reported during the same period. Case-patients commonly reported contact with reptile types from which *Salmonella* was most frequently isolated.

Salmonella is a common cause of human bacterial enteric illness in Canada (1). Most cases of human infection are associated with *Salmonella enterica* subspecies *enterica*, the main subspecies responsible for mammalian salmonellosis and one that is also frequently isolated from reptiles (2,3). Each year, a lesser number of human infections are associated with *Salmonella* subspecies primarily associated with cold-blooded animals, including reptiles, such as *S. enterica* subspecies *salamae*, *arizonae*, *diarizonae*, *houtenae*, and *indica* (2). *Salmonella* bacteria are naturally found in the gastrointestinal microbiota of many animals, including reptiles kept as pets. According to a representative national population-based survey in Canada (2014–2015) assessing food, animal and water exposures, 2.1% of Ontario residents reported contact with a reptile in the week before interview, and 1.0% reported contact with an amphibian (4). A recent review by Varela et al. (5) noted ownership of pet reptiles and amphibians is increasing in the United States; similar trends have been reported in Canada, particularly during the COVID-19 pandemic when persons were spending more time at home (6).

Reptiles (including lizards, snakes, and turtles) and amphibians (including frogs, toads, and salamanders) often carry *Salmonella* bacteria in their intestines without any signs of clinical illness and can contaminate their surrounding environments through fecal shedding (7). Improper animal husbandry is a risk factor for human illness (8). If reptiles do not receive appropriate veterinary care, are fed an inappropriate diet, are housed in inappropriate environments (e.g., inadequate temperature, overcrowded, or environments in which they are unable to express natural behaviors or receive environmental cues), or are otherwise stressed during capture and transport, shedding

Some serotypes from human case-patients were closely associated with contact with specific reptile types, including *Salmonella* Paratyphi B biovar Java (*Salmonella* Paratyphi B variant L (+) tartrate +) with snakes, *Salmonella* Agbeni with turtles, and *Salmonella* Cotham, *Salmonella* Chester, and *Salmonella* Tennessee with bearded dragons. *Salmonella* was most likely to be reported from reptiles fed a carnivorous diet. Education of reptile owners could help promote proper veterinary care and reduce transmission of zoonotic infections.

of *Salmonella* bacteria can be exacerbated, increasing the risk for transmission to humans (5,9,10). Reptiles in captivity have indeed been noted to shed *Salmonella* at higher frequencies than their wild counterparts (11). Immunosuppression from husbandry stress, in snakes for example, has also been noted to change pet oral flora from predominantly gram-positive to aerobic gram-negative bacteria, increasing oral cavity colonization by *Salmonella* and potentially leading to more gastrointestinal tract shedding (12). Further, all adult amphibians and some reptiles (e.g., snakes) are obligate carnivores and require diets of live foods. Many examples of recommended feeding sources (e.g., crickets, worms, or rodents) (13) for carnivorous amphibians and reptiles are also natural reservoirs of *Salmonella*, further increasing the risk for infection in pet owners or those involved in animal husbandry who fail to take appropriate preventive measures (5).

Children <5 years of age, elderly persons, and those with impaired immunity are particularly susceptible to *Salmonella* because of their developing or weakened immune systems (14). Several outbreaks of reptile-associated salmonellosis have been reported in Canada and the United States, including recent outbreaks of *S. enterica* serovar Vitkin (bearded dragons, 2024), *S. enterica* serovar Lome (geckos, 2020–2024), *S. enterica* serovar Muenchen (geckos, 2020–2024), *S. enterica* serovar Cotham and *S. enterica* serovar Kisarawe (bearded dragons, 2012–2014), *S. enterica* serovar Typhimurium (snakes and feeder rodents, 2012–2014), and *S. enterica* serovar Enteritidis (snakes and feeder mice, 2012–2015); children accounted for a high proportion of cases in each instance (15–20). Similarly, numerous outbreaks of *Salmonella* Typhimurium linked to pet amphibians and turtles have been reported in the United States (21), including a large outbreak associated with dwarf clawed frogs (2008–2011) and a multistate outbreak associated with pet turtles (2008) (22,23). In both instances, children accounted for a high percentage of cases (22,23).

In Ontario, persons reported to have salmonellosis are interviewed by local public health investigators to identify a potentially causative source of

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DOI: <https://doi.org/10.3201/eid3110.241803>

infection. Where multiple possible exposures are reported, awareness of serotypes known to be associated with reptiles (or feeder prey) can aid in identifying the most likely exposure and inform follow-up actions. This study aimed to explore the proportion of locally acquired human salmonellosis case-patients in Ontario during 2015–2022 who reported reptile or amphibian contact before symptom onset, to assess the reptile species and *Salmonella* serotypes reported most often among those human case-patients, and to compare those observations to reptile and amphibian veterinary isolates reported during the same time period in Ontario, thereby establishing whether the reported reptile or amphibian exposure in human case-patients was a reasonable source of acquisition on the basis of correlating veterinary data.

Methods

Epidemiology

We extracted for analysis data for all human case-patients meeting the confirmed or probable case definition for salmonellosis in Ontario (24) and reported through the provincial electronic surveillance system by local public health units with an episode date of January 1, 2015–December 31, 2022. Episode date was captured per the following hierarchy depending on available information: symptom onset date, specimen collection date, laboratory test date, or date on which the case was reported to the local public health unit.

We extracted *Salmonella* serotype, demographic data, and exposure data for all human case-patients with a yes or no response regarding contact with reptiles or amphibians during the week before symptom onset. Sporadic cases had no epidemiologic link to a known common source. Outbreak-associated cases were linked by a shared spatiotemporal or exposure event or by PulseNet Canada whole-genome sequencing (WGS) with an initial threshold of 0–10 alleles by whole-genome multilocus sequence typing, and where ≥ 2 related isolates occurred within a 60-day period (24,25).

Per the standardized Ontario questionnaire for salmonellosis, persons who report travel during the exposure period do not necessarily need to be asked about other potential exposures, including reptile or amphibian contact; however, that information can be optionally collected (26). We thus excluded persons who reported travel during the exposure period from further analysis. Removing those cases was further justified because reptile and amphibian species can vary globally, and we anticipated interactions during travel to differ from

those with pets or interactions through local petting zoos or wildlife exposure.

We compared demographic data for sporadic cases in which no travel was reported to assess differences in age or sex between those with or without reptile or amphibian contact. We performed descriptive analyses of case-patient demographics, *Salmonella* serotype, and reptile and amphibian types for a reduced dataset, which included sporadic case-patients who reported having contact with a reptile or amphibian in the week before symptom onset and who did not report travel. We used Pearson χ^2 test to assess associations between case-patients reporting any or no reptile or amphibian contact and case-patient demographics or risk factors.

Reptile and Amphibian Veterinary Isolates

We obtained Ontario reptile and amphibian veterinary isolate data reported to the Ontario Ministry of Agriculture, Food and Agribusiness (OMAFRA) through mandatory reporting by veterinary diagnostic laboratories during 2015–2022 (27). Those data reflected all reptile and amphibian specimens submitted to Ontario veterinary diagnostic laboratories from private veterinarians as part of animal health examinations in which *Salmonella* was identified. Sequencing data from veterinary *Salmonella* isolates are not routinely reported to OMAFRA; therefore, only serotype information was available for evaluation. We compared the serotypes detected with the human case data and further reviewed to assess the frequency of detection of specific serotypes by diet type and whether the animals were privately owned or from a zoological collection. We used the Pearson χ^2 test to assess associations between reptile types and reptile diet. We conducted all analyses using Microsoft Excel (Microsoft) and SAS Enterprise Guide version 8.2 (SAS Institute Inc.) with a significance level of 5% ($\alpha = 0.05$). This project did not require research ethics committee approval because the activities described herein were conducted in fulfillment of Public Health Ontario's legislated mandate "to provide scientific and technical advice and support to the health care system and the Government of Ontario in order to protect and promote the health of Ontarians" and are therefore considered public health practice, not research (28).

Results

Epidemiology

A total of 18,452 human salmonellosis cases (all serotypes) were reported in Ontario during 2015–2022

(18,296 confirmed, 246 probable). Of those case-patients, 68.8% (12,585) had a yes or no response regarding reptile or amphibian contact in the week before symptom onset; the remainder reported contact as unknown or not asked. Most cases were sporadic (92.7%, 11,668/12,585); the remainder were linked to 1 of 97 unique outbreaks (ranging in size from 1–59 cases with a median of 6 cases). Of the outbreak-associated cases, 13 were linked by WGS to a known outbreak of *Salmonella* (I) 4,[5],12:i:- associated with snakes and feeder rodents (2022), 2 were linked by WGS to a known outbreak of *Salmonella* Vitkin associated with bearded dragons (2022), and the remainder were linked to nonreptile or feeder prey exposure.

Of all case-patients, 28.2% (3,551/12,585) reported travel outside of Ontario during the week before symptom onset and were excluded from subsequent analyses. Of the remaining sporadic case-patients, 6.3% (513/8,148) reported having contact with a reptile or amphibian in the week before symptom onset (from 4.3% [27/625] of cases in 2022 to 8.1% [99/1,225] of cases in 2015) (Table 1).

Age was unknown in 2 sporadic cases with no reported reptile contact; we excluded those persons from analyses involving age. The age distribution of

sporadic case-patients differed between those who reported having contact with a reptile or amphibian during the week before symptom onset and those who reported no contact ($\chi^2 = 46.1$; $p < 0.001$) (Table 1). Of all sporadic case-patients who reported reptile or amphibian contact, 9.0% (46/513) were <1 year of age and 29.2% (150/513) were <10 years of age (Table 1). Comparatively, 3.6% of sporadic case-patients with no reported reptile or amphibian exposure were <1 year of age (278/7,635) and 21.6% were <10 years of age (1,646/7,635) (Table 1).

Reptile and Amphibian Exposures

The most common reptiles reported by sporadic case-patients with local acquisition of infection were lizards (46.4%, 238/513) followed by snakes (26.7%, 137/513) and turtles or tortoises (19.3%, 99/513), whereas 10.7% (55/513) reported contact with amphibians (Table 1). Just under 12% of case-patients reported >1 reptile type (11.9%, 61/513). Corn snakes (*Pantherophis guttatus*) were the most frequently reported species of snake (44.1%, 15/34), followed by pythons (family Pythonidae) or ball pythons (*Python regius*) (32.4%, 11/34) and boas (family Boidae) (14.7%, 5/34). However, information regarding snake species was not

Table 1. Case-patient demographics for reported confirmed and probable sporadic locally acquired human cases of salmonellosis in Ontario, Canada, 2015–2022*

Characteristic	Reptile contact		Total cases	p value (χ^2)
	Yes	No		
Sex†‡				0.37 (0.82)
M	234 (6.0)	3,637 (94.0)	3,871 (100.0)	
F	279 (6.5)	3,992 (93.5)	4,271 (100.0)	
All	513 (6.3)	7,629 (93.7)	8,142 (100.0)	
Age group, y‡§				<0.001 (46.1)
<1	46 (9.0)	278 (3.6)	324 (4.0)	
1–4	42 (8.2)	815 (10.7)	857 (10.5)	
5–9	62 (12.1)	553 (7.2)	615 (7.6)	
10–19	84 (16.4)	693 (9.1)	777 (9.5)	
20–29	109 (21.2)	939 (12.3)	1,048 (12.9)	
30–39	54 (10.5)	699 (9.2)	753 (9.2)	
40–49	52 (10.1)	696 (9.1)	748 (9.2)	
50–59	40 (7.8)	965 (12.6)	1,005 (12.3)	
60–69	13 (2.5)	853 (11.2)	866 (10.6)	
≥70	11 (2.2)	1,142 (15.0)	1,153 (14.2)	
All	513 (100.0)	7,633 (100.0)	8,146 (100.0)	
Year				
2015	99 (8.1)	1,126 (91.9)	1,225	
2016	88 (6.2)	1,328 (93.8)	1,416	
2017	80 (6.4)	1,176 (93.6)	1,256	
2018	69 (5.3)	1,236 (94.7)	1,305	
2019	71 (6.4)	1,035 (93.6)	1,106	
2020	42 (6.5)	609 (93.5)	651	
2021	37 (6.6)	527 (93.4)	564	
2022	27 (4.3)	598 (95.7)	625	
All	513 (6.3)	7,635 (93.7)	8,148	

*Values are no. or no. (%) except as indicated.

†Sex is reported in the provincial electronic surveillance system as male, female, transgender, or other. Analyses involving sex were restricted to data for case-patients with sex reported as male or female due to low counts for other variables. No case-patients with domestic acquisition of infection and reported reptile exposure reported sex other than male or female.

‡Sporadic cases only.

§Of 513 case-patients with no travel history who reported reptile exposure.

collected for most case-patients reporting snake contact (75.2%, 103/137). Almost 12% of sporadic case-patients who reported reptile or amphibian contact did not have any details available regarding species (11.9%, 61/513), and 1 person reported contact with a crocodilian (0.2%, 1/513). Of those who reported contact with lizards, most reported contact with bearded dragons (*Pogona vitticeps*) (47.1%, 112/238), followed by other lizards (order Squamata) (29.4%, 70/238), geckos (order Gekkota) (26.1%, 62/238), chameleons (family Chamaeleonidae) (5.5%, 13/238), and iguanas (family Iguanidae) (3.8%, 9/238).

Although we found no significant association between sex and whether a person reported contact with reptiles during the exposure period ($\chi^2 = 0.82$; $p = 0.37$), female patients were generally more likely than male patients to report contact with each reptile type and were significantly more likely to report contact with amphibians (Table 2). We noted a significant difference between age group and whether sporadic case-patients with local acquisition of infection reported reptile contact ($\chi^2 = 46.1$; $p < 0.001$) (Table 1). We also found an apparent association between case-patient age and reptile type. Of those reporting contact with a snake, the largest percentage (30.7%, 42/137) were 20–29 years of age (Figure 1). Conversely, children <10 years of age made up the greatest percentages of those reporting contact with an amphibian (43.6%, 24/55) or a turtle (34.3%, 34/99) (Figure 1). Children <10 years of age (26.1%, 62/238) and adults 20–29 years of age (25.6%, 61/238) each made up a similar percentage of those reporting lizard contact (Figure 1).

A total of 94 unique *Salmonella* serotypes were reported, 47.9% ($n = 45$) of which were only reported once. Most human isolates (94.9%, $n = 487$) were *S. enterica* subsp. *enterica*. The most common serotypes identified among the sporadic human case-patients reporting reptile or amphibian contact were *Salmonella* Typhimurium (15.8%, 81/513), *Salmonella* Enteritidis (14.8%, 76/513), *Salmonella* Oranienburg (5.3%, 27/513), and *Salmonella* Muenchen (4.9%, 25/513),

which are serotypes also often associated with feeder prey (Figure 2; Appendix Table, <https://wwwnc.cdc.gov/EID/article/31/10/24-1803-App1.pdf>) (29–32). Although some serotypes were associated with a range of reptile or amphibian exposures, some were more closely associated with a single type. For example, 87.5% (14/16) of *Salmonella* Paratyphi B biovar Java (also known as *Salmonella* Paratyphi B variant L [+] tartrate +) case-patients reported contact with a snake, whereas 100.0% (5/5) of *Salmonella* Chester, 81.8% (9/11) of *Salmonella* Tennessee, and 71.4% (5/7) of *Salmonella* Cotham case-patients reported contact with a bearded dragon and 80.0% (4/5) of *Salmonella* Agbeni case-patients reported contact with a turtle (Figure 2; Appendix Table). Most *S. enterica* subsp. *diarizonae* case-patients reported contact with lizards (57.1%, 8/14), as did most *S. enterica* subsp. *houtenae* case-patients (88.9%, 8/9).

Reptile and Amphibian Veterinary Isolates

A total of 46 reptile submissions where *Salmonella* was detected were reported to OMAFA by veterinary diagnostic laboratories in Ontario during 2015–2022; 35 different serotypes were isolated. No amphibian submissions were reported to OMAFA during that period. Most animals were privately owned (80.4%, 37/46) versus zoological (19.6%, 9/46), and 89.1% (41/46) could be identified to the species level. Among privately owned reptiles, the most common species with a *Salmonella* isolate were bearded dragons (16.2%, 6/37), ball pythons (13.5%, 5/37), and corn snakes (8.1%, 3/37). The most common reptile species with *Salmonella* isolated from zoological collections was the Eastern Massasauga rattlesnake (*Sistrurus catenatus* [89%, 8/9]). More *Salmonella* isolates came from snake submissions (63.0%, 29/46) than from lizard submissions (34.8%, 16/46) or chelonian submissions (2.2%, 1/46) ($\chi^2 = 16.73$; $p = 0.003$).

In this dataset, obligate carnivores were more likely to have *Salmonella* isolated (69.6%, 32/46) than

Table 2. Case-patient reptile contact by sex for reported confirmed and probable sporadic locally acquired human cases of salmonellosis in Ontario, Canada, 2015–2022*

Reptile†	Case-patient sex‡		Total cases	p value (χ^2)
	M	F		
Lizards	109 (45.8)	129 (54.2)	238	0.94 (0.006)
Snakes	58 (42.3)	79 (57.7)	137	0.22 (1.52)
Turtles	47 (47.5)	52 (52.5)	99	0.99 (0.0002)
Amphibians	17 (30.9)	38 (69.1)	55	0.01 (6.14)
Unspecified	32 (52.5)	29 (47.5)	61	>0.99 (0.00)

*Values are no. or no. (%) except as indicated.
†Of 513 case-patients with no travel history who reported reptile exposure. Sporadic cases only. Reported reptile types were not mutually exclusive.
‡Sex is reported in the provincial electronic surveillance system as male, female, transgender, or other. Analyses involving sex were restricted to data for case-patients with sex reported as male or female due to low counts for other variables. No case-patients with domestic acquisition of infection and reported reptile exposure reported sex other than male or female.

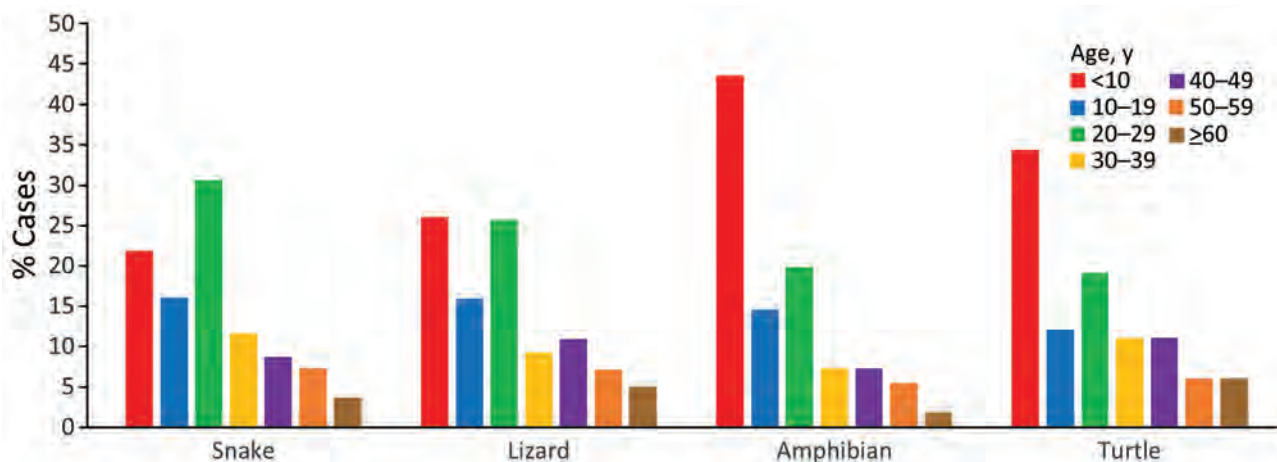


Figure 1. Age distribution of confirmed and probable sporadic locally acquired human salmonellosis case-patients who reported reptile or amphibian contact, by reptile type (n = 513), in study of reptile exposure among human cases and *Salmonella* serotypes isolated from reptiles, Ontario, Canada, 2015–2022. Reptile types reported by each case-patient were not mutually exclusive; thus, a case might be reported under >1 reptile type. The proportion of case-patients by age group is presented separately for each reptile type (i.e., percentages by age group for each reptile type sum to 100%).

were omnivores (15.2%, 7/46), herbivores (8.7%, 4/46), and species that could not be classified (6.3%, 3/46) ($\chi^2 = 14.53$; $p < 0.001$) (Figure 3). For all reptile cases, the most common *Salmonella* serotype isolated was *Salmonella* IIIa:56:z4,z23:- (15.2%, n = 7/46), followed by *Salmonella* Cotham, *Salmonella* IIIa:41:z4,z23:-, *Salmonella* IV:43:z4,z23:-, *Salmonella* IV:50:g,z51:-, and *Salmonella* Kisarawe (4.3% for each, n = 2) (Figure 3). An additional 29 serotypes were detected only once (Figure 3).

Discussion

As the popularity of reptiles kept as household pets has increased, a concurrent increase has occurred in the number of sporadic case-patients with *Salmonella* who report reptile contact before symptom onset, in addition to an increase in the number of outbreaks linked to reptiles and feeder rodents. Although only 2.1% of Ontario residents surveyed in 2014 reported having contact with a reptile in the previous week and 1.0% reported contact with an amphibian (4), 6.3% of sporadic *Salmonella* case-patients with no recent travel history reported contact with reptiles or amphibians in the week before symptom onset during the study period, reflecting a higher-than-expected frequency of reptile or amphibian contact among *Salmonella* case-patients compared with the background population. Several recent outbreaks of *Salmonella* have also been linked to reptiles (including snakes and lizards) and feeder rodents; children have been disproportionately affected (15,19).

Similar to reported trends in reptile ownership derived from online search data, lizards (particularly

bearded dragons) and snakes were commonly reported by Ontario case-patients with reptile exposures (33). The finding that *Salmonella* case-patients reporting reptile or amphibian contact were generally younger than those with no reported reptile contact likely reflects young children's increased susceptibility and increased risk for exposure to bacteria because of a combination of developing immune systems, limited hand hygiene, tendency to mouth their hands and objects, and lack of supervision during reptile contact (14). Observed associations between age and reptile type may reflect differences in pet ownership; however, low counts for some age groups resulted in insufficient study power to reliably test for a statistical difference between age groups. Recognizing the risk for *Salmonella* infection associated with pet turtle ownership (particularly for turtles with a shell <4 cm long, which children might be more likely to handle and place in their mouths), the US Food and Drug Administration banned the sale of small turtles to the public (i.e., as pets) in 1975; however, no such national ban exists in Canada, and any ownership restrictions are decided at a municipal level (34,35). Despite the US ban on turtles sold as pets, national outbreaks linked to small turtles sold illegally continue to occur, reflecting enforcement challenges associated with such bans (21).

Although the most common reptile species with detection of *Salmonella* reported to OMAFA were similar to the most common reptiles reported by human salmonellosis case-patients, the most frequent *Salmonella* serotypes isolated were dissimilar. In the reptile veterinary cases, greater diversity was observed in

the *Salmonella* serotypes isolated. In our study, this finding might be explained by the veterinary patient population included; identified serotypes might reflect those more likely to be isolated from reptiles experiencing stress or illness (and hence, reptiles undergoing veterinary examination) than isolates from apparently healthy animals sampled during human health investigation. *Salmonella* serotype richness and prevalence have been observed to increase in reptiles after shipment; those changes are typically attributed to exposure to stress, among other factors (36). Most *Salmonella* serotypes identified from reptile veterinary isolates belonged to *S. enterica* subsp. *enterica*, *arizonae*, and *diarizonae*, and within each subspecies many serotypes are known to be reptile-associated (2,37).

A carnivorous diet (10) was associated with greater risk for *Salmonella* isolation from reptile vet-

erinary cases in this dataset. Several outbreaks of human salmonellosis associated with contact with snakes and feeder rodents have been documented, which might also underlie an increased risk for veterinary salmonellosis cases in reptiles fed contaminated prey (18,38). Feeding live prey contaminated with *Salmonella*, combined with poor husbandry, poor welfare, or other concurrent physiologic stressors such as debilitation through inanition and dehydration in wild-caught reptiles, might lead to increased *Salmonella* shedding and subsequent contamination of the cage enclosure with *Salmonella* (3,5). Many omnivorous and herbivorous reptiles also consume their own fecal matter as part of their normal diet or use fecal marking as part of territoriality and home range marking, which could amplify infection.

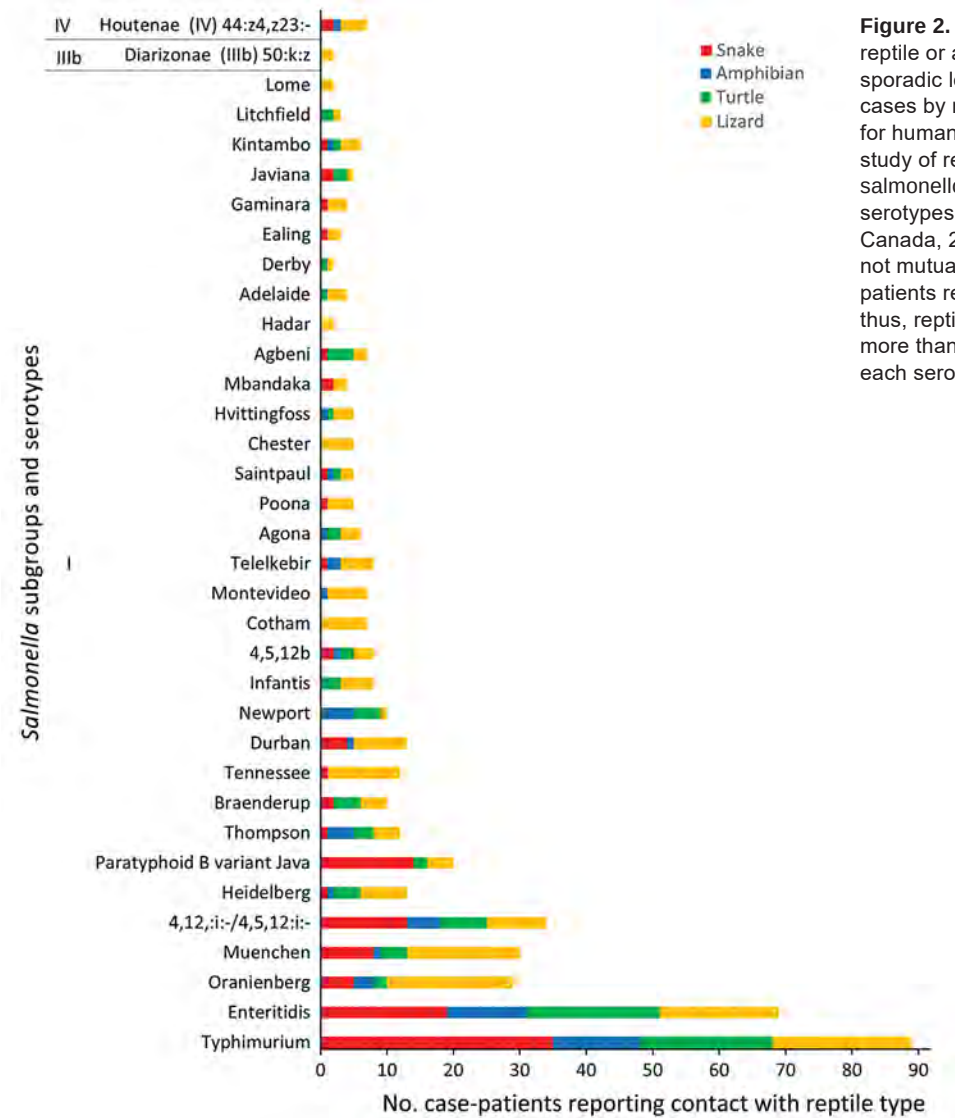


Figure 2. Major taxonomic grouping of reptile or amphibian contact reported by sporadic locally acquired salmonellosis cases by reported *Salmonella* serotype for human case-patients (n = 513) in study of reptile exposure among human salmonellosis cases and *Salmonella* serotypes isolated from reptiles, Ontario, Canada, 2015–2022. Reptile species were not mutually exclusive (i.e., some case-patients reported contact with >1 species); thus, reptile species counts may sum to more than the number of cases reported for each serotype over the study period.

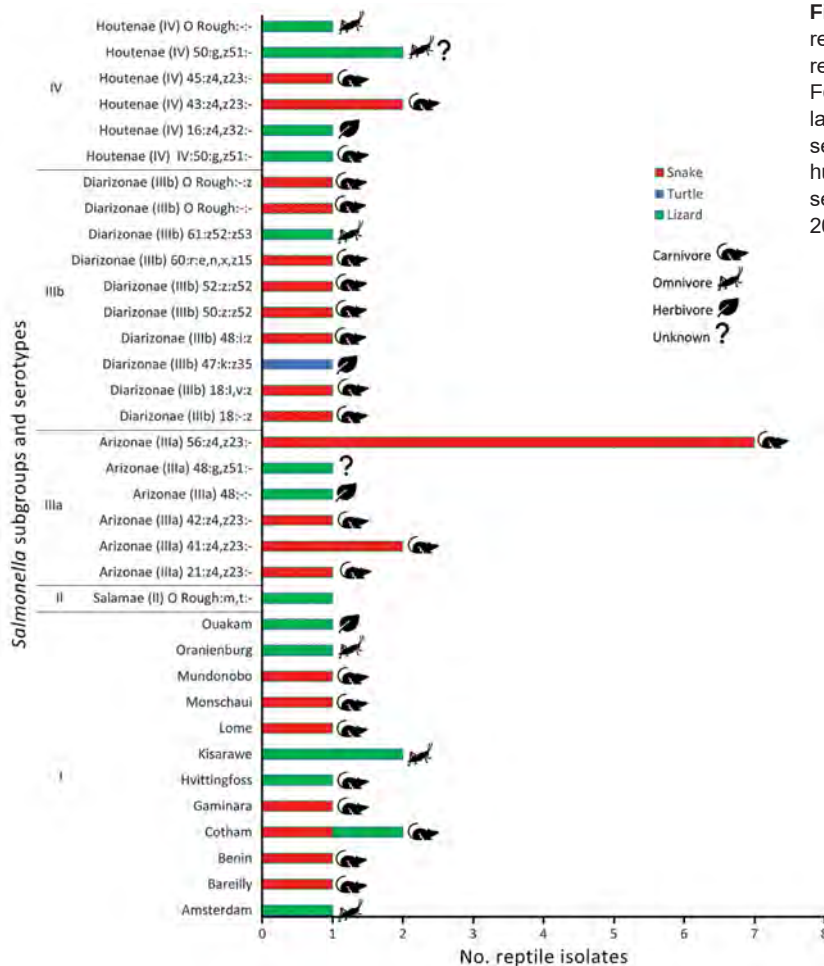


Figure 3. Major taxonomic grouping and diet of reptiles from which *Salmonella* was detected and reported to the Ontario Ministry of Agriculture, Food and Agribusiness by veterinary diagnostic laboratories in Ontario, by reported *Salmonella* serotype, in study of reptile exposure among human salmonellosis cases and *Salmonella* serotypes isolated from reptiles, Ontario, Canada, 2015–2022.

Previous research (39) noted that 48% of pet reptiles sampled from private households and pet shops were found to carry *Salmonella*, and a literature review (3) found that captive reptiles had higher rates of *Salmonella* carriage than did wild reptiles. Similar to other research, we found that *Salmonella* was isolated more frequently in snakes than in lizards or chelonians (3,11,37,39). Snakes are highly prone to *Salmonella* infections because of rodent-based diets and captive husbandry practices, which could explain their association with human disease (12). Pet owners and others in contact with reptiles should adopt precautionary measures while handling reptiles, feeder prey, and their enclosures, assuming that *Salmonella* could be present even if the reptiles appear healthy (40). Routine culture of *Salmonella* could be difficult in healthy reptiles, and treatment to reduce carriage is not possible; therefore, routine pet testing might not be advisable unless persons at risk for severe illness are frequently exposed or unless reptile handling is part of a commercial business

(12). In the event of an outbreak of human salmonellosis in which reptiles or amphibians are suspected to be the source of exposure, collection of environmental swab samples from the animal's enclosure might successfully identify the same serotype. In fact, isolating *Salmonella* from environmental specimens was found to be more reliable than isolating *Salmonella* from animal fecal specimens, possibly because of intermittent fecal shedding (15,19,40). Furthermore, as part of human outbreak management, pet owners should be encouraged to seek appropriate veterinary care, including husbandry analysis, to resolve immunocompromised health status, given pet owners might have a poor understanding of optimal health and welfare of their pets (41).

Although we identified associations between reptile contact and subsequent human illness, we did not adjust analyses for other potentially causative exposures reported during the exposure period (i.e., consumption of specific food items or other animal exposures). Thus, reptile contact should not necessarily

be inferred to be the causative exposure responsible for human illness. Data used for analyses were subject to case-patient recall and individual investigator interview and data entry practices; thus, some outbreak-associated cases might have been misreported as sporadic. Ideally, public health investigators should ensure that all *Salmonella* case-patients are asked about reptile and amphibian contact, including reptile type. If a human case or outbreak of *Salmonella* infection involving a species other than *S. enterica* is identified, potential reptile- or amphibian-associated exposure should be considered. Similarly, if reptile or amphibian exposure is reported during a *Salmonella* case or outbreak investigation and a reptile-associated serotype is identified, further testing should be considered to confirm the source of illness and inform case education and outbreak prevention.

The lack of amphibian animal salmonellosis cases in this study and overall far fewer isolations of *Salmonella* from reptiles compared with isolations in humans with exposure to reptiles suggest that *Salmonella* carriage might not always lead to illness in reptiles and amphibians, signs of illness might not always be recognized, veterinary care might not always be available, or the cause of death might not always be investigated (42). In developing educational resources for pet owners, consideration should be given to providing information to pet owners on the risks associated with both reptile ownership and handling of feeder prey such as rodents. Reminding pet owners that reptiles kept as pets should receive regular veterinary care, both to ensure the health of reptiles and to provide opportunities for ongoing pet owner education, is also crucial.

Acknowledgments

We thank the Ontario public health unit investigators involved in human case investigation and follow-up and the Ontario veterinarians involved in reptile and amphibian care and testing. We also thank Public Health Ontario's Laboratory and the Animal Health Laboratory for their routine work isolating, identifying, and sequencing *Salmonella*.

The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of Public Health Ontario; the Ministry of Agriculture, Food and Agribusiness; or the Ministry of Health.

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Comparative Epidemiology of *Salmonella enterica* Serovars Paratyphi A and Typhi Causing Enteric Fever, Bangladesh, 2018–2020

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Enteric fever remains a public health challenge. We analyzed data from a cluster-randomized Vi-tetanus toxoid conjugate vaccine trial to compare the epidemiology between *Salmonella enterica* serovars Paratyphi A, which causes paratyphoid fever, and Typhi, which causes typhoid fever. The overall incidence rate of paratyphoid fever was 27 (95% CI 23–32)/100,000 person-years (PY) and of typhoid fever was 216 (95% CI 198–236)/100,000 PY. We observed the highest incidence for both diseases in children 2–4 years of age: 72

(95% CI 41–117)/100,000 PY for paratyphoid and 887 (95% CI 715–1,088)/100,000 PY for typhoid. Lack of private toilets and safe drinking water were associated with both diseases. Prevalence of multidrug resistance was significantly higher in *Salmonella* Typhi (20.2%) than in *Salmonella* Paratyphi A (0.8%) ($p < 0.001$). Our data suggest that integrated control measures targeting water, sanitation, and hygiene measures and bivalent vaccine targeting both pathogens are promising strategies to control both diseases.

Enteric fever is caused by *Salmonella enterica* serovars Paratyphi A, B, and C, which cause paratyphoid fever, and Typhi, which causes typhoid fever. Globally, an estimated 9.3 million cases and 107,459 deaths related to typhoid and paratyphoid fevers occurred in 2021; they especially affected children living in low- and middle-income countries (1). Although *Salmonella* Typhi played a major role, *Salmonella* Paratyphi A constituted 23.3%

of enteric fever cases in South Asia countries (1). Preventing enteric fever by improving water, sanitation, and hygiene (WASH) infrastructure and practices remains challenging where resources are constrained, placing great reliance on antimicrobial therapy. Increasing multidrug resistance (MDR), defined as resistance to former first-line drugs including ampicillin, chloramphenicol, and trimethoprim/sulfamethoxazole (cotrimoxazole),

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DOI: <https://doi.org/10.3201/eid3110.241601>

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in *Salmonella* Typhi is hampering successful therapy (2–5). However, previous studies show that MDR rates declined before the emergence of fluoroquinolone-nonsusceptible *Salmonella* Paratyphi A and *Salmonella* Typhi strains that render fluoroquinolone less suitable as first-line therapy prescribed by clinicians (6–11). Furthermore, the increase in azithromycin resistance in some settings is worrisome (12,13). Given the overlapping clinical features of *Salmonella* Paratyphi A infections, laboratory diagnostics are essential for distinguishing between the pathogens to guide appropriate antimicrobial therapy. Because antimicrobial resistance (AMR) makes treatment more difficult, vaccines and WASH interventions play a vital role in prevention.

World Health Organization (WHO)-approved typhoid conjugate vaccines (TCVs) are available (14–18), and bivalent vaccines targeting both *Salmonella* Paratyphi A and *Salmonella* Typhi are in development (19). In this study, we analyzed data from a cluster randomized trial of Vi-tetanus (Vi-TT) toxoid conjugate vaccine, based on the Vi capsular polysaccharide of *Salmonella* Typhi, in urban Dhaka, Bangladesh (20), to assess epidemiologic features of enteric fever. We conducted this study as part of the original vaccine trial study (protocol no. PR-17115), which received ethical approval from the research review committee and the ethical review committee of icddr,b in Dhaka. No additional ethics submission was required. We obtained informed written consent from legal guardians of child participants and from adult participants.

Methods

Study Design and Site

We used the data from a participant- and observer-blind, cluster-randomized, controlled trial of Vi-TT vaccine effectiveness study (20), which was conducted in a densely populated urban area of wards 2, 3, and 5 of Mirpur in Dhaka (Figure 1). Vi-TT was the study vaccine, and the Japanese encephalitis (JE) vaccine was the control vaccine (21). The study area was divided into 150 geographic clusters randomly assigned to the Vi-TT or JE vaccine arms with a 1:1 ratio (20). A baseline census was conducted during February 14–March 25, 2018, to enumerate the entire population in this study area. The census was subsequently updated at 6-month intervals to capture all births, deaths, and migrations. Individual-level and household-level demographic and socioeconomic data, household geopositioning, and WASH data were collected at each census (20).

Passive Surveillance for Enteric Fever

We conducted passive surveillance during April 26, 2018–March 15, 2020, in the 8 main healthcare facilities (HCFs) serving the study population: Mirpur Field Office, Shaheed Suhrawardy Hospital, Radda MCH-FP Centre (Mirpur-10), Mirpur Adhunik Hospital, Kalshi Shishu Hospital, Kurmitola General Hospital, Kingston Hospital, and Aalok Health Care and Hospital Ltd (Figure 1) (20). We enrolled participants of all age groups living in the surveillance catchment area who had a history of fever (>48 hours) or objective fever (axillary temperature $\geq 38.0^{\circ}\text{C}$) at the time of HCF visit after they or a parent provided written informed consent. We collected blood specimens (3 mL from participants ≤ 17 years of age and 5 mL from those >17 years of age) and clinical data upon enrollment. At the time they sought clinical care, we confirmed patient identity either through household identity cards distributed during the census or via census on electronic tablets when identity cards were unavailable.

Laboratory Analysis

We performed microbiologic culture of blood samples using a standard automated BacT/ALERT method (22). When we detected a positive signal from the automated culture machine, we took subcultures on MacConkey agar plates. After overnight incubation at 37°C , we inoculated non-lactose fermenting colonies on Kligler's Iron Agar (KIA), Motile Indole Urea (MIU), and citrate tubes for biochemical testing to identify *Salmonella* spp.; we serotyped specimens with *Salmonella*-specific O and flagellar H antiserum (Denka Sieken, <https://www.denka.co.jp>) to confirm *Salmonella* Paratyphi A, B, and C and *Salmonella* Typhi. We performed antibiotic susceptibility tests (ASTs) for ampicillin, chloramphenicol, trimethoprim/sulfamethoxazole, amoxiclav, azithromycin, cefixime, ceftriaxone, gentamycin, meropenem, ciprofloxacin, and nalidixic acid using the disk diffusion method on antibiotic disks and determined resistance profile in accordance with Clinical and Laboratory Standards Institute guidelines (23).

Definitions

We defined a treatment visit for paratyphoid or typhoid fever as a visit for fever in which the enrolled patient submitted a blood culture positive for *Salmonella* Paratyphi A or *Salmonella* Typhi. We concatenated visits for fever in which the onset of symptoms occurred within 14 days of discharge from the previous visit into the same episode. We set the starting date of follow-up as the median

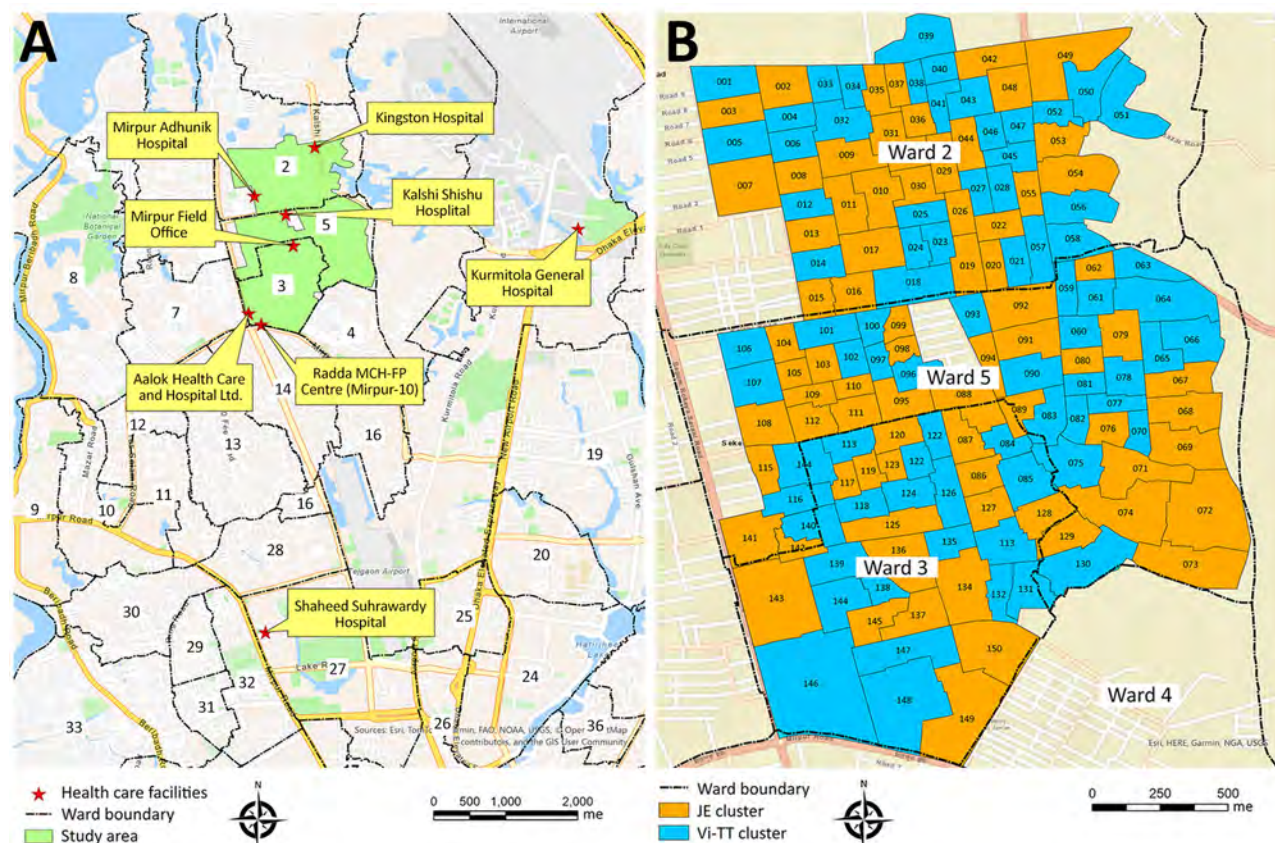


Figure 1. Study area for comparative epidemiologic study of *Salmonella enterica* serovars Paratyphi A and Typhi causing enteric fever, Dhaka, Bangladesh, 2018–2020. A) Study areas (green) and sentinel surveillance healthcare facilities (red stars). B) Clusters within the study area in Vi-TT effectiveness trial; orange shows JE cluster and blue shows Vi-TT cluster. Maps were generated using OpenStreetMap (<http://www.OpenStreetMap.org>), Esri World Street Map (http://goto.arcgisonline.com/maps/World_Street_Map), and Dhaka North City Corporation Administration Map (<https://dncc.portal.gov.bd/site/page/52cb2e96-9af8-4e8c-ab22-b542e0cc373c>); maps were digitized and updated by icddr,b. JE, Japanese encephalitis vaccine; Vi-TT, Vi-tetanus toxoid conjugate vaccine.

date (April 30, 2018) of the baseline vaccination campaign (April 15–May 15, 2018) for unvaccinated patients and the date of actual vaccination for vaccinated patients. For any persons entering our surveillance area (e.g., new births, migration into the study area) after the baseline vaccination campaign, their follow-up period started at the date of birth or date of entry.

Strategies for Analyses

We followed each participant for up to 23 months (April 15, 2018–March 15, 2020) to track the *Salmonella* Paratyphi A and *Salmonella* Typhi episodes. We considered participants residing in clusters for both Vi-TT and JE arms for analysis of *Salmonella* Paratyphi A episodes but considered only those in the JE arm for analysis of *Salmonella* Typhi episodes. We conducted analyses using both closed cohort and dynamic cohort approaches. We de-

finer the closed cohort as participants who were included in the baseline census or were present at the median date (April 30, 2018) of the baseline vaccination or both. We conducted the closed cohort analysis to evaluate the incidence rates and to measure the associations of disease occurrence with baseline features including sociodemographic and WASH characteristics. We considered only the first episodes for incidence calculation. We measured age-stratified incidence by considering the age of each patient at baseline.

The dynamic cohort referred to the entire population throughout the study period, including those added through birth, immigration after the baseline census, and reentry into the study area after earlier emigration. We conducted dynamic-cohort analysis to provide the best depiction of the disease burden with seasonality, incidence rate (overall and age-stratified), clinical features, and AMR pattern. We

calculated seasonality by exploring the monthly incidence of the diseases. For incidence calculation, we considered all episodes detected after starting the follow-up. For age-specific incidence, we used age at follow-up, expressed as age intervals; the numerator was episodes occurring in the population as it passed through the age interval of interest during follow-up, and the denominator was calendar person-time as the population passed through the age interval during follow-up. We conducted descriptive comparative analyses of the clinical features and AMR patterns using the episodes. We did not include follow-up of participants in both cohorts after the dates of death or migration out from the study area or the end of the surveillance (March 15, 2020).

Statistical Analyses

We estimated incidence rates as the total number of *Salmonella* Paratyphi A or *Salmonella* Typhi episodes (numerator) divided by the corresponding person-time of follow-up (denominator). In incidence estimation by age or month for seasonality, we considered the numerator as the total episodes from that age or calendar-time period of interest, and the denominator was the total calendar person-time at that age or calendar-time period of interest. We calculated 95% CIs of the incidence rates using the Byar method (24). To identify factors independently associated with the disease, we conducted time-to-event analysis using Cox proportional hazards model, adjusting for age, sex, and design effect. We assessed

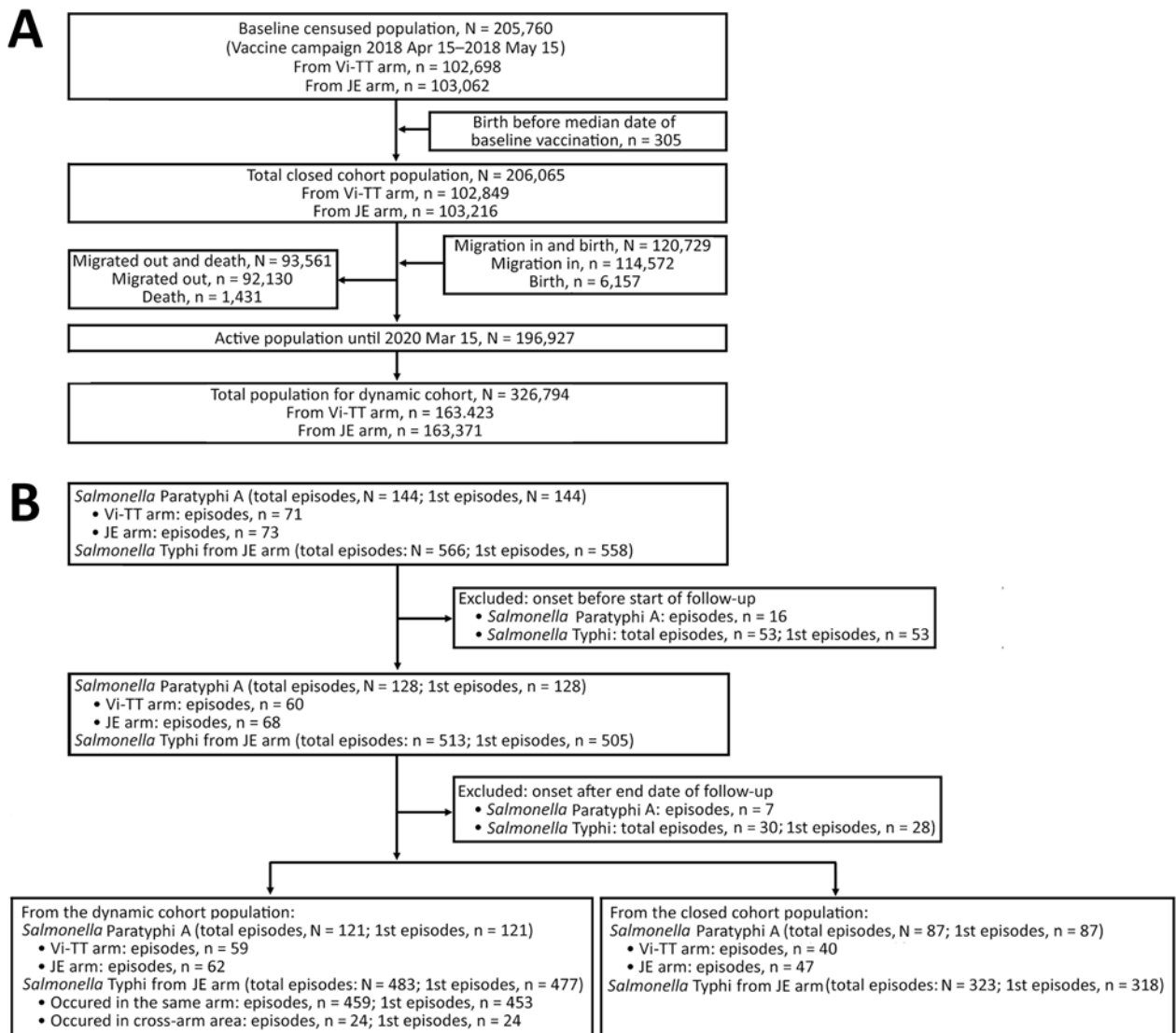


Figure 2. Selection flowcharts of the population (A) and case consort (B) in study of comparative epidemiology of *Salmonella enterica* serovars Paratyphi A and Typhi causing enteric fever, Bangladesh, 2018–2020. JE, Japanese encephalitis vaccine; Vi-TT, Vi-tetanus toxoid conjugate vaccine.

Table 1. Incidence rate of *Salmonella enterica* serovars Paratyphi A and Typhi disease from a closed cohort in epidemiologic study of *Salmonella* Paratyphi A and Typhi, Bangladesh, 2018–2020*

Characteristic	Total participants	<i>Salmonella</i> Paratyphi A				No. in JE arm	<i>Salmonella</i> Typhi			
		PY	No. cases	IR (95% CI)	p value†		PY	No. cases	IR (95% CI)	p value†
Overall	206,065	320,486	87	27 (22–33)	NA	103,216	160,790	323	201 (180–224)	NA
Cluster					0.461					NA
JE cluster	103,216	161,022	47	29 (22–38)		103,216	160,790	323	201 (180–224)	
Vi-TT cluster	102,849	159,464	40	25 (18–34)		NA	NA	NA	NA	
Age at baseline, y					<0.001					<0.001
<2	8,166	11,936	4	34 (11–80)		4,108	5,953	46	773 (573–1,021)	
2–4	12,494	19,545	14	72 (41–117)		6,284	9,809	87	887 (715–1,088)	
5 to <16	43,991	70,744	43	61 (45–81)		21,817	35,092	141	402 (340–472)	
≥16	141,414	218,260	26	12 (8–17)		71,007	109,935	49	45 (33–58)	
<16	64,651	102,225	61	60 (46–76)	<0.001	32,209	50,854	274	539 (478–605)	<0.001
≥16	141,414	218,260	26	12 (8–17)		71,007	109,935	49	45 (33–58)	
Sex					0.328					0.622
M	102,382	160,393	48	30 (22–39)		51,288	80,523	166	206 (177–239)	
F	103,683	160,093	39	24 (18–33)		51,928	80,267	157	196 (167–228)	

*IR, incidence rate, cases/100,000 persons/year; JE, Japanese encephalitis vaccine; NA, not applicable; PY, person-year; Vi-TT, Vi-tetanus toxoid conjugate vaccine.
†p values determined by likelihood ratio test from the Cox-PH model.

intraclass correlation (ICC) to investigate the clustering effects on paratyphoid and typhoid fever incidence at the levels of the randomized clusters and households. We calculated ICCs using a generalized linear mixed model under maximum-likelihood estimation via Laplace approximation with the Poisson model as the within-subjects probability model under the repeatability settings (25).

We used χ^2 test to measure the associations between categorical variables and Fisher exact test for small (<5) cell frequencies. We assessed continuous variables with *t*-test or nonparametric Mann-Whitney U test if the data violated distributional assumptions. We considered a 2-tailed test at a 5% level of

significance for all statistical analysis. We performed analysis with R statistical software version 4.3.3 (The R Project for Statistical Computing, <https://www.r-project.org>) using the epiR version 2.0.78 package to estimate the incidence rate and iccCounts for the calculation of ICC.

Results

Study Population and Episodes of Paratyphoid and Typhoid Fever

We enumerated a total of 205,760 participants during the baseline census, of which 102,698 were from the Vi-TT arm and 103,062 were from the JE arm. For

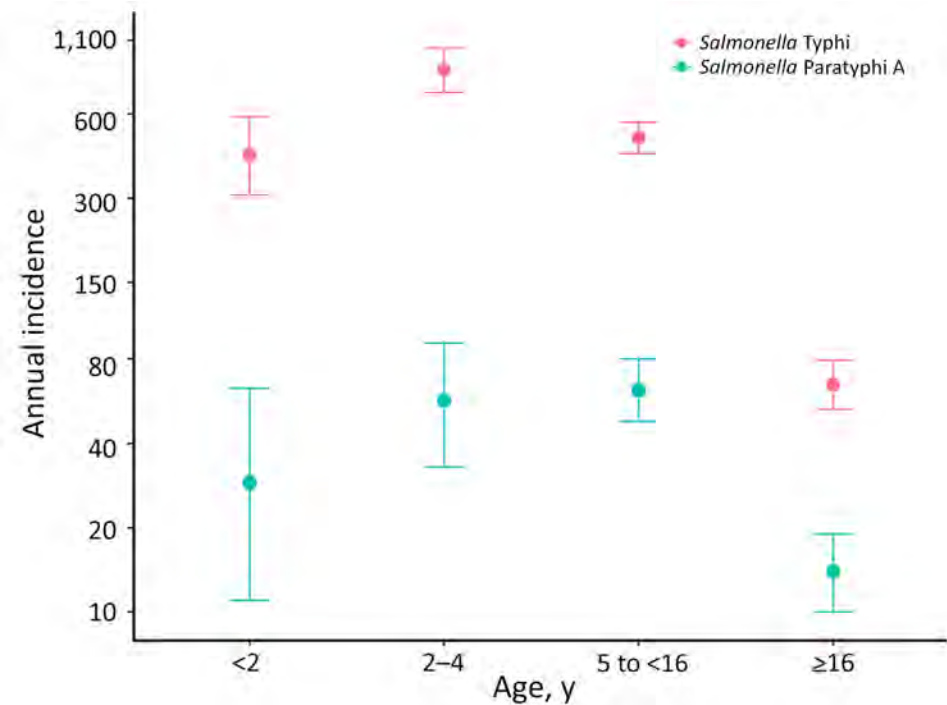


Figure 3. Annual incidence rates of *Salmonella enterica* serovar Paratyphi A and Typhi, stratified by age at follow-up, from the dynamic cohort in study of comparative epidemiology of *Salmonella* Paratyphi A and *Salmonella* Typhi causing enteric fever, Bangladesh, 2018–2020. Dots represent incidence rate, defined as no. cases/100,000 person-years. Error bars indicate 95% CIs.

the closed cohort, we considered 206,065 participants, 205,760 from the baseline population and 305 new births before the median date of the baseline vaccination. After the subsequent censuses during the study period, we added 120,729 participants, 6,157 from birth and 114,572 from immigration. As a result, we considered a total of 326,794 participants for the dynamic cohort.

Throughout the surveillance of the dynamic cohort, we found 144 episodes of *Salmonella* Paratyphi A from both the Vi-TT and JE arms and 566 episodes of *Salmonella* Typhi only from the JE arm. After excluding the episodes that occurred between the end of the study period and the start of follow-up, the total number of *Salmonella* Paratyphi A cases reported from the dynamic cohort was 121 and the total of *Salmonella* Typhi episodes was 483. Of the 483 episodes of *Salmonella* Typhi, 6 were recurrent, whereas all *Salmonella* Paratyphi A episodes were first episodes. In the closed cohort, the total number of first episodes of *Salmonella* Paratyphi A was 87 and of *Salmonella* Typhi was 323. (Figure 2).

Incidence and Seasonality of Paratyphoid and Typhoid Fever

The overall incidence rates (IRs) of paratyphoid for closed and dynamic cohorts were similar, with slightly different CIs: closed cohort IR 27/100,000 PY (95% CI 22–33/100,000 PY); dynamic cohort IR 27/100,000 PY (95% CI 23–32/100,000 PY). Moreover, we observed the highest incidence for both cohorts in children <16 years of age: closed cohort IR 60/100,000 PY (95% CI 46–76/100,000 PY); dynamic cohort IR 57/100,000 PY (95% CI 45–71/100,000 PY). The highest incidence for the closed cohort was among children 2–4 years of age

(IR 72/100,000 PY [95% CI 41–117/100,000 PY]) followed by 5 to <16 years (IR 61/100,000 PY [95% CI 45–81/100,000 PY]), <2 years (IR 34/100,000 PY [95% CI 11–80/100,000 PY]), and ≥16 years (IR 12/100,000 PY [95% CI 8–17/100,000 PY]). The highest incidence rate for the dynamic cohort was among children 5 to <16 years of age (IR 62/100,000 PY [95% CI 48–80/100,000 PY]) followed by 2–4 years of age (IR 57/100,000 PY [95% CI 33–91/100,000 PY]), <2 years of age (IR 29/100,000 PY [95% CI 11–63/100,000 PY]), and ≥16 years of age (IR 14/100,000 PY [95% CI 10–19/100,000 PY]) (Table 1; Figure 3; Appendix Table 1, <https://wwwnc.cdc.gov/EID/article/31/10/24-1601-App1.pdf>).

In comparison, the overall incidence rate of typhoid for the closed cohort was 201/100,000 PY (95% CI 180–224/100,000 PY) and for the dynamic cohort 216/100,000 PY (95% CI 198–236/100,000 PY). We observed the highest incidence in children <16 years of age: closed cohort IR 539/100,000 PY (95% CI 478–605/100,000 PY) and dynamic cohort IR 558/100,000 PY (95% CI 504–616/100,000 PY). Among the children <16 years of age, the highest incidence for both cohorts was among children 2–4 years of age; closed cohort IR was 887/100,000 PY (95% CI 715–1,088/100,000 PY) and dynamic cohort IR 863/100,000 PY (95% CI 716–1,031/100,000 PY).

For the closed cohort, the incidence rate among children <2 years of age was 773/100,000 PY (95% CI 573–1,021/100,000 PY), among children 5 to <16 years was 402/100,000 PY (95% CI 340–472/100,000 PY), and among patients >16 years was 45/100,000 PY (95% CI 33–58/100,000 PY). For the dynamic cohort, the incidence rate among children <2 years of age was

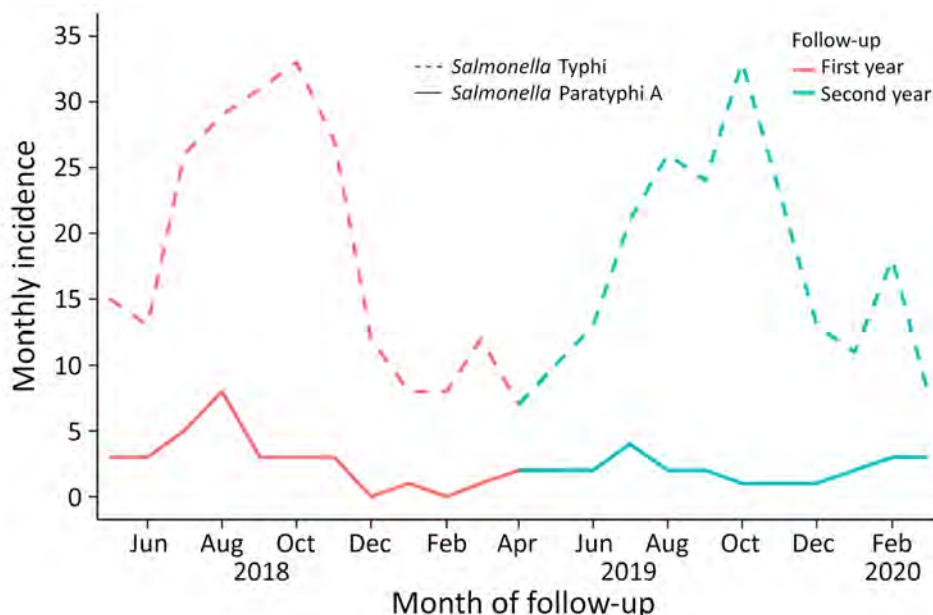


Figure 4. Seasonality of *Salmonella enterica* serovar Paratyphi A and Typhi incidence from the dynamic cohort in study of comparative epidemiology of *Salmonella* Paratyphi A and *Salmonella* Typhi causing enteric fever, Bangladesh, 2018–2020.

430/100,000 PY (95% CI 308–586/100,000 PY), among children 5 to <16 years was 494/100,000 PY (95% CI 433–561/100,000 PY), and among patients >16 years was 65/100,000 PY (95% CI 53–79/100,000 PY). The seasonality of paratyphoid fever incidence was not apparent, whereas typhoid fever peaked in the postmonsoon period (July–August) (Figure 4; Appendix Table 2).

Sociodemographic and WASH Characteristics Related to Paratyphoid and Typhoid Fever

The risk for paratyphoid fever was ≈ 3 times higher (hazard ratio 2.77 [95% CI 1.65–4.66]; $p < 0.001$) and for typhoid fever 1.5 times higher (hazard ratio 1.55 [95% CI 1.22–1.98]; $p < 0.001$) among members of

households having no private toilet compared with members of households that had a private toilet (Tables 2, 3). Moreover, the risk for paratyphoid fever was 2 times higher (hazard ratio 2.1 [95% CI 1.19–3.72]; $p = 0.011$) and for typhoid 1.4 times higher (hazard ratio 1.4 [95% CI 1.07–1.82]; $p = 0.013$) higher among members of households having no safe source of drinking water compared with those with a safe source of drinking water. We found a significant clustering effect (ICC 0.00090 [95% CI 0.00014–0.00165]) of typhoid fever at randomized cluster levels, which was considered for the adjustment in the Cox-PH model to evaluate possible risk factors, but found no clustering effect at household cluster

Table 2. Associations between sociodemographic and WaSH characteristics and the occurrence of *Salmonella* Paratyphi A disease in closed cohort in epidemiologic study of *Salmonella enterica* serovar Paratyphi A and Typhi, Bangladesh, 2018–2020*

Characteristic	No. participants	PY	No. cases	IR (95% CI)	Crude HR	p value	Adjusted HR†	p value
Religion								
Muslim	203,593	316,714	86	27 (22–33)	Referent	NA	Referent	NA
Other	2,472	3,772	1	27 (2–124)	0.98 (0.14–7.06)	0.986	1.04 (0.14–7.43)	0.973
Monthly expenditure								
Below median	100,505	153,458	46	30 (22–40)	Referent	NA	Referent	NA
Median or above	105,560	167,028	41	25 (18–33)	0.83 (0.54–1.26)	0.381	0.81 (0.53–1.24)	0.332
Toilet facility: private								
Y	85,915	141,424	18	13 (8–20)	Referent	NA	Referent	NA
N	120,150	179,062	69	39 (30–48)	2.88 (1.71–4.84)	<0.001	2.77 (1.65–4.66)	<0.001
Adult toilet: flush toilet								
Y	10,374	16,489	4	24 (8–58)	Referent	NA	Referent	NA
N	195,691	303,997	83	27 (22–34)	1.09 (0.4–2.96)	0.871	1.03 (0.38–2.81)	0.952
Child toilet: flush toilet								
Y	853	1,386	0	0 (0–178)	Referent	NA	Referent	NA
N	205,212	319,099	87	27 (22–33)	1,210,612.65 (0– ∞)	0.994	1,393,520.45 (0– ∞)	0.994
Source of drinking water: private tap, well, or pump; bottled water and water vendor								
Y	61,017	99,004	14	14 (8–23)	Referent	NA	Referent	NA
N	145,048	221,482	73	33 (26–41)	2.24 (1.26–3.96)	0.006	2.1 (1.19–3.72)	0.011
Treated cleaning water								
Y	889	1,387	0	0 (0–178)	Referent	NA	Referent	NA
N	205,176	319,098	87	27 (22–33)	1,210,682.57 (0– ∞)	0.994	1,132,383.86 (0– ∞)	0.994
Hand wash before taking meal								
Y	145,554	227,911	54	24 (18–31)	Referent	NA	Referent	NA
N	60,511	92,575	33	36 (25–49)	1.49 (0.96–2.29)	0.072	1.45 (0.94–2.24)	0.090
Hand wash after defecation								
Y	200,637	312,047	87	28 (22–34)	Referent	NA	Referent	NA
N	5,428	8,439	0	0 (0–29)	0 (0– ∞)	0.994	0 (0– ∞)	0.993
Handwashing water available in household								
Y	200,101	311,181	82	26 (21–33)	Referent	NA	Referent	NA
N	5,964	9,305	5	54 (20–118)	2.02 (0.82–4.99)	0.126	2.02 (0.82–4.99)	0.126
Handwashing soap available in household								
Y	202,351	314,711	87	28 (22–34)	Referent	NA	Referent	NA
N	3,714	5,775	0	0 (0–43)	0 (0– ∞)	0.992	0 (0– ∞)	0.992
Waste disposal place: fixed disposal								
Y	193,217	300,307	84	28 (22–34)	Referent	NA	Referent	NA
N	12,848	20,179	3	15 (4–40)	0.53 (0.17–1.67)	0.278	0.49 (0.16–1.56)	0.227
Distance to drinking water source: shorter than median distance								
Y	101,145	156,461	42	27 (20–36)	Referent	NA	Referent	NA
N	104,920	164,025	45	27 (20–36)	1.02 (0.67–1.56)	0.921	0.99 (0.65–1.51)	0.964
Water filter available in household								
Y	23,196	37,234	9	24 (12–44)	Referent	NA	Referent	NA
N	182,869	283,252	78	28 (22–34)	1.11 (0.56–2.21)	0.770	1.08 (0.54–2.15)	0.834

*HR, hazard ratio; IR, incidence rate, cases/100,000 persons/y; NA, not applicable; PY, person-years; WASH, water sanitation and hygiene.

†Adjusted by age and sex.

Table 3. Associations between sociodemographic and WaSH characteristics and the occurrence of *Salmonella* disease in the closed cohort in epidemiologic study of *Salmonella enterica* serovar Typhi Paratyphi A and Typhi, Bangladesh, 2018–2020*

Characteristic	Participants	PY	No. cases	IR (95% CI)	Crude HR	p value	Adjusted HR†	p value
Religion								
Muslim	102,187	159,289	323	203 (182–226)	Referent	NA	Referent	NA
Others	1,029	1,501	0	0 (0–164)	0 (0–∞)	>0.999	0 (0–∞)	>0.999
Monthly expenditure								
Below median	51,644	78,813	149	189 (160–221)	Referent	NA	Referent	NA
Median or above	51,572	81,976	174	212 (182–246)	1.15 (0.91–1.45)	0.234	1.18 (0.94–1.49)	0.160
Toilet facility: private								
Y	43,869	72,368	105	145 (119–175)	Referent	NA	Referent	NA
N	59,347	88,422	218	247 (215–281)	1.67 (1.31–2.13)	<0.001	1.55 (1.22–1.98)	<0.001
Adult toilet: flush toilet								
Y	5,002	8,055	18	223 (137–346)	Referent	NA	Referent	NA
N	98,214	152,735	305	200 (178–223)	0.89 (0.54–1.45)	0.630	0.85 (0.52–1.4)	0.533
Child toilet: flush toilet								
Y	410	689	1	145 (13–677)	Referent	NA	Referent	NA
N	102,806	160,101	322	201 (180–224)	1.44 (0.2–10.32)	0.717	2.23 (0.31–16.05)	0.425
Source of drinking water: private tap, well or pump; water vendor								
Y	32,110	52,266	78	149 (119–185)	Referent	NA	Referent	NA
N	71,106	108,524	245	226 (199–255)	1.53 (1.17–1.99)	0.002	1.4 (1.07–1.82)	0.013
Treated cleaning water								
Y	314	499	1	200 (18–934)	Referent	NA	Referent	NA
N	102,902	160,291	322	201 (180–224)	1 (0.14–7.14)	0.999	1.01 (0.14–7.23)	0.992
Hand wash before taking meal								
Y	71,253	112,173	220	196 (171–223)	Referent	NA	Referent	NA
N	31,963	48,616	103	212 (174–256)	1.07 (0.83–1.38)	0.594	1.05 (0.82–1.35)	0.707
Hand wash after defecation								
Y	100,523	156,548	314	201 (179–224)	Referent	NA	Referent	NA
N	2,693	4,241	9	212 (105–387)	0.87 (0.43–1.76)	0.698	0.81 (0.4–1.65)	0.568
Handwashing water available in household								
Y	99,578	155,046	308	199 (177–222)	Referent	NA	Referent	NA
N	3,638	5,744	15	261 (153–420)	1.31 (0.74–2.3)	0.358	1.3 (0.74–2.29)	0.363
Handwashing soap available in household								
Y	101,581	158,246	318	201 (180–224)	Referent	NA	Referent	NA
N	1,635	2,543	5	197 (75–431)	0.88 (0.34–2.28)	0.787	0.86 (0.33–2.22)	0.749
Waste disposal place: fixed disposal								
Y	97,714	152,047	307	202 (180–225)	Referent	NA	Referent	NA
N	5,502	8,743	16	183 (109–290)	0.92 (0.53–1.62)	0.782	0.85 (0.48–1.49)	0.574
Distance to drinking water source: shorter than median distance								
Y	53,075	82,428	167	203 (174–235)	Referent	NA	Referent	NA
N	50,141	78,362	156	199 (170–232)	1.01 (0.8–1.27)	0.926	0.97 (0.77–1.22)	0.821
Water filter available in household								
Y	11,804	19,125	30	157 (108–221)	Referent	NA	Referent	NA
N	91,412	141,665	293	207 (184–232)	1.31 (0.89–1.92)	0.168	1.27 (0.87–1.87)	0.218

*HR, hazard ratio; IR, incidence rate, cases/100,000 persons/year; PY, person-years; WASH, water sanitation and hygiene.

†Adjusted by age and sex.

levels (Table 4). Moreover, we observed no significant clustering effect of paratyphoid fever at either the household or the randomized cluster levels.

Clinical Characteristics of Paratyphoid and Typhoid Fever

We found no significant differences in clinical features between paratyphoid and typhoid patients, even when stratified by age groups (Table 5). The mean

temperature recorded at enrollment for paratyphoid patients was 37.8°C and for typhoid patients, 37.9°C. We observed high fever, defined as an axillary temperature of ≥40°C, in 2/121 (1.7%) of paratyphoid and 3/483 (0.6%) of typhoid patients when they sought care. Five (4.1%) of 121 paratyphoid patients and 24 (5.0%) of 483 typhoid patients were admitted to a hospital. Of those, 35/121 (28.9%) paratyphoid and 109/483 (22.6%) typhoid patients had a history of antimicrobial

Table 4. Clustering of *Salmonella enterica* serovars Paratyphi A and Typhi infection in epidemiologic study, Bangladesh, 2018–2020*

Clustering level	<i>Salmonella</i> Paratyphi A		<i>Salmonella</i> Typhi	
	No. clusters	ICC (95% CI)	No. clusters	ICC (95% CI)
Randomized clusters	150	0.00028 (–0.00013 to 0.00069)	75	0.00090 (0.00014–0.00165)
HH clusters	50,688	0.00000012 (–1 to 1)	25,478	0.00000010 (–1 to 1)

*HH, household; ICC, intracluster correlation

Table 5. Clinical findings of paratyphoid and typhoid cases by age group from cases detected in the dynamic cohort in epidemiologic study, Bangladesh, 2018–2020*

Clinical finding	Paratyphoid cases from all clusters, n = 121	Typhoid cases from JE clusters, n = 483	p value
Overall	121 (100)	483 (100)	
Mean body temperature, °C (±SD)	37.8 (±0.95)	37.9 (±0.93)	0.165†
High fever, ≥40°C	2 (1.7)	3 (0.6)	0.263‡
Antibiotic taken in past 2 weeks	35 (28.9)	109 (22.6)	0.177
Admitted to hospital	5 (4.1)	24 (5.0)	0.883
Median duration of hospitalization, d (IQR)	6 (5–6)	9.5 (5–13)	0.139§
Gastrointestinal tract diseases¶	29 (24.0)	147 (30.4)	0.198
Lower respiratory tract diseases#	33 (27.3)	130 (26.9)	>0.999
Upper respiratory tract diseases**	12 (9.9)	67 (13.9)	0.316
Neurologic diseases‡‡	13 (10.7)	39 (8.1)	0.450
Rash/new lesion	1 (0.8)	3 (0.6)	>0.999‡
<5 years of age	22 (18.2)	189 (39.1)	
Mean body temperature, °C (±SD)	37.8 (±1.04)	37.9 (±0.92)	0.677†
High fever, ≥40°C	2 (9.1)	0 (0.0)	0.010‡
Antibiotic taken in past 2 weeks	8 (36.4)	39 (20.6)	0.159
Admitted to hospital	1 (4.5)	8 (4.2)	>0.999‡
Median duration of hospitalization, d (IQR)	4 (4–4)	10.5 (10–13)	0.241§
Gastrointestinal tract diseases¶	4 (18.2)	59 (31.2)	0.324‡
Lower respiratory tract diseases#	8 (36.4)	59 (31.2)	0.803
Upper respiratory tract diseases**	5 (22.7)	33 (17.5)	0.753
Neurologic diseases‡‡	1 (4.5)	5 (2.6)	0.488‡
Rash/new lesion	0 (0.0)	0 (0.0)	>0.999‡
≥5 years of age	99 (81.8)	294 (60.9)	
Mean body temperature, °C (±SD)	37.8 (±0.93)	37.9 (±0.95)	0.221†
High fever (≥40°C)	0 (0.0)	3 (1.0)	0.575‡
Antibiotic taken in last 2 weeks	27 (27.3)	70 (23.8)	0.578
Admitted to hospital	4 (4.0)	16 (5.4)	0.792‡
Median duration of hospitalization, d (IQR)	6 (6–6)	8 (5–13)	0.669§
Gastrointestinal tract diseases¶	25 (25.3)	88 (29.9)	0.446
Lower respiratory tract diseases#	25 (25.3)	71 (24.1)	0.932
Upper respiratory tract diseases**	7 (7.1)	34 (11.6)	0.282
Neurologic diseases‡‡	12 (12.1)	34 (11.6)	>0.999
Rash/new lesion	1 (1.0)	3 (1.0)	>0.999‡

*Values are no. (%) except as indicated. p values derived from χ^2 test except as indicated.

†By t-test for mean difference.

‡By Fisher exact test.

§By Mann-Whitney U test.

¶Gastrointestinal includes any of the following: abdominal pain, nausea, vomiting, diarrhea.

#Lower respiratory tract includes any of the following: cough, wheeze, tachypnea, respiratory distress.

**Upper respiratory tract includes any of the following: sore throat, coryza, ear pain, enlarged tonsils, painful/swollen lymph nodes in head/neck, stridor.

‡‡Neurological includes any of the following: headache, photophobia, neck stiffness, seizure.

drug intake during the 2 weeks before seeking care at the medical facility. Gastrointestinal (abdominal pain, nausea, vomiting, diarrhea) and lower respiratory tract (cough, wheeze, tachypnea) symptoms were equally common among the paratyphoid and typhoid patients. Among paratyphoid patients, 29/121 (24.0%) experienced gastrointestinal symptoms and 33/121 (27.3%) lower-respiratory symptoms; among typhoid patients, 147/483 (30.4%) experienced gastrointestinal symptoms and 130/483 (26.9%) lower-respiratory symptoms. All patients recovered, and none experienced severe complications such as intestinal hemorrhage, perforation, or encephalopathy.

AMR Patterns

One (0.8%) of the 121 *Salmonella* Paratyphi A isolates was MDR; the rest were susceptible to the first-line antimicrobial drugs ampicillin, chloramphenicol, and

trimethoprim/sulfamethoxazole. In contrast, 97/480 (20.2%) *Salmonella* Typhi isolates were MDR ($p<0.001$ for the difference in occurrence between *Salmonella* Paratyphi A and *Salmonella* Typhi). Of note, azithromycin resistance was significantly more frequent ($p<0.001$) among *Salmonella* Paratyphi A isolates (14/121 [11.6%]) than among *Salmonella* Typhi isolates (9/483 [1.9%]). However, we did not observe resistance to any third-generation cephalosporin tested for either pathogen. Almost all *Salmonella* Paratyphi A (119/121 [98.3%]) and *Salmonella* Typhi (456/483 [94.4%]) isolates were resistant to nalidixic acid and had intermediate resistance to ciprofloxacin (119/121 [98.3%] *Salmonella* Paratyphi A and 411/483 [85.1%] *Salmonella* Typhi isolates) (Table 6).

Discussion

In an urban slum population in Dhaka, Bangladesh, we identified a moderate incidence of paratyphoid

fever (defined as 10–100/100,000 PY) and a high incidence of typhoid fever (defined as >100/100,000 PY) (26). The lack of private toilets and safe drinking water was one of the key risk factors for both diseases. Our findings highlight a high prevalence of both diseases in the population predominantly affecting the same age group and sharing similar risk factors; integrated control and preventive measures might include the use of bivalent vaccines for both typhoid and paratyphoid fevers and improved WASH. We observed a higher burden of MDR in *Salmonella* Typhi, whereas *Salmonella* Paratyphi A remained largely susceptible to former first-line drugs but exhibited higher azithromycin resistance. Both pathogens exhibited intermediate resistance to ciprofloxacin.

This study is one of the few detailed comparative epidemiologic investigations on paratyphoid and typhoid fever in Bangladesh undertaken concurrently for both diseases. We found a higher incidence of typhoid fever than paratyphoid fever, consistent with previous studies (1,27,28). The Surveillance for Enteric Fever in Asia Project in 2016–2019 (27) estimated a crude incidence of typhoid fever as 103/100,000 PY and of paratyphoid fever as 16/100,000 PY in Bangladesh. A population-based study under the STRATAA (Strategic Typhoid Alliance across Africa and Asia) consortium also conducted in Dhaka in 2016–2018 (28) showed crude incidences of typhoid as 161/100,000 PY and paratyphoid as 42/100,000 PY. However, during the TCV efficacy study, the incidence of typhoid fever in Nepal (342/100,000 PY) (29) and Bangladesh (213/100,000 PY) (20) was higher than in Malawi (182.7/100,000 PY) (30) in the control group.

Previous studies from Dhaka noted that some environmental factors, such as the preceding rainy season, higher temperature, higher rainfall, or water

level of nearby water sources, were associated with a higher incidence of typhoid fever (31,32). Estimating the typhoid prevalence is challenging, given that the factors influencing blood culture positivity can vary widely. Existing studies have shown a strong correlation between enteric fever incidence and poor sanitation and limited access to clean drinking water (33,34) which aligns with our WASH risk factor analysis. Although establishing optimal WASH infrastructure is resource heavy, our findings suggest that public health interventions to improve private toilets and safe drinking water sources should be considered as a near-term investment for tackling both diseases.

The STRATAA study showed the highest reported typhoid incidence in Kathmandu and Dhaka among the 5–9-year age group (28), whereas in this study, we found that children <16 years of age had the highest risk for enteric fever. This finding suggests a bivalent vaccine against both pathogens could be targeted for children <16 years of age. Among children <16 years of age, the highest incidence for both diseases was among children 2–4 years of age, emphasizing that immunization programs with a bivalent vaccine should prioritize infants <2 years of age, as has been done with the implementation of TCV in typhoid-endemic countries (20,29,30).

AMR constitutes a compelling driver for the development of new-generation bivalent vaccines as well as improved WASH interventions. Although first-line antimicrobial drugs remain effective for treating paratyphoid fever in our setting, MDR *Salmonella* Typhi is highly prevalent. Because the 2 diseases are difficult to distinguish based on clinical features, the selection of appropriate first-line antimicrobial drugs is difficult in many settings (35). Moreover, nalidixic acid resistance and ciprofloxacin intermediate resistance in both pathogens, as well as

Table 6. Antimicrobial resistance of *Salmonella enterica* serovars Paratyphi A and Typhi isolates from the dynamic cohort in epidemiologic study, Bangladesh, 2018–2020*

Drug	No. resistant (%)		p value
	<i>Salmonella</i> Paratyphi A isolates from all clusters, n = 121	<i>Salmonella</i> Typhi isolates from JE clusters, n = 483	
Ampicillin	1/121 (0.8)	194/482 (40.2)	<0.001
Chloramphenicol	2/121 (1.7)	120/479 (25.1)	<0.001
Trimethoprim/sulfamethoxazole	1/120 (0.8)	122/482 (25.3)	<0.001
Amoxiclav	0/121 (0.0)	38/482 (7.9)	<0.001
Azithromycin	14/121 (11.6)	9/483 (1.9)	<0.001
Cefixime	0/121 (0.0)	0/483 (0.0)	>0.999
Ceftriaxone	0/121 (0.0)	0/483 (0.0)	>0.999
Gentamicin	0/121 (0.0)	2/482 (0.4)	>0.999
Meropenem	0/121 (0.0)	0/482 (0.0)	>0.999
Ciprofloxacin†	1/121 (0.8)	57/483 (11.8)	<0.001
Nalidixic acid	119/121 (98.3)	456/483 (94.4)	0.229
MDR for ampicillin, chloramphenicol, trimethoprim/sulfamethoxazole	1/121 (0.8)	97/480 (20.2)	<0.001

*p values determined by Fisher exact test. MDR, multidrug resistance.

†Intermediate result for *Salmonella* Paratyphi A, 119/121 (98.3%); for *Salmonella* Typhi, 411/483 (85.1%).

the substantial level of resistance to azithromycin in *Salmonella* Paratyphi A, indicate that empiric use of these drugs for treating enteric fever might not be effective in most patients (2,9–11,36). Furthermore, third-generation cephalosporin-resistant *Salmonella* Typhi has been reported in Pakistan (37). Our study found both organisms to be sensitive to cephalosporin, but Pakistan's rapid cephalosporin resistance spread signals a need for vigilant monitoring and prudent antimicrobial use.

TCVs have been approved for implementation in the Expanded Program of Immunization of Bangladesh. However, TCVs do not provide cross-protection against *Salmonella* Paratyphi A. After a period of TCV implementation, the prevalence of paratyphoid fever may increase, perhaps because of strain replacement, as was observed in China with the Vi-polysaccharide typhoid vaccine (38). In aggregate, our findings thus strongly support preventing both diseases by devising effective bivalent vaccines and WASH interventions (19,39,40).

The first limitation of our study was its 2-year duration; seasonality analysis would be strengthened with a longer surveillance period. Second, some cases might have been missed because we enrolled a fraction of eligible participants from the 8 healthcare facilities. Third, the available clinical data were categorized according to systems, not on the basis of individual symptoms or signs, which limited our ability to identify distinguishing clinical features. Fourth, the WASH data was based on a simple questionnaire and could have missed important information. Last, our study was limited to an urban setting in Dhaka, so the findings might not be generalizable to rural areas or other geographic locations. Despite all those limitations, the strength of this study was the prospective, comprehensive, and concurrent surveillance of a large study population for both paratyphoid and typhoid fever, together with repeated censuses, which enabled us to analyze the dynamic cohort in a highly mobile population along with closed cohort from the baseline study population.

In conclusion, we found that, although the incidence of *Salmonella* Typhi in the study area in Dhaka is greater than that of *Salmonella* Paratyphi A, the effect of *Salmonella* Paratyphi A is not negligible, especially in children. Vaccination with a bivalent vaccine should be programmatically feasible given the similar age-specific patterns of incidence, and the similarities of WASH factors associated with the risk for each pathogen suggest that simple WASH interventions might be effective against both pathogens.

Acknowledgments

We thank the governments of Bangladesh and Canada for providing unrestricted support. We acknowledge the support from International Vaccine Institute, Kenya Medical Research Institute, and Armauer Hansen Research Institute.

This study was funded by the Bill and Melinda Gates Foundation (grant no. OPP1151153). This publication was made possible through a grant from the Bill & Melinda Gates Foundation (grant no. INV-062435).

Author contributions: J.D.C., F.Q., S.K., and K.Z. contributed to the conception and study design. S.I.A.R., M.G.F., S.E.P., F.K., S.A., and K.H. drafted the manuscript. S.I.A.R., M.G.F., S.E.P., F.K., S.A., K.H., F.A., J.D.C., and X.L. led the analysis and interpretation. J.D.C., F.Q., and A.J.P. supervised and monitored the original study activities. All authors have read and approved the final version of this report and had final responsibility for the decision to submit it for publication.

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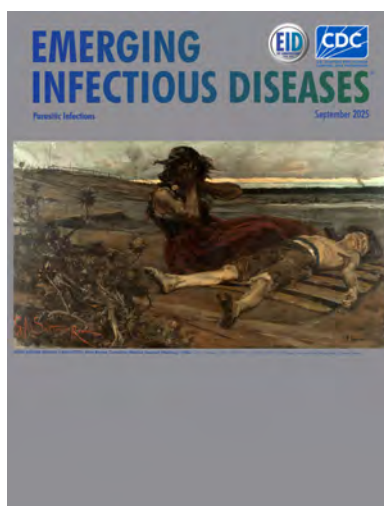
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September 2025

Parasitic Infections

- Chagas Disease, an Endemic Disease in the United States
- Severe Group A *Streptococcus* Infection among Children, France, 2022–2024
- Rickettsioses as Underrecognized Cause of Hospitalization for Febrile Illness, Uganda
- Epidemiology of Chikungunya Hospitalizations, Brazil, 2014–2024
- Drivers of Crimean-Congo Hemorrhagic Fever in Natural Host and Effects of Control Measures, Bulgaria
- Increased Incidence of *Candida auris* Colonization in Early COVID-19 Pandemic, Orange County, California, USA
- Differences in Lyme Disease Diagnosis among Medicaid and Medicare Beneficiaries, United States, 2016–2021
- *Theileria luwenshuni* and Novel *Babesia* spp. Infections in Humans, Yunnan Province, China
- Detection of Multiple Nosocomial *Trichosporon asahii* Transmission Events via Microsatellite Typing Assay, South America
- *Sporothrix brasiliensis* Treatment Failure without Initial Elevated Itraconazole MICs in Felids at Border of Brazil
- Insights into Infant Strongyloidiasis, Papua New Guinea



- Attachment Patterns of Avian Influenza H5 Clade 2.3.4.4b Virus in Respiratory Tracts of Marine Mammals, North Atlantic Ocean
- Related Melioidosis Cases with Unknown Exposure Source, Georgia, USA, 1983–2024
- CYP2D6 Genotype and Primaquine Treatment in Patients with Malaria, Venezuela
- Gastric Submucosal Tumor in Patient Infected with *Diocetophyme renale* Roundworm, South Korea, 2024

- Rapidly Progressing Melioidosis Outbreak in City Center Zoo, Hong Kong, 2024
- Genetic Characterization of *Orientia tsutsugamushi*, Bhutan, 2015
- Novel Henipavirus, Salt Gully Virus, Isolated from Pteropid Bats, Australia
- Modeling Case Burden and Duration of Sudan Ebola Virus Disease Outbreak in Uganda, 2022
- Detection of Rat Lungworm (*Angiostrongylus cantonensis*) in Rats and Gastropods, Italy
- Imported Malaria and Congenital Acquisition in Infant, Portugal, 2024
- Monkeypox Virus Clade IIa Infections, Liberia, 2023–2024
- Detection of Rat Lungworms in Invasive Mollusks, Georgia, USA, 2024
- Characterization of Emerging Human *Dirofilaria repens* Infections, Estonia, 2023
- Zoonotic Rat Lungworm *Angiostrongylus cantonensis* in Black Rats, Houston, Texas, 2024
- Human Babesiosis Caused by *Babesia venatorum*, Russia, 2024
- Pediatric Case Report and Overview of Autochthonous Tick-Borne Encephalitis, Belgium

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Prolonged Monkeypox Virus Infections, California, USA, May 2022–August 2024

Samuel Schildhauer, Kayla Saadeh, Robert E. Snyder, Eric C. Tang, Eric Chapman, Deanna A. Sykes, Philip Peters, Kathleen Jacobson, Jessica Watson, Kelly A. Johnson

Monkeypox virus (MPXV) infection typically lasts 14–28 days. Prolonged MPXV infection, in which symptoms or test positivity last ≥ 28 days, has been documented but not fully characterized. We used the California Department of Public Health (California, USA) mpox registry to compare prolonged (≥ 28 days) and nonprolonged (< 28 days) mpox cases by demographics, HIV status, and JYNNEOS vaccination status. Of 6,469 cases, 82 (1.3%) were prolonged. Persons with prolonged MPXV infections were more likely to be Black or African American (prolonged, 20.7%, vs. nonprolonged, 11.6%) and to have HIV (prolonged, 61.0%, vs. nonprolonged, 39.9%). Among persons with HIV, prolonged infections were more likely among those with lower (< 200) CD4 counts (prolonged, 10.0%, vs. nonprolonged, 3.9%) or not engaged in HIV care (prolonged, 46.0%, vs. nonprolonged, 18.1%). No prolonged infections occurred in persons who received 2 JYNNEOS vaccine doses. Groups disproportionately affected by prolonged mpox should be prioritized for mpox vaccine education and outreach.

Mpx (formerly known as monkeypox) is a disease caused by the monkeypox virus (MPXV), a large, enveloped, double-stranded DNA orthopoxvirus that is closely related to smallpox (1). Although mpox has been recognized as endemic in West and Central Africa since 1970, occasional outbreaks have occurred outside of sub-Saharan Africa (1). In May 2022, an unprecedented global outbreak of MPXV (clade IIb) infections began, causing $> 102,000$ cases by November 2024

(2,3); of those cases, 34,349 were reported in the United States and 6,643 were reported in the state of California (2,4). Because of the novel geographic spread of mpox, decreasing smallpox vaccine-induced immunity, and continuing endemic transmission in many countries around the world, a need exists to understand more about this virus, including viral transmission dynamics and clinical characteristics of infections (5,6).

MPXV infection typically causes self-limited systemic symptoms (e.g., fever, fatigue, and lymphadenopathy) and a rash (7,8). Although the duration of MPXV viral DNA detection by lesion-based testing varies, the median is 14–28 days from initial lesion manifestation (8–11). Although uncommon, some persons may experience prolonged MPXV infections, in which viral DNA is detected for ≥ 28 days, lesions persist for ≥ 28 days, or both (12–15), and previous publications have suggested that lesions can persist for up to 205 days (15,16). Persons with immunocompromising conditions, especially HIV infection, are at increased risk for severe mpox manifestations, including hospitalization (11,17). Case studies of clinically severe MPXV infections in immunocompromised persons demonstrate that more severe infections often occur in tandem with prolonged infections (11,17). However, clinically mild MPXV infections also can be prolonged in immunocompromised persons, suggesting the ability of their immune systems (perhaps combined with medical interventions) to partially control but not clear the virus (11,12,14,17).

Persons experiencing prolonged infections represent an epidemiologically and clinically important group; not only do they suffer from prolonged pain and a heightened risk for severe symptoms or hospitalization compared with persons in whom mpox symptoms do not last as long, but they also are infectious for longer periods and may present opportunities for more frequent mutations of MPXV (12,16,18).

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DOI: <https://doi.org/10.3201/eid3110.250507>

Understanding the prevalence of prolonged MPXV infections and the sociodemographic and clinical characteristics associated with persons who have prolonged infections can inform clinical care and public health policy, which could include expanding vaccination and messaging efforts. For these reasons, we sought to identify and characterize prolonged mpox cases in California by using the California Department of Public Health (CDPH) mpox case registry. We compared demographic and clinical characteristics between persons with prolonged and nonprolonged MPXV infections to identify populations who may be at a higher risk for prolonged MPXV infection.

Methods

Data Sources

All reactive orthopoxvirus or MPXV results are reportable to CDPH per statute. We matched California's mpox surveillance registry to the statewide HIV surveillance registry to obtain HIV status and related clinical information. We also matched the mpox surveillance registry to the California Immunization Registry to determine status of vaccination with the 2-dose JYNNEOS (<https://jynneos.com>) mpox vaccine.

Definitions

Given the abundance of literature stating that the typical clinical course of mpox lasts 14–28 days (7–11), we defined prolonged mpox cases as MPXV infections lasting ≥ 28 days between either 2 positive MPXV diagnostic PCR results or self-reported onset of symptoms and a subsequent positive MPXV diagnostic PCR result. Information on symptoms associated with disease onset and progression, including rash, fever, chills, lymphadenopathy, pruritis, and conjunctivitis, were reported by patients and documented by their healthcare providers. We defined nonprolonged mpox cases as illness in persons infected with MPXV who did not have a 28-day interval between either 2 positive test results or symptom onset and a positive test result. CDPH assesses possible MPXV reinfections through clinical follow-up and epidemiologic investigation; we excluded from this analysis all persons with probable MPXV reinfection, as defined by the Centers for Disease Control and Prevention (CDC) and confirmed by CDPH (7). We calculated the duration of infection for each mpox case as the amount of time in days between reported onset of symptoms or initial MPXV-positive PCR result and the last recorded MPXV-positive PCR result.

All mpox patients self-reported data on sex, race or ethnicity, and sexual orientation. Among patients

with HIV, we aggregated CD4 lymphocyte counts into ranges (<200 , 200–499, or ≥ 500 cells/mm³), consistent with the CDC classification system for HIV infection (19,20). We also aggregated HIV viral load as <200 or ≥ 200 copies/mL of blood. We limited CD4 and HIV viral load analyses to persons with HIV (PWH) and MPXV co-infection for whom these datapoints were available within 365 days before the earliest of either mpox symptom onset or testing date. PWH with no CD4 or viral load laboratory results within the last year may be out of HIV care and, as a result, more likely to be immunocompromised because of HIV infection; therefore, we considered them in a separate category. We classified JYNNEOS vaccination status as unvaccinated, 1-dose vaccinated, 2-dose vaccinated, or post-exposure vaccinated (if the vaccine was administered in the 13 days before symptom onset date or first specimen collection date of their MPXV infection, in which case, based on the incubation period for mpox, we assumed they had a recent exposure and had received the JYNNEOS vaccine as postexposure prophylaxis).

We modified an established mpox symptom severity score by Zucker et al. (21) to include data available in the CDPH mpox case registry (e.g., the degree of rash spread around the body, the number of mucosal sites affected, and the level of care required for the case) (Appendix Table 1, <https://wwwnc.cdc.gov/EID/article/31/10/25-0507-App1.pdf>). We did not include 3 of the severity score parameters from Zucker et al. (21) because of a lack of data availability (i.e., confluent lesions with diameter ≥ 2 cm, treatment for bacterial superinfection, and pain or analgesia requirement). The severity score uses a point system, whereby points accumulate for the severity of each of these categories; our modified severity score can result in a minimum of 0 points and a maximum of 15.

Analysis

We analyzed prolonged and nonprolonged mpox cases in California that occurred during May 12, 2022–August 31, 2024, by comparing the case-patients' demographic and clinical characteristics, JYNNEOS vaccination status, and HIV status (including CD4 counts and viral loads). We calculated percentages for each category among prolonged, nonprolonged, and total cases. To investigate the possible effect of HIV status on the association between race or ethnicity and prolonged mpox cases, we stratified race or ethnicity by HIV status (Appendix Table 2). We then calculated 95% CIs by using the exact Clopper-Pearson method for prolonged mpox cases (because of small cell sizes), and we used the Wilson method elsewhere. We calculated 95% CIs for age and severity score by

using the Wald method. We considered nonoverlapping 95% CIs between prolonged and nonprolonged mpox cases as an indication of statistical significance for a given category.

Records with a missing illness onset date often had more missing values across all variables. Accordingly, we conducted a sensitivity analysis to see if results changed after excluding cases with missing onset dates. We did not conduct sensitivity analyses on data pertaining to HIV. We performed all analyses by using R version 4.3.2 (The R Project for Statistical Computing, <https://www.r-project.org>).

Ethics

We conducted our study as an activity of public health surveillance. Therefore, the California Health and Human Services Agency Committee for the Protection of Human Subjects determined that our analysis was not human subjects research.

Results

Overall, 6,469 mpox cases were reported in California during May 12, 2022–August 31, 2024. Most mpox patients self-identified as male (6,093 [94.2%]) and gay, lesbian, or same-gender loving (3,998 [61.8%]); 41.9%

(2,710) self-identified as Hispanic or Latino. A total of 82 (1.3%) prolonged cases and 6,387 (98.7%) nonprolonged cases were reported. The longest duration of infection identified was 345 days; 21 (25.6%) of the 82 prolonged infections lasted ≥ 50 days.

In terms of sociodemographic characteristics, a larger percentage of patients with prolonged infections (20.7% [95% CI 12.57%–31.11%]; 17/82) identified as Black or African American compared with patients with nonprolonged infections (11.6% [95% CI 10.84%–12.41%]; 741/6,387), a finding that persisted after we stratified for HIV status (Appendix Table 2). Although 95% CIs overlapped among persons experiencing homelessness, a notably higher percentage of patients with prolonged infections also reported having experienced homelessness while infected with MPXV (9.8% [95% CI 4.31%–18.32%]; 8/82) compared with patients with nonprolonged infections (4.2% [95% CI 3.7–4.68]; 266/6,387). We observed no statistically significant differences in age, sex, or sexual orientation between patients with prolonged versus nonprolonged mpox infections (Table 1).

Regarding clinical characteristics, among all 6,649 patients with mpox in California during the study period, 306 (4.7%) were hospitalized, 5,534 (85.5%) had

Table 1. Demographic characteristics of 6,469 patients with prolonged and nonprolonged monkeypox virus infections, California, USA, May 12, 2022–August 31, 2024*

Characteristic	Prolonged		Nonprolonged		Total	
	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)
Sex						
M	76	92.7 (84.75–97.27)	6,017	94.2 (93.61–94.75)	6,093	94.2 (93.59–94.73)
F	3	3.7 (0.76–10.32)	153	2.4 (2.05–2.8)	156	2.4 (2.06–2.81)
No option specified	2	2.4 (0.3–8.53)	150	2.3 (2–2.75)	152	2.3 (2.01–2.75)
Declined to answer or unknown	1	1.2 (0.03–6.61)	67	1.0 (0.83–1.33)	68	1.1 (0.83–1.33)
Sexual orientation						
Gay or lesbian	49	59.8 (48.34–70.44)	3,949	61.8 (60.63–63.01)	3,998	61.8 (60.61–62.98)
Bisexual	10	12.2 (6.01–21.29)	558	8.7 (8.07–9.45)	568	8.8 (8.11–9.49)
Heterosexual	8	9.8 (4.31–18.32)	504	7.9 (7.25–8.58)	512	7.9 (7.28–8.6)
Declined to answer	4	4.9 (1.34–12.02)	190	3.0 (2.59–3.42)	194	3.0 (2.61–3.44)
Orientation not listed	0	0.0 (0.0–4.4)	112	1.8 (1.46–2.11)	112	1.7 (1.44–2.08)
Unknown	11	13.4 (6.89–22.74)	1,074	16.8 (15.92–17.75)	1,085	16.8 (15.88–17.7)
Race or ethnicity						
Hispanic or Latino	36	43.9 (32.96–55.3)	2,674	41.9 (40.66–43.08)	2,710	41.9 (40.69–43.1)
White	19	23.2 (14.56–33.8)	1,867	29.2 (28.13–30.36)	1,886	29.2 (28.06–30.27)
Black or African American†	17	20.7 (12.57–31.11)	741	11.6 (10.84–12.41)	758	11.7 (10.96–12.52)
Asian	1	1.2 (0.03–6.61)	349	5.5 (4.93–6.05)	350	5.4 (4.89–5.99)
Other	2	2.4 (0.3–8.53)	122	1.9 (1.6–2.28)	124	1.9 (1.61–2.28)
Multiple races	0	0.0 (0.0–4.4)	87	1.4 (1.11–1.68)	87	1.3 (1.09–1.66)
AI/AN	1	1.2 (0.03–6.61)	23	0.4 (0.24–0.54)	24	0.4 (0.25–0.55)
NHOPi	0	0.0 (0.0–4.4)	29	0.5 (0.32–0.65)	29	0.4 (0.31–0.64)
Unknown	6	7.3 (2.73–15.25)	495	7.8 (7.12–8.43)	501	7.7 (7.12–8.42)
Housing status						
Homeless	8	9.8 (4.31–18.32)	266	4.2 (3.7–4.68)	274	4.2 (3.77–4.75)
Not homeless	36	43.9 (32.96–55.3)	2,286	35.8 (34.62–36.98)	2,322	35.9 (34.73–37.07)
Declined to answer	2	2.4 (0.3–8.53)	90	1.4 (1.15–1.73)	92	1.4 (1.16–1.74)
Unknown	36	43.9 (32.96–55.3)	3,745	58.6 (57.42–59.84)	3781	58.4 (57.24–59.64)
Mean age, y (95% CI)	38.32 (37.24–39.39)		37.16 (37.04–37.29)		3.29 (3.27–3.31)	
Total	82		6,387		6,469	

*AI/AN, American Indian or Alaska Native; IQR, interquartile range; NHOPi, Native Hawaiian or other Pacific Islander.

†Indicates nonoverlapping 95% CIs (statistical significance).

not been vaccinated with the JYNNEOS vaccine, 80 (1.2%) were fully vaccinated with 2 JYNNEOS doses, and 2,599 (40.2%) were PWH (Table 2). The mean severity score among all case-patients was 3.29.

A larger percentage of patients with prolonged mpox infections were hospitalized (25.6% [95% CI 16.6%–36.44%]; 21/82) compared with patients with nonprolonged infections (4.5% [95% CI 3.98%–5.0%]; 285/6,387). We observed no statistically significant differences between the severity score or vaccination status of patients with prolonged versus nonprolonged infections, but no patients with prolonged infection had received 2 doses of the JYNNEOS vaccine. A higher percentage of prolonged MPXV infections occurred in PWH (61.0% [95% CI 49.57%–71.56%]; 50/82) compared with nonprolonged MPXV infections (39.9% [95% CI 38.71%–41.12%]; 2,549/6,387). Among all mpox infections in PWH, most were in patients with a CD4 count >500 cells/mm³ (1,488/2,599 [57.3%]) and a viral load <200 copies/mL (1,857/2,599 [71.5%]) (Table 3).

Among PWH, a higher percentage of patients with prolonged mpox infections had a CD4 count <200 (10.0% [95% CI 3.33%–21.81%]; 5/50) or had no CD4 laboratory results available (46.0% [95% CI 31.81%–60.68%]; 23/50) compared with patients with nonprolonged infections, among whom only 3.9% (95% CI 3.24%–4.75%; 100/2,549) had a CD4 count <200 and 18.1% (95% CI 16.64%–19.63%; 461/2,549) had no CD4 laboratory results available. In terms of viral suppression, a significantly lower percentage of patients with prolonged infections (42.0% [95% CI 28.19%–56.75%]; 21/50) had an HIV viral load <200 compared with patients with nonprolonged infections (72.0% [95% CI 70.25%–73.74%]; 1,836/2,549). Furthermore, a significantly higher percentage of patients with prolonged infections (46.0% [95% CI 31.81%–60.68%]; 23/50) had

no HIV viral load information available compared with patients with nonprolonged infections (19.0% [95% CI 17.55%–20.6%]; 485/2,549).

We determined duration of MPXV infection by using the period between symptom onset date to specimen collection date for most mpox cases (5,360/6,469 [82.9%]). Information on illness onset date was missing for 1,109 (17.15%) mpox cases, so we calculated the duration of infection by using the period between positive MPXV PCR test results. Results from a sensitivity analysis indicated that excluding cases with a missing illness onset date did not affect the significance of any findings (Appendix Table 3, 4).

Discussion

As MPXV continues to circulate worldwide, the epidemiologic and clinical features of prolonged MPXV infection remain poorly understood. In California, during May 2022–August 2024, prolonged MPXV infections were rare: only 82 persons (1.27%) were found to have MPXV infections for ≥28 days. Although more patients with prolonged infections were hospitalized than were patients with nonprolonged infections, most persons with prolonged infections reported mild symptoms, and patients with prolonged and nonprolonged cases had comparable symptom severity scores. Prolonged MPXV infections disproportionately affected Black or African American persons, persons experiencing homelessness, and PWH (especially PWH with uncontrolled HIV or who may be out of care). All 3 findings merit further consideration.

First, when analyzing demographic characteristics, we found that patients with prolonged MPXV infections disproportionately identified as Black or African American compared with patients with nonprolonged infections, an observation that remained consistent when we stratified by HIV status. Those results align

Table 2. Clinical characteristics of 6,469 patients with prolonged and nonprolonged monkeypox virus infections, California, USA, May 12, 2022–August 31, 2024

Characteristic	Prolonged		Nonprolonged		Total	
	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)
Hospitalization						
Yes*	21	25.6 (16.6–36.44)	285	4.5 (3.98–5.0)	306	4.7 (4.24–5.27)
No*	49	59.8 (48.34–70.44)	4,889	76.5 (75.49–77.57)	4,938	76.3 (75.28–77.35)
Unknown	12	14.6 (7.8–24.17)	1,213	19.0 (18.05–19.97)	1,225	18.9 (18–19.91)
JYNNEOS vaccination status						
Unvaccinated	76	92.7 (84.75–97.27)	5,458	85.5 (84.57–86.3)	5,534	85.5 (84.67–86.38)
1-dose vaccinated	2	2.4 (0.3–8.53)	287	4.5 (4.01–5.03)	289	4.5 (3.99–5.0)
Postexposure vaccinated	4	4.9 (1.34–12.02)	562	8.8 (8.13–9.52)	566	8.7 (8.09–9.46)
2-dose vaccinated	0	0.0 (0.0–4.4)	80	1.3 (1.01–1.56)	80	1.2 (0.99–1.54)
HIV status						
HIV-positive*	50	61.0 (49.57–71.56)	2,549	39.9 (38.71–41.12)	2,599	40.2 (38.99–41.38)
HIV-negative*	32	39.0 (28.44–50.43)	3,838	60.1 (58.88–61.29)	3,870	59.8 (58.62–61.01)
Mean symptom severity score (95% CI)		3.51 (3.3–3.72)		3.28 (3.26–3.31)		3.29 (3.27–3.31)
Total	82		6,387		6,469	

*Indicates nonoverlapping 95% CIs (statistical significance).

Table 3. CD4 count and viral load among 2,599 persons with HIV among patients with prolonged and nonprolonged monkeypox virus infections, California, USA, May 12, 2022–August 31, 2024

Value	Prolonged		Nonprolonged		Total	
	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)
CD4 count						
<200	5	10.0 (3.33–21.81)	100	3.9 (3.24–4.75)	105	4.0 (3.35–4.87)
200–499	8	16.0 (7.17–29.11)	514	20.2 (18.65–21.77)	522	20.1 (18.59–21.67)
≥500*	14	28.0 (16.23–42.49)	1,474	57.8 (55.9–59.73)	1,488	57.3 (55.34–59.14)
Out of care*	23	46.0 (31.81–60.68)	461	18.1 (16.64–19.63)	484	18.6 (17.17–20.17)
Viral load						
<200*	21	42.0 (28.19–56.79)	1,836	72.0 (70.25–73.74)	1,857	71.5 (69.68–73.15)
≥200	6	12.0 (4.53–24.31)	228	8.9 (7.9–10.12)	234	9.0 (7.96–10.17)
Out of care*	23	46.0 (31.81–60.68)	485	19.0 (17.55–20.6)	508	19.5 (18.07–21.12)
Total	50		2,549		2,599	

*Indicates nonoverlapping 95% CIs (statistical significance).

with prior analyses in California and across the United States, which have shown that the incidence of mpox in general has been higher among Black or African American persons, who also were less likely to be vaccinated with the JYNNEOS vaccine than those from other racial or ethnic groups (4,22,23). An analysis of an mpox outbreak in Los Angeles, California, for example, found that no Black or African American persons with mpox were fully vaccinated with the JYNNEOS vaccine, compared with 57.0% of White persons with mpox (23). Those differences, at least in part, probably are influenced by social determinants of health, including inadequate access to health care, leading to poor vaccination uptake and a higher risk for not only acquiring mpox but also having more prolonged infections (24,25), as we observed in our analysis.

Second, a disproportionate number of prolonged cases occurred in PWH, especially among persons with low CD4 counts and persons who lacked HIV laboratory testing in the preceding 12 months, which may signal that a person is out of HIV care (26). For many persons who are on antiretroviral therapy and have well-controlled HIV, MPXV infections are mild and without serious sequelae (19). However, PWH who are not virologically suppressed may experience prolonged illness, debilitating illness, or both because of MPXV infection (14–16), a finding confirmed by our study. Our results indicate that clinicians should remain vigilant to the possibility of prolonged MPXV infection in persons with persistent symptoms, particularly persons with advanced HIV infection. Those persons also represent a group for whom MPXV antiviral and immunoglobulin therapies may be considered to prevent or treat severe or prolonged MPXV infection, although the effectiveness of those therapies is still being researched (11,14,15,27).

Third, a notably higher proportion of prolonged mpox infections occurred in persons who were experiencing homelessness, regardless of whether they were living with HIV, although overlapping 95%

CIs indicate some uncertainty around those estimates. The relationship between social determinants of health (e.g., housing status) and MPXV infection may follow that of other infectious diseases, in which persons experiencing homelessness are at increased risk for contracting infectious diseases and having more severe disease (28,29). Such populations often consist of persons who have underlying health conditions and experience worse health outcomes because of limited access to healthcare (including vaccination) that is driven at least in part by housing insecurity (28–31). Dedicated outreach for such groups, including interventions such as mobile vans and street medicine teams with the ability to offer mpox education and vaccination, may be helpful.

Regarding mpox prevention, the 2-dose JYNNEOS vaccine series prevents severe mpox disease and reduces the odds of hospitalization among persons with mpox compared with persons who are unvaccinated (32). We observed no prolonged infections among persons who had received 2 doses of JYNNEOS vaccine in our sample, which is additional evidence that getting the 2 recommended vaccine doses protects against severe disease, including prolonged infections. Persons who are at risk for adverse outcomes related to MPXV infection, including prolonged infections, such as Black or African American persons, gay persons, bisexual persons, men who have sex with men, and PWH, especially those with advanced HIV infection, thus should be prioritized for community-based vaccine education and outreach (27,33,34).

One limitation of our analysis is that we might have underestimated the duration of prolonged MPXV infections because that variable was dependent on having either a reported symptom onset date or a person having been tested multiple times for MPXV, which is not routine in California. Similarly, we might have underestimated the number of prolonged infections if healthcare providers did not obtain repeat MPXV testing of patients with ongoing symptoms or rashes.

Prolonged infections may be overrepresented among persons with inadequate access to healthcare, given that these groups may be less likely to seek care for mild illness because of structural barriers and less favorable social determinants of health, which have been linked to worse health outcomes in various other contexts (24,25,29,35). In addition, mpox cases in persons who were hospitalized or were experiencing homelessness, living in congregate settings (e.g., skilled nursing facilities or shelters), or both could have been overrepresented among persons with prolonged MPXV infections because they may be more likely to be tested multiple times. Our analysis also was subject to missing information; data that contributed to the severity score, such as lesion prevalence, were lacking for multiple cases, leading to the possible underestimation of severity and possibly decreasing the metric's utility. As a result, the mean severity score of 3.51 among prolonged and 3.28 among nonprolonged mpox cases primarily came from 1 point in active lesion prevalence (1–9 lesions), 1 point in extent of body involvement (1–3 parts), and 1 point for use of outpatient services. Our analysis could not differentiate between hospitalizations that occurred in intensive care units and those that did not. The use of overlapping 95% CIs as an indicator for statistical significance instead of p values is conservative and might have led to underestimation of statistical differences in certain circumstances. We also could not assess orthopoxvirus antiviral use among prolonged cases compared with nonprolonged cases because the primary mechanism for accessing antiviral therapy during the study period was through a blinded placebo-controlled clinical trial, in which patients' assigned treatment groups were not reported to public health surveillance. In addition, passive surveillance systems may inadequately capture data on important confounding variables such as socioeconomic status or healthcare access. Furthermore, we could not evaluate the infectivity of persons with positive MPXV PCR results. Because a lesion is necessary for testing in the United States, it is presumed that all persons with prolonged infections had lesions; however, we did not evaluate whether the lesions contained replication-competent virus.

In conclusion, our analysis compared prolonged versus nonprolonged MPXV infections, characterizing persons who may be at increased risk for prolonged infections, which can improve clinical care and public health practice. During the study period, prolonged mpox cases were rare in California and were more common among persons who identified as Black or African American, persons who experienced homelessness, and in PWH, especially PWH who had

uncontrolled HIV infection. Those groups, who often face barriers to healthcare access, should be prioritized for JYNNEOS vaccination, alongside other groups at risk for severe disease. Furthermore, ensuring that PWH who have mpox are adequately linked to ongoing HIV treatment may be an opportunity to reduce poor health outcomes, including prolonged mpox disease.

This work was supported, in part, by CDC's Cooperative Agreement for Emergency Response: Public Health Crisis Response (TP22-2201) (grant no. NU90TP922197).

The findings and conclusions in this manuscript are those of the authors and do not necessarily represent the views or opinions of CDPH or the California Health and Human Services Agency.

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Differences in COVID-19 Fatality Rates among Ethnic Groups, Hawaii, USA, 2020–2022

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Asian American, Native Hawaiian, and Pacific Islander populations have been underrepresented in research on COVID-19 outcomes. We conducted a cross-sectional study of 5,494 electronic medical records of patients in a large tertiary care health system in the ethnically diverse state of Hawaii, USA. We compared fatality rates for hospitalized patients on the basis of race or ethnicity, age, vaccination status, and sequential organ failure assessment (SOFA) score at admission. Fatality rates varied between racial and ethnic groups but were associated with increasing age across all groups. Fatality rates were closely associated with increasing SOFA score and were inversely associated with the number of COVID-19 vaccinations received. We found that Asian and Pacific Islander groups experienced higher rates of in-hospital death and that death was strongly associated with increased age and SOFA score and with ≤ 1 COVID-19 vaccination. Clinicians should be aware of these outcomes when treating COVID-19 patients from these ethnic groups.

The COVID-19 pandemic has been a major cause of illness and death globally, and the World Health Organization reported >770 million confirmed cases and >7 million deaths by February 2024 (1). Although differences in health outcomes and deaths among racial and ethnic groups have been a topic of research for many years, the COVID-19 pandemic magnified those differences. Several prior studies demonstrated that racial and ethnic minority groups in the United States experience disproportionately high rates of COVID-19–related hospitalization and in-hospital death (2). Examining the association between demographic factors and COVID-19 outcomes is pivotal to enhancing clinical

decision-making, allocating resources, and prioritizing interventions.

Multiple studies have demonstrated differences in COVID-19 outcomes between racial minority groups and White persons. However, few studies have disaggregated minority groups into ethnic subgroups, particularly Asian, Native Hawaiian, and Pacific Islander groups (2). One study demonstrated that COVID-19 test positivity and hospitalization rates were highest in Filipino, Pacific Islander, and other Asian subgroups but did not find notable variation in intensive care unit (ICU) admission or death (3). Because socioeconomic determinants of health, such as income and education level (4), and underlying conditions, such as diabetes mellitus and obesity, differ greatly between those ethnic subgroups (5), further investigation of outcomes among those subgroups is warranted.

The population of Hawaii includes a higher percentage of ethnic groups that are often underrepresented in studies conducted in the continental United States, namely Native Hawaiian, Pacific Islander, and Asian American populations (6). Prior studies showed that those groups had disproportionately higher rates of COVID-19 than other groups in Hawaii and in the continental United States (7). Native Hawaiian and Pacific Islander populations also were found to have lower rates of COVID-19 vaccination than non-Hispanic White and Asian groups (8) and were underrepresented in studies regarding the uptake and efficacy of COVID-19 vaccinations (9). Comparing outcomes between vaccinated and unvaccinated persons in ethnic groups can provide valuable insights for shaping public health vaccination strategies and resource allocation within communities encompassing those populations. We investigated differences in outcomes among underrepresented racial and ethnic groups by reviewing COVID-19 fatality rates among hospitalized patients in a large tertiary care health-care system in Hawaii, USA.

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DOI: <https://doi.org/10.3201/eid3110.241070>

Methods

We conducted a retrospective study of hospitalized patients with COVID-19 diagnosed via PCR and treated at a large tertiary care in-hospital provider in Hawaii during February 2020–August 2022. We collected deidentified data directly from the electronic medical record (EMR) of Queen's Medical Center Health System, which includes 745 inpatient beds across 3 islands and is the main hospital on Oahu, serving as the quaternary referral center for the state of Hawaii.

We reviewed a total of 5,900 patient medical records; we excluded 406 records that were repeat admissions of individual patients, leaving 5,494 unique patients. As a part of the health system's policy, all patients admitted for hospitalization were screened for SARS-CoV-2, and we included all hospitalized patients who tested positive within the study timeframe. We categorized patient age groups as 10–20, 21–30, 31–40, 41–50, 51–60, 61–70, and ≥ 71 years of age.

We calculated fatality rates by dividing the number of deaths by the total number of patients in a particular age or ethnic group, then multiplying by 100 to obtain a percentage. We investigated the association between death and race or ethnicity, age, sequential organ failure assessment (SOFA) score, and vaccination status in ICU and non-ICU settings.

We categorized patients by self-reported race or ethnicity according to the Hawaii Department of Health classifications: White, Native Hawaiian, Pacific Islander, Filipino, Japanese, Chinese, other Asian, Black, and other. The Pacific Islander group consisted primarily of persons who identified as Samoan. The other group included persons who identified as Puerto Rican, Mexican, Native American, Central American, Cambodian, Alaska Native, South American, Kosraean (persons from the Federated States of Micronesia), and persons who declined to respond. The other Asian group included persons who identified as Korean, Vietnamese, Laotian, Thai, Asian/East Indian, Middle Eastern, and other Asian groups.

The SOFA score (10) is a clinical score to assess a patient's degree of organ failure on the basis of partial pressure of oxygen in blood, fraction of inspired oxygen, need for mechanical ventilation, platelet count, Glasgow coma score (11), serum bilirubin, mean arterial pressure, and serum creatinine. SOFA scores range from 0 to 24, and higher scores are associated with more severe illness and increased risk for death. For patients in this study, the EMR automatically calculated the SOFA score as if all parameters for SOFA were populated within patient records.

We defined vaccinated persons as those who received ≥ 1 of the available SARS-CoV-2 vaccinations

before admission or during their hospital stay. Available vaccines were BNT162b2 (Pfizer-BioNTech, <https://www.pfizer.com>), Ad26.COV2.S (Johnson & Johnson/Janssen, <https://www.janssen.com>), and mRNA-1273 (Moderna, <https://www.moderna.com>). The deidentified dataset contained vaccine records adjudicated with the State of Hawaii COVID-19 vaccine registry. We quantified the total number of vaccinations regardless of the type of vaccine received.

To investigate the association between ethnicity and fatality rates, we performed age-specific regressions and stratified by vaccination status. We also performed regressions for the fatality rate and number of vaccinations received. In addition, we performed SOFA score-specific regressions of fatality rates to assess the association between SOFA score and death, and we adjusted for race or ethnicity and vaccination status.

Results

Of 5,494 patients included, 17% were Pacific Islander, 25% were Filipino, 13% were Native Hawaiian, 4% were other Asian, 5% were Chinese, 10% were Japanese, 20% were White, 2% were Black, and 3% were other (Table). Among our cohort, we noted 612 deaths, an overall fatality rate of 11.1%, of which Filipino persons comprised 28%, Pacific Islanders 16%, White persons 15%, Native Hawaiian persons 13%, Japanese persons 11%, Chinese persons 8%, other Asian persons 6%, other 2%, and Black persons 1% (Table). We noted the highest in-hospital fatality rates (15.9%) among persons of other Asian ethnicity, followed by Chinese (15.8%), Japanese (12.8%), Filipino (12.7%), Native Hawaiian (10.8%), Pacific Islander (10.3%), White (8.3%), Other (8.3%), and Black (4.8%) (Figure 1).

We found fatality rates were positively associated with increasing age across all racial and ethnic groups (Figure 2). Age-adjusted specific fatality rates were highest among Chinese persons and overlapped in Native Hawaiian, Filipino, and Pacific Islander persons. In the oldest age group, ≥ 71 years, Chinese persons had the highest fatality rates and White persons had the lowest.

We also found that SOFA scores were positively associated with fatality rates. However, only 562 (10.2%) patients had a SOFA score calculated at admission; most (89.8%) did not. Among the patients who had a SOFA score, mean scores were highest (12.26) among Chinese persons, followed by persons in the other Asian (11.96), Japanese (11.93), Native Hawaiian (11.76), Pacific Islander (11.57), Filipino (11.41), other (11.10), White (11.03), and Black (9.86) groups. However, the differences in mean SOFA score between ethnic groups were not statistically

Table. Clinical characteristics and underlying conditions in a study of differences in COVID-19 fatality rates among ethnic groups, Hawaii, USA, 2020–2022*

Characteristics	Total	Race or ethnicity								
		Black	Chinese	Filipino	Native Hawaiian	Japanese	Other Asian	Other ethnicity	Pacific Islander	White
Total no.	5,494 (100)	105 (2)	292 (5)	1,379 (25)	733 (13)	523 (10)	226 (4)	181 (3)	935 (17)	1,120 (20)
Sex										
M	3,036 (55)	66 (63)	163 (56)	806 (58)	370 (51)	278 (53)	108 (48)	101 (56)	496 (53)	648 (58)
F	2,458 (45)	39 (37)	129 (44)	573 (42)	363 (49)	245 (47)	118 (52)	80 (44)	439 (47)	472 (42)
Mean age, y	59.3	50.9	64.3	62.5	54.2	69.8	60.4	52.3	52.1	60.1
No. deaths	612 (11)	5 (5)	46 (16)	175 (13)	79 (11)	67 (13)	36 (16)	15 (8)	96 (10)	93 (8)
Fatality rate, %†	11.1	4.8	15.8	12.7	10.8	12.8	15.9	8.3	10.3	8.3
Underlying conditions										
Diabetes	1,801 (33)	19 (18)	89 (31)	485 (35)	263 (36)	176 (34)	70 (31)	51 (28)	421 (45)	227 (20)
Hypertension	2,348 (43)	36 (34)	122 (42)	675 (49)	336 (46)	248 (47)	80 (35)	54 (30)	407 (44)	390 (35)
Atrial fibrillation	505 (9)	6 (6)	31 (11)	138 (10)	83 (11)	48 (9)	8 (4)	8 (4)	63 (7)	120 (11)
CHF	750 (14)	10 (10)	36 (12)	182 (13)	149 (20)	71 (14)	21 (9)	6 (3)	132 (14)	143 (13)
Obesity	1,974 (36)	34 (32)	70 (24)	315 (23)	381 (52)	101 (14)	36 (16)	61 (34)	596 (64)	380 (34)
Malignancy	43 (0.7)	2 (1.9)	2 (0.7)	8 (0.6)	6 (0.8)	8 (1.5)	2 (0.9)	1 (0.5)	4 (0.4)	10 (0.9)

*Values are no. (%) except as indicated. CHF, congestive heart failure.
†Calculated by dividing the number of deaths by the total number of patients per group, then multiplying by 100.
‡Considered body mass index ≥30.

significant, and SOFA score at admission was linearly correlated with increasing fatality rates ($R^2 = 0.94$; $p < 0.05$) (Figure 3). We found that unvaccinated patients in every age group had higher fatality rates than did vaccinated patients (Figure 4). Age-adjusted specific mortality rates in the unvaccinated group were higher than in the vaccinated group. Although fatality rates were positively associated with increasing age among vaccinated and unvaccinated groups, the fatality rate increased exponentially with age in the unvaccinated group compared with the vaccinated group. We found that the fatality rate was inversely associated with the number of COVID-19 vaccinations received, and an increased number of vaccinations correlated with lower fatality rate ($R^2 = 0.84$) (Figure 5); that relationship was also true for patients who received mechanical ventilation. Although the fatality rate was

higher in the group that received mechanical ventilation than the overall study sample, the linear regression coefficient was similar between that group and the overall study sample ($R^2 = 0.81$ vs. 0.84 ; $p < 0.05$). Fatality rates were also higher in the unvaccinated group compared with the vaccinated group across all ethnicities. Vaccinated Filipino persons had the highest risk reduction (RR) at 3.91, followed by Black (RR = 2.77), Japanese (RR = 2.62), other Asian (RR = 2.55), Chinese (RR = 2.4), other (RR = 2.35), Pacific Islander (RR = 2.09), and White persons (RR = 1.27) (Figure 6).

Discussion

We investigated in-hospital fatality rates in Hawaii, a state with a high percentage of Asian, Native Hawaiian, and Pacific Islander populations that are underrepresented in national COVID-19 research (12). We attempted to further disaggregate Asian and Pacific

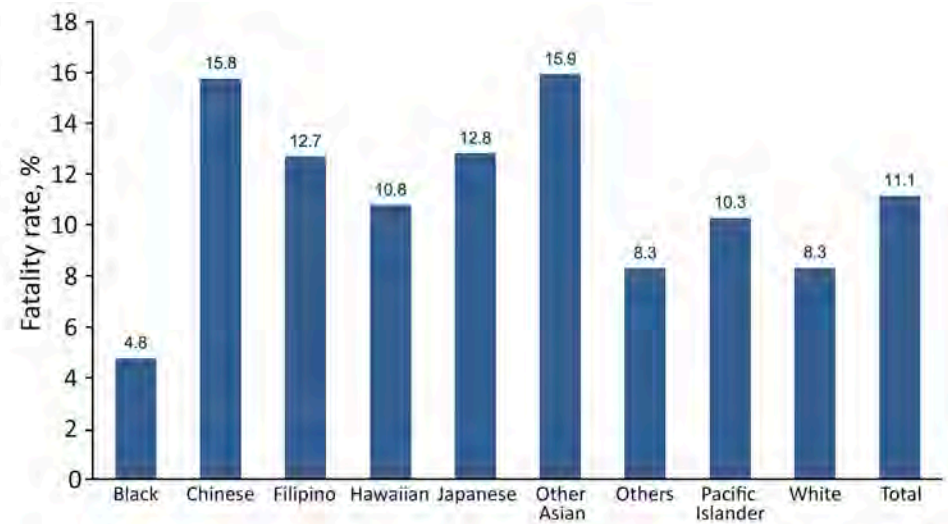


Figure 1. Overall fatality rates in study of differences in COVID-19 fatality rates among ethnic groups, Hawaii, USA, 2020–2022.

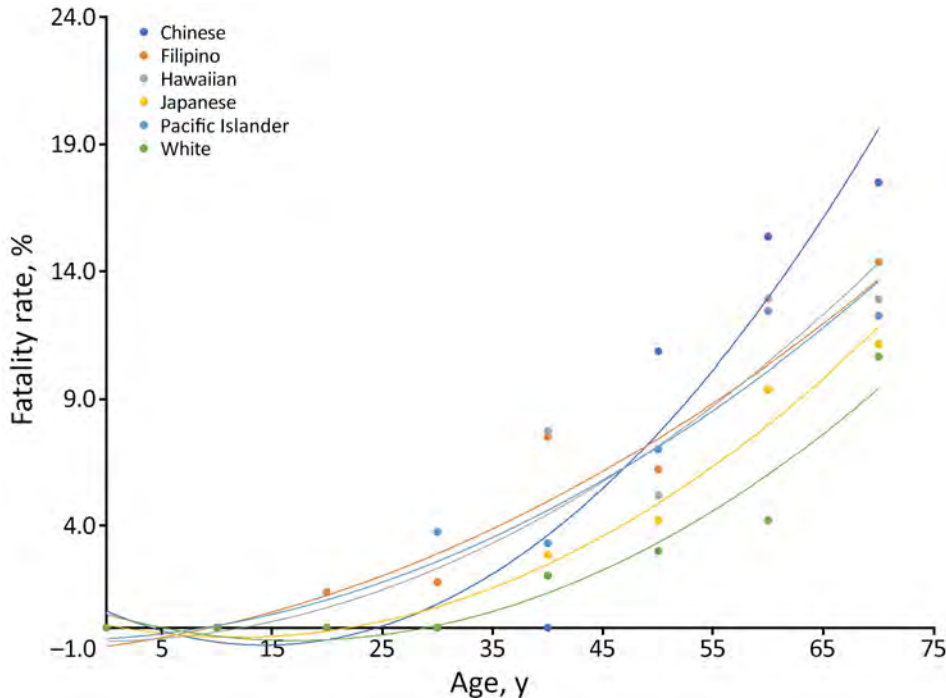


Figure 2. Fatality rates by age in study of differences in COVID-19 fatality rates among ethnic groups, Hawaii, USA, 2020–2022. Lines show polynomial trends among each racial or ethnic group; dots indicate average per 10-year age group. Black dotted line indicates mean fatality rate.

Islander populations into more specific subgroups of Japanese, Native Hawaiian, Filipino, Chinese, and other Asian groups. We found that the highest ICU fatality rates were among the Chinese and other Asian groups and that rates differed greatly between ICU and non-ICU settings. However, the total number of hospitalized persons from those groups was smaller than admissions among the Filipino, Native Hawaiian, and Pacific Islander populations. That finding is consistent with findings from previous studies in the United States and the United Kingdom that show higher ICU admission and fatality rates among Asian populations (13,14). However, our study also demonstrated that less-represented

ethnic groups, including Native Hawaiian, Pacific Islander, and Filipino persons, also have higher in-hospital fatality rates than White persons. Despite the differences in race and ethnicity, overall hospital fatality rates in this study were lower than those in the continental United States (15). In addition, >95% of hospitalized patients in this study were insured, suggesting that difference in access to medical care is not the predominant factor in fatality rate differences.

The findings of our study are also aligned with that of previous studies showing that SOFA score is a reliable prognostic indicator of illness and death in COVID-19 patients (16,17). Although 89.8% of

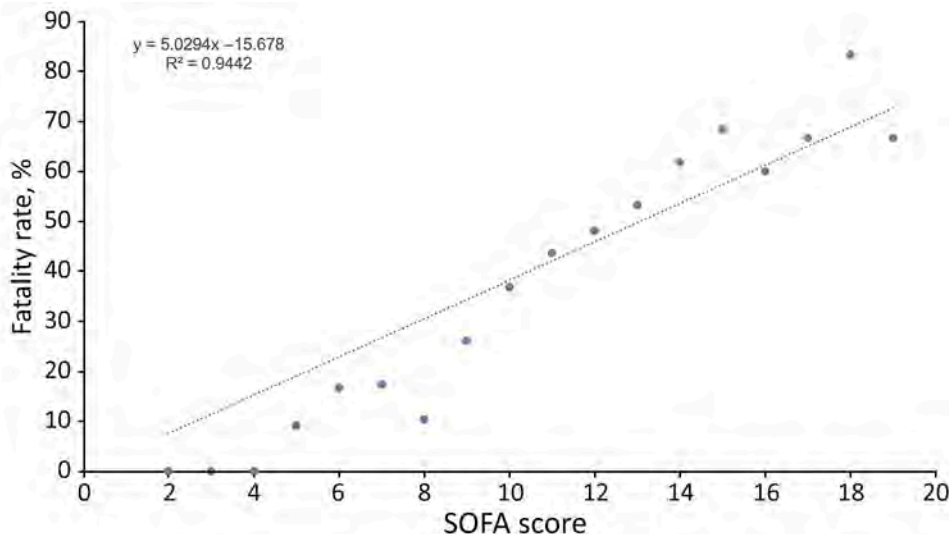


Figure 3. Correlation between SOFA score and death in a study of differences in COVID-19 fatality rates among ethnic groups, Hawaii, USA, 2020–2022. We used SOFA scores collected at admission for 562/5,494 (10.2%) patients included in the study. Dotted line indicates trend in fatality rate by SOFA score; dots indicate average fatality rate by SOFA score. We found higher SOFA scores strongly correlated with higher fatality rates. SOFA, sequential organ failure assessment.

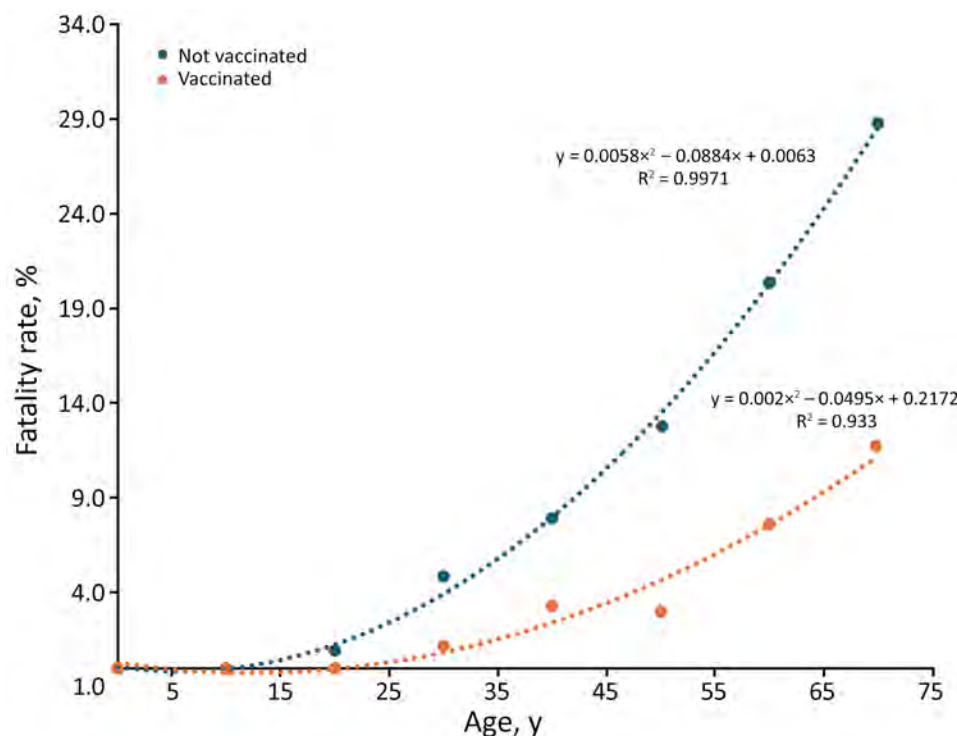


Figure 4. Fatality rates by vaccination status and age in a study of differences in COVID-19 fatality rates among ethnic groups, Hawaii, USA, 2020–2022. Dotted lines show trends among vaccinated (orange) versus unvaccinated (blue) patients; dots indicate average fatality rate per 10-year age group.

hospitalized patients in our study did not have a SOFA score, among those that did, we found that a SOFA score at admission was strongly correlated with fatality rate. We considered evaluating other scores, such as the Charlson Comorbidity Index (18), the COVID-19 severity index (19), and the MuLBSTA score for viral pneumonia mortality (20), to predict fatality rates among patients with diagnosed COVID-19. However, the Charlson Comorbidity Index is designed to predict long-term rather than acute death (18), and the MuLBSTA score and COVID-19 severity

index do not incorporate many of the markers of systemic organ failure that are included in the SOFA score (19,20). Therefore, we concluded that the SOFA score, which is widely used in hospital settings, was a more appropriate tool for assessing fatality rates and accounting for underlying conditions in hospitalized COVID-19 patients.

An analysis of variance showed that the average SOFA score did not differ greatly among ethnic groups despite substantial differences in fatality rates among groups. Further analyses also showed that differences in fatality rates between ethnic groups persisted even when adjusting for SOFA score, suggesting other health differences influenced fatality rates. Those findings suggest that differences in fatality rates between different racial and ethnic groups are influenced by additional ethnicity-related factors aside from SOFA score, echoing previous findings (21). We also found the association between SOFA score and fatality rate persisted when adjusted for age, vaccination status, and underlying conditions. A 2019 study measuring SOFA scores within racial differences in COVID-19 patients found that Black non-Hispanic patients were more likely than White non-Hispanic patients to have a SOFA score ≥ 6 (22). Other studies also suggest that higher SOFA scores are tied to increased mortality prediction rates and are higher than in non-White populations (23). Those findings suggest that widespread use of SOFA in clinical

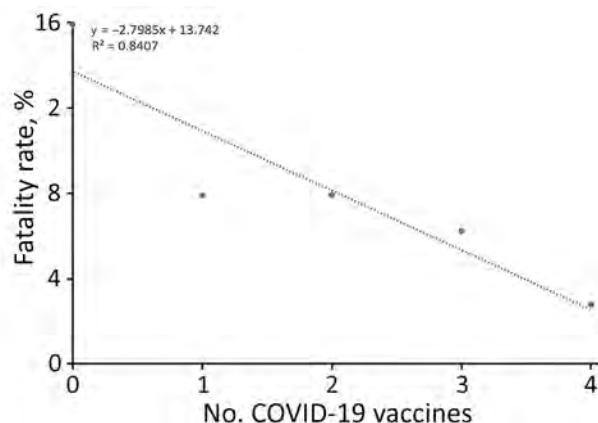


Figure 5. Fatality rates by vaccination status in a study of differences in COVID-19 fatality rates among ethnic groups, Hawaii, USA, 2020–2022. Dotted line show trends per number of COVID-19 vaccines received; dots indicate average fatality rate per vaccine group.

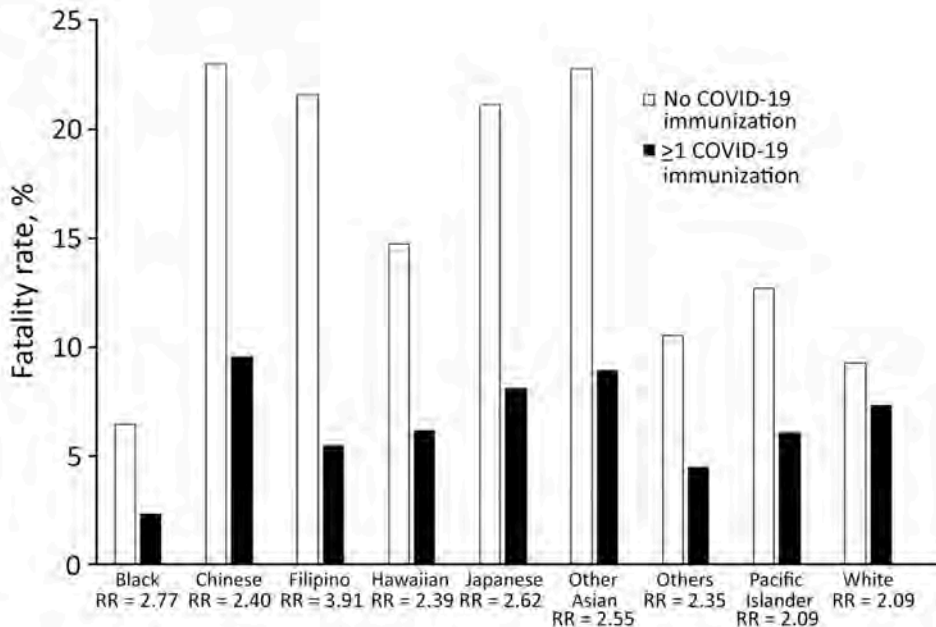


Figure 6. Fatality rates and RR by vaccination status in a study of differences in COVID-19 fatality rates among ethnic groups, Hawaii, USA, 2020–2022. Graph shows differences in fatality rates among racial and ethnic groups on the basis of vaccination status and overall RR for each group for persons vaccinated against COVID-19. RR, risk reduction.

workflows could help care teams identify patients at higher risk for death during the admission process.

We found that fatality rates increased exponentially with age and decreased with confirmed vaccination status and increasing number of vaccinations. Those age-related findings are consistent with previous studies, such as the National Hospital Care Survey, which measured inpatient COVID-19 mortality rates during March 18, 2020–December 27, 2022, in 40 US hospitals (15,24). Those data showed consistently higher fatality rates for hospitalized patients ≥ 60 years of age compared with hospitalized patients 30–59 or 0–29 years of age. Our findings also align with findings from other studies that showed fatality rates among vaccinated hospitalized persons were lower worldwide (25,26). In our results, we also stratified vaccination status with age and found that fatality rates increased with both advancing age and lack of vaccination. Previous studies have shown that COVID-19 death rates among patients ≤ 65 years of age were higher among Hispanic and non-White non-Hispanic patients than in other ethnic groups (27).

Our data illustrate the benefits of COVID-19 vaccination across all racial and ethnic groups. However, we found that stratifying by ethnicity further elucidated differences in RR, showing that Filipino persons had the highest RR and White persons had the lowest. Paired with the disparate findings in fatality rates, those findings seem to suggest that lack of COVID-19 vaccination disproportionately affects certain minority populations. The benefits of COVID-19 vaccination can be profound, and Filipino, Native

Hawaiian, and Pacific Islander populations that have the lowest vaccination rates in Hawaii could see the highest benefits. Lack of trust in providers and state government continues to be a major issue in getting those ethnic groups fully vaccinated (28).

Our findings can possibly be attributed to more underlying conditions in patients with advanced age, non-White ethnicities, or societal inequalities influencing social determinants of health. As has been established by previous research, racial and ethnic minority groups experienced the brunt of the COVID-19 pandemic in higher hospitalization and fatality rates (29).

Our study also shows that increased number of vaccinations is associated with decreased fatality rates, which aligns with studies showing decreased fatality among persons who had at least a 2-dose vaccination series with or without a COVID-19 booster (30–32). Furthermore, our study showed that vaccination nearly equalized the fatality rate across racial and ethnic groups, and those groups also had similar RR. Those results emphasize the critical role of vaccination in preventing severe illness and speak to the comparable efficacy of the available vaccinations across ethnic groups, all of which support the benefit of continued COVID-19 vaccination even in the face of vaccine hesitancy.

The first limitation of this study is the complex and multifaceted reasons for differences in illness and death between ethnic groups. Although socioeconomic factors, including limited access to healthcare, higher prevalence of underlying health conditions, and disparities in social determinants of health, can play a role, we did not examine nor adjust for those

factors in our study (13,14,33). Recognizing and addressing those differences is crucial in working toward equitable health outcomes. The second limitation is that the study included patients hospitalized with COVID-19 as their primary diagnosis and those who were incidentally diagnosed with COVID-19 after admission; thus, many patients could have had other acute medical conditions associated with illness and death. Third, all data used in this study was collected from a single hospital system exclusive to the state of Hawaii (albeit the largest hospital system in Hawaii) and therefore are not generalizable to the country. In addition, sample sizes for some groups within study (for example, Black) were relatively small and might not reliably reflect the broader populations from which they were derived. Finally, SOFA scores were only calculated in patients for whom all SOFA score parameters were populated within the EMR. Therefore, all analyses on the basis of SOFA score include only a small percentage of the overall study sample, which might introduce selection bias.

Despite its limitations, this study included a diverse sample, extended timeframe, and objective confirmation of COVID-19 diagnosis. The diverse population represented in this study enabled analysis of COVID-19 outcomes in ethnic groups that are underrepresented in national data. The study also featured >2 years of data and spanned multiple SARS-CoV-2 variants, accounting for discrepancies in outcomes between variants that have been shown to have different rates of hospitalization and death (34,35). In addition, confirmation of COVID-19 diagnosis via standardized hospital PCR testing minimizes interference from differing accuracy between multiple testing modalities. Overall, our study was robust in showing that fatality rates in COVID-19 patients differed by ethnicity, age, SOFA score, and vaccination status.

In conclusion, we found that Chinese, Japanese, Filipino, Native Hawaiian, and Pacific Islander patients with COVID-19 had higher in-hospital fatality rates than White patients. Our investigation revealed a strong positive correlation between in-hospital fatality rate and age, consistent with the existing literature. In addition, we determined that SOFA score was predictive of in-hospital death across all racial and ethnic groups. Our findings reaffirmed the association between COVID-19 vaccination and decreased in-hospital fatality rates across various racial and ethnic groups and that risk for in-hospital fatality decreased as number of COVID-19 vaccinations increased. Clinicians should consider those factors when treating COVID-19 patients of those racial and ethnic groups.

Acknowledgments

We thank the Queen's Health System's Office of Research and Development for providing access to the data used for this study.

This work was supported by the National Institutes of Health National Institute on Minority Health and Health Disparities (grant no. U54MD007601).

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Effect of Seasonal Influenza Vaccines on Avian Influenza A(H5N1) Clade 2.3.4.4b Virus Infection in Ferrets

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Highly pathogenic avian influenza A(H5N1) clade 2.3.4.4b viruses have infected >1,000 herds of dairy cattle and hundreds of poultry flocks in the United States since the beginning of 2024. Seventy human cases have been reported during that period, mainly through occupational exposure. Although prior influenza A(H1N1) pdm09 virus infection has been shown to confer protection against influenza A(H5N1) clade 2.3.4.4b virus infection in the ferret model, it remains unclear if influenza vaccines, known to elicit a less potent and narrower cross-reactive immune response, can achieve a similar effect. In this article, we demonstrate that immunization with commercially available human seasonal influenza vaccines also confers partial protection against disease caused by H5N1 clade 2.3.4.4b virus in ferrets, which is partially associated with the presence of cross-reactive antibodies targeting H5N1 virus antigens.

Human seasonal influenza A viruses (IAVs) are endemic in humans, causing 9.3–41 million cases, 120,000–710,000 hospitalizations, and 6,300–52,000 deaths annually in the United States (1). Humans might also be infected by IAVs with zoonotic potential circulating in many species, including wild and domestic birds and mammals. Although human infections with zoonotic IAVs are sporadic, the viruses have the potential for sustained transmission in humans, potentially leading to global pandemics with relatively high morbidity and mortality rates, as seen in 1918, 1957, 1968, and 2009 (2,3).

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DOI: <https://doi.org/10.3201/eid3110.250668>

Most adult humans have been repeatedly infected with or vaccinated against multiple seasonal influenza A and B viruses and have antibody and T-cell responses against IAV components, including the major surface glycoproteins hemagglutinin (HA) and neuraminidase (NA). Although antigenic drift in HA and NA enables IAVs to reinfect persons despite immunity, immune responses that are even partially matched to the infecting virus are believed to confer some protection against disease (4). Zoonotic strains of IAV often express HA subtypes that are distantly related to endemic seasonal strains, and the general population typically exhibits low levels of or no HA head binding antibodies to potential pandemic viruses (5; Z.-N. Li et al., unpub. data, <https://www.medrxiv.org/content/10.1101/2025.03.30.25323419v1>).

Since the first documented human infection with highly pathogenic avian influenza (HPAI) A(H5N1) virus was reported in China and Hong Kong in 1997, this virus subtype has remained a major public health concern because of its ability to cross species barriers and infect humans (6,7), often causing severe or fatal disease. Originally found in Asia, HPAI H5N1 viruses have spread widely in birds, likely because of wild bird migration routes. HPAI H5N1 viruses have undergone extensive evolution and countless reassortments, leading to many different clades on the basis of HA sequence. Of those clades, clade 2.3.4.4b has emerged as the most widespread in recent years. In 2021, HPAI H5N1 clade 2.3.4.4b viruses began to spread widely across the Americas (8,9), continuing to diversify, and leading to outbreaks in commercial and backyard poultry across the United States. In 2024 and 2025, clade 2.3.4.4b virus of the

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B3.13, D1.1, and D1.3 genotypes caused infections in dairy cows and poultry in North America (10,11). As of April 10, 2025, there have been 70 H5N1 human infections with those clades reported and 1 death (12). Of note, in contrast to the severe disease that has historically been associated with H5N1 virus infections outside of the United States (13,14), most of the human infections with the clade 2.3.4.4b B3.13 and D1.1 genotypes have been relatively mild (15,16), manifesting primarily as conjunctivitis and mild respiratory symptoms.

Ferrets are a small-animal model used for human IAV infection because they often show similar clinical manifestations and severity of infection with IAV strains, without needing to adapt the virus to ferrets (17). However, in contrast to the mild disease in humans, ferrets infected with clade 2.3.4.4b viruses have typically shown severe or lethal infections (18,19). One possible explanation for this contrasting severity is that humans might have cross-protective immunity to this clade of viruses because of prior infection with human seasonal strains of IAV, whereas the laboratory ferrets used are immunologically naive to all strains of influenza. Several groups have demonstrated that prior infection of ferrets with human influenza A(H1N1)pdm09 (pH1N1) virus confers major cross-protection against 2.3.4.4b B3.13 viruses (20,21; P.H. Bringle et al., unpub. data, <https://www.biorxiv.org/content/10.1101/2024.10.23.619695v1>). However, the mechanisms underlying that cross-protection remain unclear.

Although vaccination against human seasonal IAV remains the most effective way of protecting against seasonal influenza virus infection, it is unclear whether seasonal influenza vaccine immunity could confer cross-protection against H5N1 viruses. In this article, we report the results of testing the ability of several human seasonal vaccines to protect ferrets against disease induced by clade 2.3.4.4b B3.13 IAV infection.

Materials and Methods

Vaccines, Ferrets, and Viruses

The vaccines used in this study were for the 2024–25 Northern Hemisphere influenza season. The vaccines were the egg-based trivalent inactivated influenza vaccine (IIV3) Fluarix (GlaxoSmithKline Biologicals, <https://www.gsk.com>), the live attenuated influenza vaccine (LAIV) FluMist (AstraZeneca, <https://www.astrazeneca.com>), and the recombinant hemagglutinin vaccine (RIV) Flublok (Sanofi, <https://www.sanofi.com>). Six-month-old male fitch ferrets (Triple F Farms, <https://www.tripleffarmsresearch.com>)

were housed in Duo-Flo Bioclean units (Lab Products, Inc., <https://labproductsllc.com>) at Centers for Disease Control and Prevention (CDC) animal facilities. Animal studies were approved by CDC's Institutional Animal Care and Use Committee and were conducted in American Association for Accreditation for Laboratory Animal Care accredited animal facilities.

We inoculated ferrets with the manufacturers' recommended human doses of 0.5 mL IIV3 intramuscularly (IM) (containing 15 µg HA from each virus strain), 0.5 mL of RIV IM (containing 45 µg HA of each strain), or 0.2 mL of LAIV intranasally (IN) (containing 10^{6.5–7.5} fluorescent focus units of live attenuated influenza virus reassortant of each strain). IIV3 also contains NA from each virus, although the quantity of NA included is not regulated and varies between virus strains (22). The RIV does not contain NA.

We challenged ferrets with A/Michigan/90/2024 A(H5N1) (MI/24) (GISAID [<https://gisaid.org>] accession no. EPI_ISL_19162802), a clade 2.3.4.4b, B3.13 genotype virus isolated from a human experiencing mild disease (23), which has been shown to cause moderate, nonfatal disease in laboratory ferrets (24). We conducted all HPAI preparation and animal infection experiments in Biosafety Level 3 containment laboratories, including enhancements regulated by the US Department of Agriculture and the Federal Select Agent Program.

Ferret Immunization and Challenge

We confirmed ferrets to be initially serologically negative for current circulating human influenza viruses (pH1N1, H3N2, and influenza B) by hemagglutination inhibition assay (HAI). We immunized groups of 6 ferrets 2 times at 3-week intervals IM with RIV or with phosphate-buffered saline (PBS) and IN with LAIV. The IIV3 group did not achieve serologic titers that are correlated with protection after 2 immunizations and therefore received a third IM immunization, 3 weeks apart. We used a separate mock-immunized control group for those animals. Because the outcomes of infection in both mock-immunized groups were statistically similar (body weight, $p = 0.093$; temperature, $p = 0.96$; viral shedding, $p = 0.64$), we have presented them as 1 merged group. We challenged ferrets by IN inoculation under anesthesia, with 10⁶ plaque-forming unit (PFU) MI/24 diluted in PBS in 1 mL volume, 24–25 days after final immunization. We monitored ferrets daily for clinical signs of infection, including body weight, temperature, and lethargy (25,26). We calculated

the relative inactivity index for each group on the basis of a previously published scoring system (27). We collected nasal wash samples for virus titers at 1–4, 6, and 8 days postinoculation (DPI).

Hemagglutination Inhibition Assay

We conducted HAI assays by using a World Health Organization kit (International Reagent Resource, <https://www.internationalreagentresource.org>) against A/Victoria/2570/2019 (H1N1)pdm09 and A/Delaware/01/2021(H3N2), as previously described (28). We treated ferret serum with a receptor-destroying enzyme (Denka Seiken, <https://www.denka.co.jp>). We then serially diluted the treated serum samples 2-fold with PBS, mixed with virus diluted to 8 hemagglutination units; we then incubated the samples at room temperature with 0.5% turkey red blood cells in PBS. We conducted HAI against MI/24 virus by using 1% horse red blood cells in PBS as previously described (29). We expressed HAI titers as the reciprocal of the highest dilution of the serum samples completely inhibiting hemagglutination.

Enzyme-Linked Lectin Assay

We assessed the neuraminidase inhibition (NAI) activity of ferret immune serum against A/Wisconsin/67/2022 A(H1N1)pdm09, A/Massachusetts/18/2022 A(H3N2), and A/Texas/37/2024 A(H5N1) (clade 2.3.4.4b, genotype B3.13) by using an enzyme-linked lectin assay (ELLA) with a fetuin-based method, as previously described (30). We added serum and virus to a 96-well plate precoated with 25 µg/mL of fetuin (Sigma-Aldrich, <https://www.sigmaaldrich.com>), followed by overnight incubation at 37°C. We then quantified NAI activity by using horseradish peroxidase-labeled peanut lectin with O-phenylenediamine dihydrochloride substrate. We calculated the percentage of inhibition by using the formula $100 \times (\text{OD virus only} - \text{OD test sample}) / \text{OD virus only}$. We assigned samples below the limit of detection a titer of 10.

Multiplex Influenza Antibody Detection Assay

We performed a high-throughput multiplex influenza antibody detection assay (MIADA), as described previously (31). We included HA globular head, HA ectodomain, HA stalk, NA ectodomain, and nucleoprotein (NP) antigens from IAVs and the HA globular head from influenza B viruses (Appendix Table, <http://wwwnc.cdc.gov/EID/article/31/10/25-0668-App1.pdf>). In a 96-well black-wall plate, we added 50 µL of microsphere suspension (2,000 microspheres/antigen) to each well in assay buffer. We added diluted ferret serum (1:200)

in duplicate. We incubated the plates for 60 minutes, washed, and then added 100 µL of protein A-phycoerythrin conjugate. After another 60-minute incubation and washing, we read the plates by using a Bio-Plex MAGPIX Multiplex Reader (Bio-Rad Laboratories, <https://www.bio-rad.com>). We calculated median fluorescence intensities by using GraphPad version 7.05 (GraphPad Software Inc., <https://www.graphpad.com>).

Virus Titration

At 3 DPI, we euthanized a subset of ferrets ($n = 3$ /group) and collected tissue samples from the upper respiratory tract (nasal turbinate, ethmoid turbinate, soft palate, and trachea), lower respiratory tract (lung), gastrointestinal tract (intestine), olfactory bulb, and brain tissues. We then determined viral loads in the tissues and nasal wash (NW) by using standard plaque formation assays in MDCK cells (limit of detection is 10 PFU/mL or PFU/g of tissues).

Statistical Analysis

We analyzed body weights and NW titers with a linear mixed model with repeated measures by using R version 4.4.0 with lmerTest version 3.1.3 (The R Project for Statistical Computing, <https://www.r-project.org>). We analyzed the tissue virus titers and antibody titers by using analysis of variance with Dunnett's posthoc test for multiple comparisons by using GraphPad version 7.05.

Results

Serologic Response to Vaccination

Before challenge with A(H5N1), we evaluated the antibody responses against HA and NA antigens of seasonal IAV A/Wisconsin/67/2022 A(H1N1)pdm09, A/Massachusetts/18/2022 A(H3N2), and clade 2.3.4.4b A(H5N1) virus (MI/24 or TX/22) in the serum of vaccinated ferrets and the control group by using HAI assay or ELLA. Among the 3 vaccinated groups, 2 doses of LAIV or RIV vaccination elicited robust HAI titers against H1 HA (Table 1; Figure 1), whereas the IIV3 vaccinated group exhibited much lower titers even after a second vaccine booster (Table 1; Figure 1). HAI titers against the H3N2 virus were generally lower than those against the pH1N1 virus, and IIV3 groups had no detectable HAI titers against H3N2 virus. The HAI antibody titer against MI/24 virus was below detectable levels in all 3 vaccinated groups (Table 1; Figure 1).

In naive ferrets immunized with LAIV, the production of functional NAI antibodies against N1 from

Table 1. Serologic response to vaccination in ferrets in study of the effect of seasonal human influenza vaccination on pathogenesis of human influenza A(H5N1) clade 2.3.4.4b virus in the ferret model*

Vaccine group	IAV lineage	Vaccine strain	Prechallenge HAI GMT	Prechallenge ELLA GMT
LAIV	A(H1N1)pdm09	NO/22	806 (606–1074)	320 (236–434)
	A(H3N2)	TH/22	718 (573–901)	71 (54–95)
	A(H5N1)	NA	<10	<10
RIV	A(H1N1)pdm09	WV/22	905 (668–1227)	<10
	A(H3N2)	MA/22	202 (128–317)	<10
	A(H5N1)	NA	<10	<10
IIV3	A(H1N1)pdm09	VIC/22	45 (30–68)	<10
	A(H3N2)	TH/22	<10	<10
	A(H5N1)	NA	<10	18.8 (13.0–27.3)
MOCK	A(H1N1)pdm09	NA	<10	<10
	A(H3N2)	NA	<10	<10
	A(H5N1)	NA	<10	<10

*Values in parentheses are 95% CIs. n = 6 ferrets per vaccine group, n = 12 ferrets in the mock group. The vaccines contained antigens recommended for the 2024–25 Northern Hemisphere influenza season. ELLA, enzyme-linked lectin assay; GMT, geometric mean titer; HAI, hemagglutination inhibition assay; IAV, influenza A virus; IIV3, Fluarix trivalent inactivated influenza vaccine (GlaxoSmithKline Biologicals, <https://www.gsk.com>); LAIV, FluMist live attenuated influenza vaccine (AstraZeneca, <https://www.astrazeneca.com>); MA/22, A/Massachusetts/18/2022 A(H3N2); NA, not available; NO/22, A/Norway/31694/2022 A(H1N1)pdm09; RIV, Flublok vaccine (Sanofi, <https://www.sanofi.com>); TH/22, A/Thailand/8/2022 A(H3N2); WV/22, A/West Virginia/30/2022 A(H1N1)pdm09; VIC/22, A/Victoria/4897/2022 A(H1N1)pdm09.

pH1N1 virus was readily detected by ELLA (geometric mean titer [GMT] of 319.9 [95% CI 236.0–433.6]), whereas those immunized with IIV3 had an undetectable NAI antibody response to N1 from pH1N1 virus

(Figure 2). Ferrets immunized with LAIV had a detectable antibody response against N2 NA, whereas those immunized with IIV3 did not. IIV3-immunized ferrets had detectable responses to N1 from H5N1

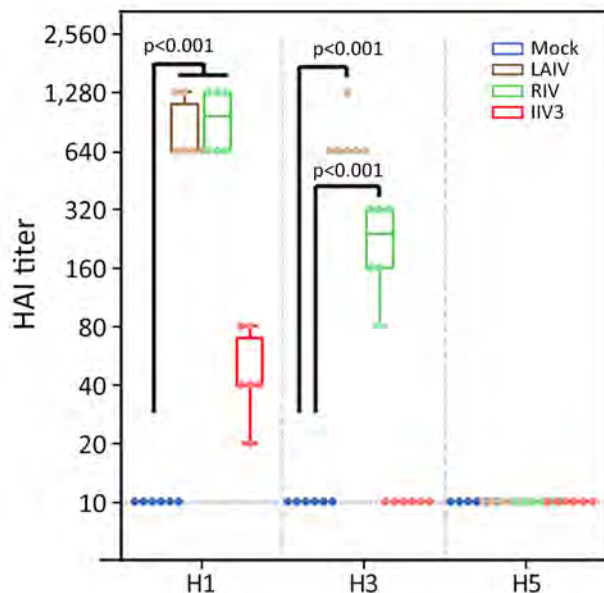


Figure 1. Hemagglutination-inhibiting antibody titers measured by using HAI in study of the effect of seasonal influenza vaccines on influenza A(H5N1) clade 2.3.4.4b virus infection in ferrets. n = 6 ferrets per vaccinated group, n = 12 ferrets in the mock vaccinated group. Colored dots indicate individual values, horizontal lines within boxes represent geometric mean titers, box tops and bottoms indicate interquartile ranges, and error bars indicate limits of the distribution. Dotted gray horizontal line indicates limit of detection. H1, A/Victoria/2570/2019(H1N1)pdm09; H3, A/Delaware/01/2021(H3N2); H5, A/Michigan/90/2024(H5N1); HAI, hemagglutination inhibition assay; IIV3, Fluarix trivalent inactivated influenza vaccine (GlaxoSmithKline Biologicals, <https://www.gsk.com>); LAIV, FluMist live attenuated influenza vaccine (AstraZeneca, <https://www.astrazeneca.com>); RIV, Flublok recombinant influenza vaccine (Sanofi, <https://www.sanofi.com>).

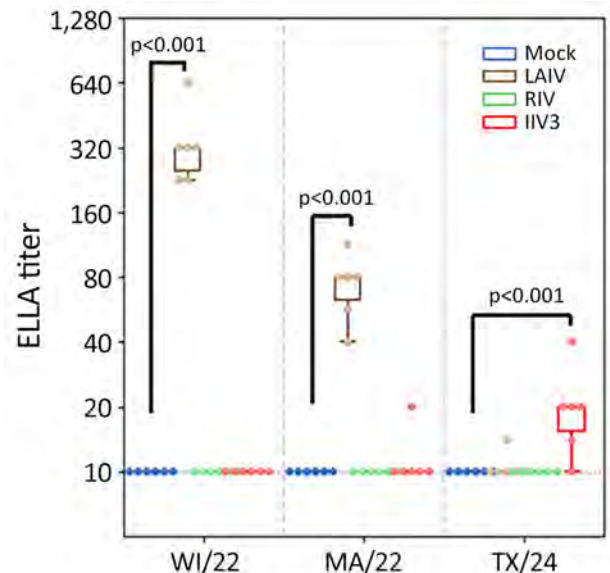
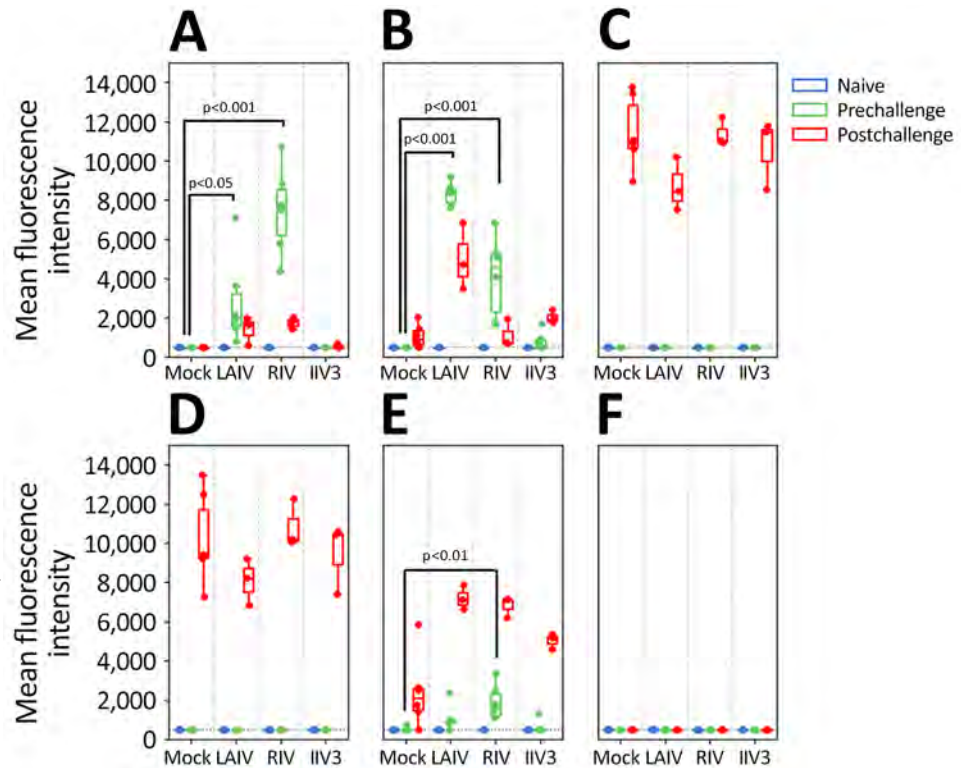


Figure 2. Neuraminidase-inhibiting antibody titers measured by using ELLA in study of the effect of seasonal influenza vaccines on influenza A(H5N1) clade 2.3.4.4b virus infection in ferrets. n = 6 ferrets per the vaccinated group, n = 12 ferrets in the mock vaccinated group. Colored dots indicate individual values, horizontal lines within boxes represent geometric mean titers, box tops and bottoms indicate interquartile ranges, and error bars indicate limits of the distribution. Dotted gray horizontal line indicates limit of detection. ELLA, enzyme-linked lectin assay; IIV3, Fluarix trivalent inactivated influenza vaccine (GlaxoSmithKline Biologicals, <https://www.gsk.com>); LAIV, FluMist live attenuated influenza vaccine (AstraZeneca, <https://www.astrazeneca.com>); MA/22, A/Massachusetts/18/2022(H3N2); RIV, Flublok recombinant influenza vaccine (Sanofi, <https://www.sanofi.com>); TX/24, A/Texas/37/2024(H5N1); WI/22, A/Wisconsin/67/2022(H1N1)pdm09).

Figure 3. Hemagglutinin-binding antibody titers measured by using multiplex influenza antibody detection assay in study of the effect of seasonal influenza vaccines on influenza A(H5N1) clade 2.3.4.4b virus infection in ferrets. *n* = 6 ferrets per vaccinated group, *n* = 12 ferrets in the mock vaccinated group. A) Globular head of hemagglutinin H1 from influenza A/Wisconsin/67/2022(H1N1pdm09). B) Globular head of hemagglutinin H3 from A/Massachusetts/18/2022(H3N2). C) Globular head of hemagglutinin H5 from A/Texas/37/2024(H5N1). D) Ectodomain of hemagglutinin H5 from A/Texas/37/2024(H5N1). E) Stalk of hemagglutinin H1 from A/Michigan/45/2015(H1N1pdm09). F) Stalk of hemagglutinin H3 from A/Singapore/INFIMH-16-0019/2016(H3N2). Colored dots indicate individual values, horizontal lines within boxes represent geometric mean titers, box tops and bottoms indicate interquartile ranges, and error bars indicate limits of the distribution. Dotted gray horizontal line indicates limit of detection. IIV3, Fluorix trivalent inactivated influenza vaccine (GlaxoSmithKline Biologicals, <https://www.gsk.com>); LAIV, FluMist live attenuated influenza vaccine (AstraZeneca, <https://www.astrazeneca.com>); RIV, Flublok recombinant influenza vaccine (Sanofi, <https://www.sanofi.com>).



Colored dots indicate individual values, horizontal lines within boxes represent geometric mean titers, box tops and bottoms indicate interquartile ranges, and error bars indicate limits of the distribution. Dotted gray horizontal line indicates limit of detection. IIV3, Fluorix trivalent inactivated influenza vaccine (GlaxoSmithKline Biologicals, <https://www.gsk.com>); LAIV, FluMist live attenuated influenza vaccine (AstraZeneca, <https://www.astrazeneca.com>); RIV, Flublok recombinant influenza vaccine (Sanofi, <https://www.sanofi.com>).

(GMT 18.8 [95% CI 13.0–27.3]; *p* = 0.005 compared with mock-immunized ferrets). Titers against N1 from H5N1 exceeded the limit of detection of 10 in only 1 of 6 LAIV-immunized animals (Figure 2). Because NA is not present in RIV, no anti-NA response was detected in ferrets immunized with this vaccine.

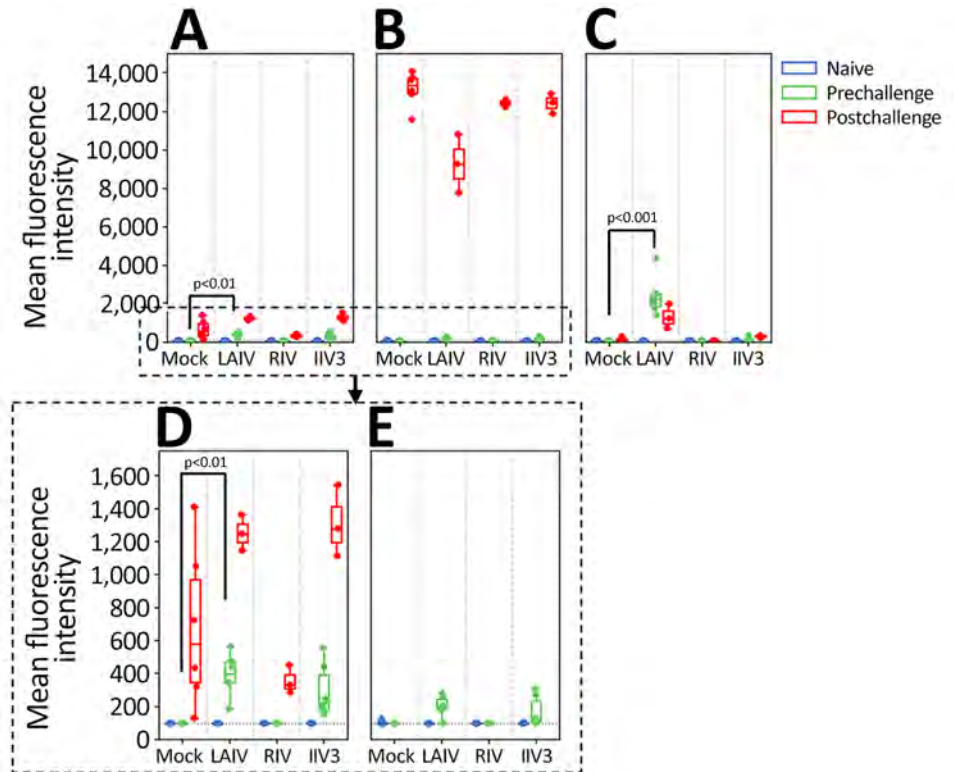
In addition, we tested ferret serum from prevaccination and prechallenge and postchallenge time points in each group with MIADA to assess levels of binding antibodies against a range of antigens, including HA globular head, ectodomain, stalk, and NA ectodomain of pH1N1, H3N2, H5N1 viruses, and HA globular heads of influenza B virus and NP antigen. In agreement with HAI and NAI antibody titers, antibodies binding the H1 and H3 HA globular head and N1 and N2 NA ectodomains were readily detected in LAIV-immunized ferrets (Figures 3, 4). RIV-immunized ferrets also had antibodies targeting H1 and H3 HA. In addition, LAIV and RIV groups elicited detectable binding antibodies targeting group 1 (H1), but not group 2 (H3), HA stalk regions after 2 vaccinations, whereas these antibodies were not detected in the IIV3 group even after 3 vaccinations (Figure 3). Three of 6 ferrets immunized with LAIV and 2 of 6 immunized with IIV3 had detectable levels of cross-

reactive binding antibodies to N1 of H5N1 (LAIV, GMT 223.3 [95% CI 194.5–252.0]; *p* = 0.37 relative to mock-immunized ferrets; IIV3, GMT 228.5 [95% CI 190.8–266.2]; *p* = 0.23) (Figure 4). Ferrets immunized with LAIV and IIV3 also possessed detectable NP binding antibody before challenge, which markedly increased postchallenge (Figure 5). As expected, RIV-immunized animals did not develop antibodies against NP or cross-reactive H5N1 NA antibodies before challenge (Figures 4, 5). Antigens used in the MIADA are provided (Appendix Table), and the results of the assay for all 27 antigens are also provided (Appendix Figure).

Vaccine Protection Against H5N1 Disease

Twenty-four days after receiving a vaccine booster (LAIV and RIV), or 25 days after the second vaccine booster (IIV3), we inoculated ferrets with 10^6 PFU of H5N1 MI/24 and observed them for clinical signs of disease. Mock-immunized ferrets showed signs of moderate disease as previously described (24), including sporadic nasal discharge, sneezing, mild lethargy, transient fever (average maximum temperature rise 2.2°C above baseline), and a maximum weight loss of 15.3% (95% CI 12.5%–18.1%)

Figure 4. Neuraminidase antibody titers measured by using multiplex influenza antibody detection assay in study of the effect of seasonal influenza vaccines on influenza A(H5N1) clade 2.3.4.4b virus infection in ferrets. *n* = 6 ferrets per vaccinated group, *n* = 12 ferrets in the mock vaccinated group. A) Neuraminidase N1 from A/Wisconsin/67/2022(H1N1pdm09). B) Neuraminidase N1 from A/Texas/37/2024(H5N1). C) Neuraminidase N2 from A/Massachusetts/18/2022(H3N2). D) Expanded view of neuraminidase N1 from A/Wisconsin/67/2022(H1N1pdm09) to more clearly show the cross-reactive responses postvaccination. E) Expanded view of neuraminidase N1 from A/Texas/37/2024(H5N1) to more clearly show the cross-reactive responses postvaccination. Colored dots indicate individual values, horizontal lines within boxes represent geometric mean titers, box tops and bottoms indicate interquartile ranges, and error bars indicate limits of the distribution. Dotted gray horizontal line indicates limit of detection. IIV3, Fluarix trivalent inactivated influenza vaccine (GlaxoSmithKline Biologicals, <https://www.gsk.com>); LAIV, FluMist live attenuated influenza vaccine (AstraZeneca, <https://www.astrazeneca.com>); RIV, Flublok recombinant influenza vaccine (Sanofi, <https://www.sanofi.com>).



(Table 2). In contrast, ferrets immunized with LAIV displayed milder symptoms with less frequent nasal discharge and sneezing, characterized by a lower average maximum temperature rise and lethargy score, along with a maximum weight loss of 6.2% (95% CI 2.5%–9.9%; *p* = 0.0292). Those vaccinated with RIV and IIV3 also demonstrated limited protection and reduced frequency of nasal discharge and sneezing; although their maximum weight loss of 9.3% (95% CI 6.2%–12.3%) in the RIV group and 13.6% (95% CI 8.9%–18.2%) in the IIV3 group were not significantly less than the mock-immunized group (RIV, *p* = 0.083; IIV3, *p* = 0.84). Each group recovered significantly more weight than did mock-immunized groups after

7 DPI (on day 7, *p* = 7.9×10^{-7} for LAIV, *p* = 1.6×10^{-7} for RIV, and *p* = 0.006 for IIV3) (Figure 6, panel A).

Vaccine Protection Against H5N1 Virus Replication and Shedding

Next, we evaluated the effect of seasonal influenza vaccination on viral replication and shedding after infection with H5N1 MI/24 virus in ferrets. As we demonstrated (Figure 6, panel B), ferrets in the mock group shed infectious virus above a GMT of $10^{5.2}$ PFU/mL through 4 DPI and maintained a GMT of $\approx 10^{4.2}$ PFU/mL at 6 DPI, whereas ferrets vaccinated with LAIV exhibited significantly lower level of infectious virus in the NW (4 DPI, *p* = 2×10^{-5}), with

Table 2. Pathogenicity of A(H5N1) influenza virus in naive and vaccinated ferrets in study of the effect of seasonal human influenza vaccination on pathogenesis of human influenza A(H5N1) clade 2.3.4.4b virus in the ferret model*

Vaccine group	Mean maximum weight loss, %	Mean temperature rise, °C	RII	Mean log ₁₀ peak titer
Mock	15.3 (12.5–18.1)	2.2 (2.0–2.4)	1.4	5.8 (5.4–6.2)
LAIV	6.2 (2.5–9.9)	1.2 (0.7–1.7)	1.0	4.5 (3.9–5.0)
RIV	9.3 (6.2–12.3)	1.2 (1.1–1.4)	1.1	5.6 (5.3–5.9)
IIV3	13.6 (8.9–18.2)	1.2 (1.0–1.4)	1.1	5.9 (5.5–6.3)

*Values in parentheses are 95% CIs. *n* = 6 ferrets per vaccine group, *n* = 12 ferrets in the mock group. The maximum values for each individual ferret were used to calculate maximum weight loss, temperature, and virus titer in nasal washes. IIV3, Fluarix vaccine (GlaxoSmithKline Biologicals, <https://www.gsk.com>); LAIV, FluMist vaccine (AstraZeneca, <https://www.astrazeneca.com>); RII, relative inactivity index; RIV, Flublok vaccine (Sanofi, <https://www.sanofi.com>).

GMT of $10^{3.4-4.2}$ PFU/mL on 1–4 DPI, and cleared the virus by 6 DPI (Figure 6, panel B). Ferrets vaccinated with IIV3 and RIV shed comparable amounts of virus through 4 DPI; however, both groups displayed a major reduction in viral shedding by 6 DPI, with no detectable virus present by 8 DPI. Beyond virus shedding in the NW specimens, the MI/24 virus was capable of replicating in both the upper and lower respiratory tract, as well as extrapulmonary tissues such as the olfactory bulb and brain, which was consistent with previous findings (24). Compared with the mock group, all 3 vaccinated groups exhibited similar levels of viral replication in the ethmoid turbinate, soft palate, the olfactory bulb, and brain tissues at 3 DPI. Of note, ferrets in the LAIV group had significantly reduced infectious virus levels in the nasal turbinates ($p = 0.004$, relative to the mock-immunized group), trachea ($p = 0.004$), and lung ($p = 0.043$); only 1 of 3 LAIV-vaccinated ferrets had low levels of virus ($10^{1.8}$ PFU/g) in the trachea, and none of the ferrets in this group showed detectable virus in the lung (Figure 6, panel C). In contrast, both IIV3 and RIV vaccination did not result in substantial effects on viral replication or detection frequency across respiratory tract and extrapulmonary tissues (Figure 6, panel C).

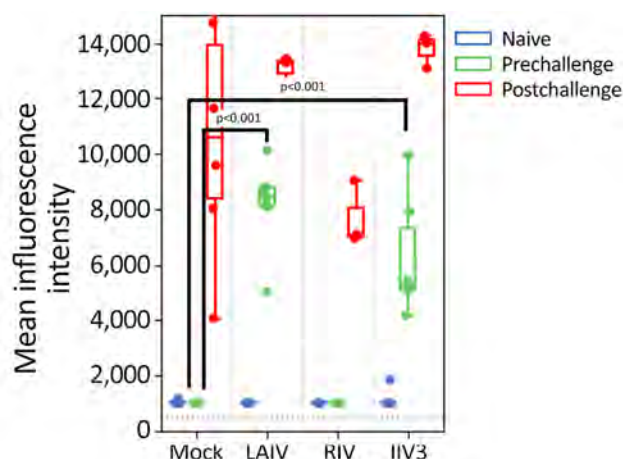


Figure 5. Nucleoprotein antibody titers for A/Brisbane/10/2007(H3N2) virus measured by using multiplex influenza antibody detection assay in study of the effect of seasonal influenza vaccines on influenza A(H5N1) clade 2.3.4.4b virus infection in ferrets. $n = 6$ ferrets per vaccinated group, $n = 12$ ferrets in the mock vaccinated group. Colored dots indicate individual values, horizontal lines within boxes represent geometric mean titers, box tops and bottoms indicate interquartile ranges, and error bars indicate limits of the distribution. Dotted gray horizontal line indicates limit of detection. IIV3, Fluorix trivalent inactivated influenza vaccine (GlaxoSmithKline Biologicals, <https://www.gsk.com>); LAIV, FluMist live attenuated influenza vaccine (AstraZeneca, <https://www.astrazeneca.com>); RIV, Flublok recombinant influenza vaccine (Sanofi, <https://www.sanofi.com>).

Discussion

In this article, we demonstrate that immunization of naïve ferrets with commercially available vaccines against human seasonal influenza confers some cross-protection against infection and disease caused by HPAI H5N1 virus. This evidence of cross-protection is consistent with previous observations that prior infection of ferrets with pH1N1 virus provides a degree of cross-protection against subsequent infection with HPAI H5N1 virus (21). Although human seasonal influenza vaccines confer protection against disease without the risks associated with natural influenza virus infection, the immunity that is induced in vaccinated persons is less potent and less cross-reactive than immunity induced by natural infection (32–34). Our findings indicate that despite minimal detectable cross-reactive anti-H5 responses, commercially available influenza vaccines can reduce disease severity in immunologically naïve H5N1-infected ferrets.

Vaccination of naïve ferrets with LAIV, RIV, or IIV3 induced serologic responses against the vaccine antigens, as expected. No cross-reactive response against H5 was detected after vaccination (Figures 1, 3). H5 and H1 HAs are in the same phylogenetic group and cross-reactive antibodies that recognize both H1 and H5 were identified, which includes both antibodies that react with the relatively conserved stalk region of HA (35) and some that interact with the less conserved globular head (36–38). Both LAIV and RIV immunization did induce binding antibodies against the stalk region from H1 (Figure 3). Titers of those antibodies increased after H5N1 infection, whereas those against the globular head region of H1 and H3 waned, demonstrating that serologic cross-reactivity between H5 and H1 is mainly limited to the stalk region (Figure 3).

H5N1 viruses share an NA subtype with the pH1N1, and antibodies against N1 from both human H1N1 and HPAI H5N1 have been identified after infection with pH1N1 (39–41). Because of the shared NA subtype, substantial cross-reactive binding antibodies to N1 NA of H6N1 2.3.4.4b A have been detected in the human population (5; Z.-N. Li et al., unpub. data). Some ferrets vaccinated with LAIV and IIV3 did develop low but detectable cross-reactive binding antibodies against N1 from H5N1 clade 2.3.4.4b virus, and antibodies against N1 from pH1N1 virus were somewhat boosted by H5N1 infection (Figures 2, 4), again demonstrating cross-reactivity between these antigens. The RIV vaccine contains no NA and therefore did not induce any anti-NA response.

Despite the low cross-reactive response induced by the vaccines, vaccination still conferred some

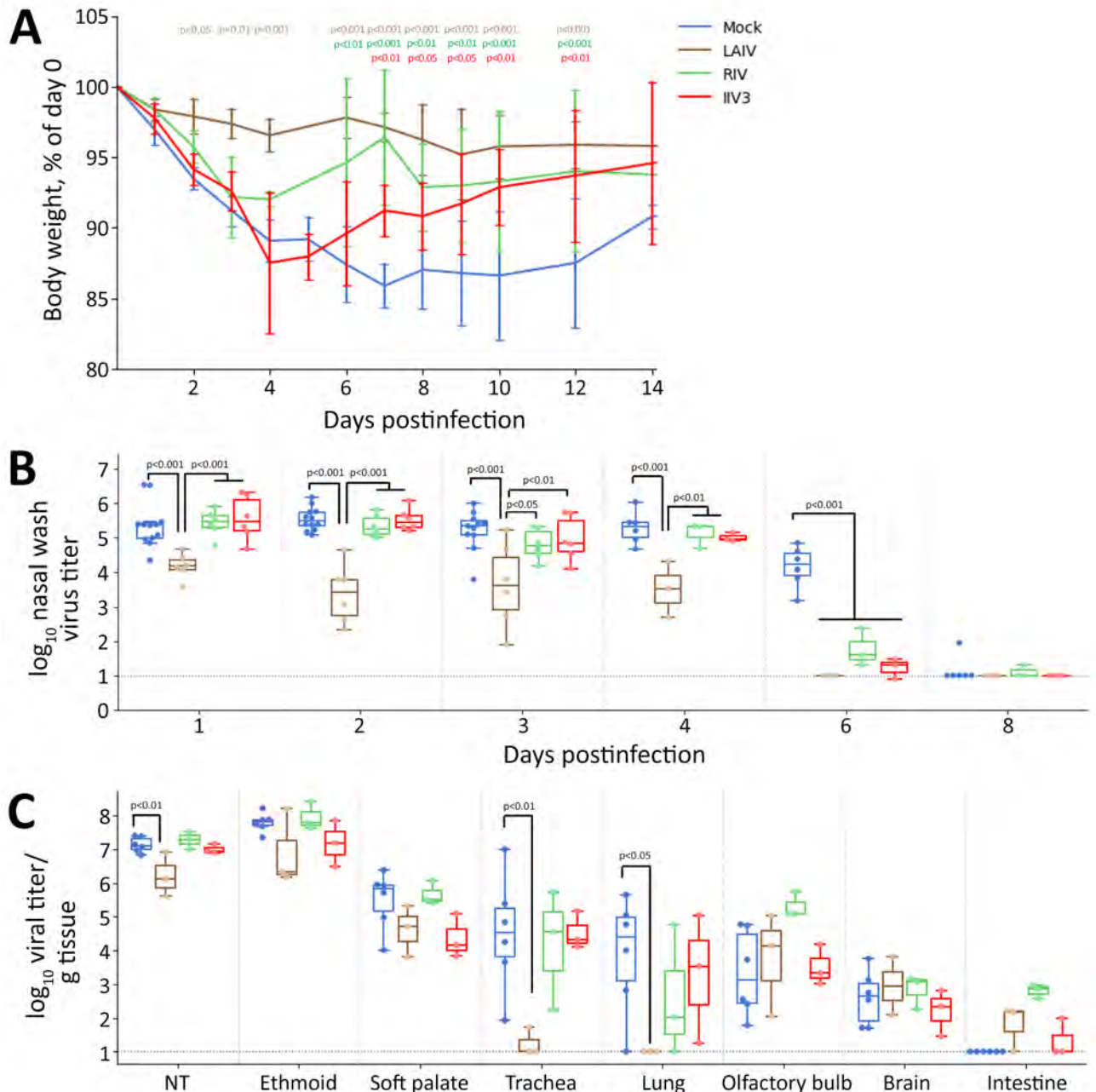


Figure 6. Body weight and influenza A(H5N1) clade 2.3.4.4b viral titers from nasal wash and tissue samples collected during study of the effect of seasonal influenza vaccines on clade 2.3.4.4b H5N1 virus infection in ferrets. $n = 6$ ferrets per vaccinated group, $n = 12$ ferrets in the mock vaccinated group. A) Body weights were monitored for 14 days postinfection and scaled to each ferret's weight on day 0. We assessed p values relative to the mock-infected group by using a linear mixed model with repeated measures in R with lmerTest version 3.1.3 (The R Project for Statistical Computing, <https://www.r-project.org>); p values are indicated in colors matching the relevant body weight traces. Lines are plotted as means $\pm$ SDs. B) We collected nasal washes with phosphate buffered saline and measured the amount of virus by standard plaque formation assays. We assessed p values by using a linear mixed model with repeated measures in R with lmerTest version 3.1.3. C) Three days after infection, we euthanized 3 ferrets per immunized group and 6 ferrets in the mock control group. We measured the amount of virus present in each tissue sample by standard plaque formation assay and assessed p values relative to the mock-infected group. In panels B and C, colored dots indicate individual values, horizontal lines within boxes represent geometric mean titers, box tops and bottoms indicate interquartile ranges, and error bars indicate limits of the distribution. Dotted gray horizontal line indicates limit of detection. IIV3, Fluarix trivalent inactivated influenza vaccine (GlaxoSmithKline Biologicals, <https://www.gsk.com>); LAIV, FluMist live attenuated influenza vaccine (AstraZeneca, <https://www.astrazeneca.com>); RIV, Flublok recombinant influenza vaccine (Sanofi, <https://www.sanofi.com>).

protection against H5N1 disease in ferrets. LAIV, which induced both antistalk antibodies and cross-reactive anti-N1 antibodies, gave the strongest protection against disease and reduced virus shedding. RIV, which induced antistalk HA antibodies but no anti-NA response, also provided some protection against weight loss and reduced the duration of virus shedding. IIV3, which induced only low levels of antistalk HA antibodies but did induce cross-reactive N1 antibodies, provided limited protection against weight loss and reduced the duration of virus shedding.

In addition, both LAIV and IIV3 were able to induce antibodies against NP, which is relatively conserved across different IAV subtypes, and NP antibodies might also contribute to cross-protection (42). Cell-mediated immunity against influenza might be more broadly cross-protective against disease than antibodies because T-cell responses often target highly conserved internal proteins such as NP (43). Although both LAIV and IIV3 immunization induce T-cell responses, cell-mediated immune responses to inactivated vaccines are expected to be low and to target mainly the less conserved HA and NA, because NP and other internal proteins are only present at low and variable levels. The same is true for RIV, which does not include any influenza antigens other than HA.

Immunization with LAIV leads to some replication of the attenuated vaccine virus in the nasal cavity and induces localized inflammation and cytokine expression (44). Although we waited for 24 days after the last immunization before challenging ferrets with H5N1, it is possible that low-level localized inflammation might have contributed some nonspecific protection against infection. However, the IM-administered RIV vaccine also conferred major protection against disease, although its effect on virus shedding was more limited, suggesting that nonspecific local immunity alone was not required for cross-protection.

The findings that both prior human seasonal IAV infection and vaccination with commercially available human seasonal influenza vaccines confers some cross-protection against disease caused by the B3.13 genotype H5N1 in the ferret model is intriguing. However, unlike most adult humans who receive seasonal influenza vaccination, the laboratory ferrets used in this study are completely naive to influenza, and their response to the vaccines might not fully reflect the adult human response. The function and extent of cross-protection between immunity to human seasonal viruses and potentially zoonotic HPAI requires further investigation, particularly given the complex landscape of existing IAV immunity in the general population and its potential influence on

the immune response elicited by seasonal influenza vaccination. This consideration is especially true for LAIV. Despite the cross-protection against the H5N1 virus observed in LAIV-vaccinated ferrets, the ability of LAIV to replicate in the upper respiratory tract of humans with existing immunity and to elicit antibody response is limited (45).

In conclusion, this study demonstrates that seasonal influenza vaccination can potentially provide cross-protection against the B3.13 genotype of H5N1 virus. Although the applicability to humans is currently unknown, revealing this benefit in the naive ferret model is a crucial step to further exploring the benefits of seasonal influenza vaccination in reducing the effects of HPAI H5N1 in human populations.

About the Author

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August 2025

Mpox and Other Viral Diseases

- A Roadmap of Primary Pandemic Prevention Through Spillover Investigation
- Preparedness and Response Considerations for High-Consequence Infectious Disease
- Emergence of Clade Ib Monkeypox Virus—Current State of Evidence
- Surveillance of Viral Respiratory Infections within Maximum-Security Prison, Australia
- Transmission Dynamics of Highly Pathogenic Avian Influenza A(H5N1) and A(H5N6) Viruses in Wild Birds, South Korea, 2023–2024
- Variance among Public Health Agencies' Boil Water Guidance



- Estimated COVID-19 Periodicity and Correlation with SARS-CoV-2 Spike Protein S1 Antigenic Diversity, United States
- Group A *Streptococcus* among American Indian Persons, White Mountain Apache Tribal Lands, United States, 2016–2019
- Multidisciplinary Tracking of Highly Pathogenic Avian Influenza A(H5N1) Outbreak in Griffon Vultures, Southern Europe, 2022
- *Scheffersomyces spartinae* Fungemia among Pediatric Patients, Pakistan, 2020–2024
- ACE2 Receptor Usage across Animal Species by SARS-CoV-2 Variants
- Recombinant Myxoma Virus in European Brown Hares, 2023–2024
- Rapid Emergence and Evolution of SARS-CoV-2 Intrahost Variants among COVID-19 Patients with Prolonged Infections, Singapore

**EMERGING
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Spotted Fever Group Rickettsioses among Hospitalized Patients and Circulation of *Rickettsia* in Ticks, Kazakhstan, 2019

Yekaterina V. Bumburidi, Dmitriy V. Berezovskiy, Bakhytkul T. Zhakipbayeva, Roberta Z. Horth, Alexander J. Millman, William L. Nicholson, Galina E. Zemtsova, Victoria Seffren, Elina R. Maltseva, Zhanna A. Berdygulova, Yekaterina O. Ostapchuk, Yuliya V. Perfilyeva, Andrey M. Dmitrovskiy, Zhansulu K. Dukayeva, Zhanna Zh. Shapiyeva

Testing for spotted fever group rickettsioses (SFGR) and the criteria for identifying suspected patients are not routinely used in Kazakhstan. In 2019, we performed a cross-sectional study in 6 sentinel hospitals in the Pavlodar region. We tested 105 hospitalized patients with SFGR-like symptoms by using PCR or indirect immunofluorescence antibody assay and identified 62 cases of SFGR. Most (78%) cases of disease were caused by *Rickettsia sibirica* and *R. raoultii*. Cutaneous

signs (eschar or rash) were found in 87% of SFGR patients; 79% had a rash, 48% had an eschar, and 13% had neither. Testing of suspected rickettsia cases resulted in a 27% increase in laboratory-detected SFGR over the mean of the previous 3 years (62 vs. 49). Broadening the case definition by including fever, headache, or myalgia and expanding routine testing for suspected cases of SFGR could contribute to improved case detection and earlier treatment.

Spotted fever group rickettsioses (SFGR) are tick-borne diseases that are typically characterized by nonspecific symptoms such as fever, headache, and muscle pain caused by closely related bacteria. A rash or eschar (a black scab) is present in some but not all infections, although manifestations vary depending on the rickettsia species. That variability in clinical manifestations creates challenges for diagnosing SFGR. SFGR are treatable; however, untreated SFGR cases can result in severe complications and death in otherwise healthy persons. The severity of rickettsiosis depends on the *Rickettsia* species with which the patient is infected (1–3).

Tickborne rickettsial bacteria cannot survive on their own in the environment; they depend on a complex lifecycle involving small animal hosts, such as rodents, and can be transmitted transovarially, with ticks serving as both the primary vector

and reservoirs. Infected ticks transmit spotted fever group *Rickettsia* to humans through bites. Although SFGR are globally distributed, the actual burden of illness remains unknown because of underrecognition, limited testing, and underreporting. The geographic expansion and increase in cases of SFGR worldwide can be attributed to increased human-animal contact resulting from land-use changes, climate variations, improved clinical awareness of tickborne diseases, and the increased availability of novel molecular diagnostic techniques (4–7).

Rickettsial diseases are notifiable in Kazakhstan. By the end of 2019, SFGR were recognized as endemic in 4 regions (East Kazakhstan, Kyzylorda, Pavlodar, and North Kazakhstan). Several different *Rickettsia* species have been identified in ticks in Kazakhstan, including *R. sibirica*, *R. raoultii*, *R. slovaca*,

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Z.A. Berdygulova, Y.O. Ostapchuk, Y.V. Perfilyeva, A.M. Dmitrovskiy); Pavlodar Regional Hospital named after Sultanov, Pavlodar, Kazakhstan (Z.K. Dukayeva); Scientific-Practical Center for Sanitary-Epidemiological Expertise and Monitoring, National Center for Public Healthcare, Ministry of Health, Almaty (Z.Z. Shapiyeva)

DOI: <https://doi.org/10.3201/eid3110.250037>

R. aeschlimannii-like organism, and *R. conorii* (8–12). In addition, *R. sibirica*, *R. raoultii*, and *R. heilongjiangensis* were identified in Russia in a region bordering the Pavlodar oblast in Kazakhstan (13,14).

The Pavlodar region has the second highest reported rate of rickettsioses in Kazakhstan. Unlike Kyzylorda, the region with the highest reported burden, no studies have documented circulating rickettsial diseases in Pavlodar (15). On the basis of data available in the national surveillance system (Infectious Diseases in the Republic of Kazakhstan), during 2016–2018 the Pavlodar region reported 146 clinically identified cases of SFGR. Because SFGR signs and symptoms can be nonspecific and routine laboratory confirmation is not available in Kazakhstan, cases are often missed or misdiagnosed. Therefore, the true burden of SFGR is likely higher than reported. We assessed the clinical and epidemiologic characteristics of the disease in humans (including disease burden, clinical features, and etiologic agent) and the molecular identification of *Rickettsia* species in ticks collected from the study region.

Methods

Study Design

We conducted a cross-sectional study during April–October 2019 among persons hospitalized with signs and symptoms consistent with SFGR. We selected 2 regional infectious disease hospitals in the city of Pavlodar (including a pediatric hospital), which serve as referral hospitals, and 4 district hospitals that reported rickettsial disease cases in 2016–2018. Participants ≥ 6 years of age were recruited during April–October, the period when the risk for seasonal tickborne transmission was highest on the basis of active surveillance for ticks in the region.

Enrollment and Inclusion Criteria

Participants were persons hospitalized during the study period with symptoms consistent with *Rickettsia*-like illnesses (suspected cases of rickettsiosis), such as fever ($\geq 38^{\circ}\text{C}$) and ≥ 1 of the following clinical signs or symptoms: rash, eschar, headache, myalgia, anemia, thrombocytopenia, hepatic transaminase elevation, or clinician suspicions of rickettsiosis (16). Participants provided written informed consent; for persons < 18 years of age, the primary caregiver consented. We excluded persons who were not residents of the Pavlodar region (residence was defined as having lived ≤ 2 weeks outside the Pavlodar region during the study period before disease onset) and children < 6 years of age.

Data Collection

Clinicians at the sentinel sites were trained to collect data at the time of patient's inclusion to apply the standard case definition of *Rickettsia*-like illness and to notify the health department of new suspected cases of rickettsiosis. Upon receiving notifications, trained surveillance officers visited the hospitals and obtained written informed consent from hospitalized patients or their primary caregivers to participate in the study. Surveillance officers completed paper case investigation forms containing demographic, clinical, laboratory, and behavioral data. They also abstracted clinical and laboratory data from the medical and laboratory records and entered data into EpiInfo 7.2 (<https://www.cdc.gov/epiinfo>).

Specimen Collection

We collected acute-phase samples, including rash or eschar biopsies or swab specimens and serum samples, at admission (17,18). We collected second serum samples (convalescent) 2–3 weeks after admission from patients with no rash or eschar or whose rash or eschar samples were PCR negative. We collected second serum samples at the hospital before discharge; however, if duration of hospitalization was < 2 weeks, we invited patients by phone to the territorial polyclinic for specimen collection. We collected rash and eschar biopsies and swab samples under sterile conditions.

We stored specimens in refrigerators (4°C) at the hospital for up to 24 hours before being transported to the Pavlodar regional laboratory for PCR testing (rash and eschar samples) and freezing (at -20°C). Samples were subsequently sent to the Central Reference Laboratory for sequencing.

Laboratory Testing of Human Specimens

PCR Testing and Sequencing

We extracted DNA from rash and eschar specimens using the QIAGEN DNA Mini Kit (QIAGEN, <https://www.qiagen.com>) according to the manufacturer's instructions. We used a real-time genus-specific *Rickettsia* (pan-*Rickettsia*) assay targeting the 23S rRNA as a screening assay (19). We further tested specimens positive for rickettsial DNA in the pan-*Rickettsia* assay by using a seminested PCR to amplify and subsequently sequence a fragment of the gene encoding the protein rOmpA, appropriate for differentiation of 21 *Rickettsia* species, including the most commonly reported species circulating in the investigated region (20). We used the human housekeeping glyceraldehyde 3-phosphate

dehydrogenase (GAPDH) gene, yielding a 157-bp fragment as an internal control (21). We reported PCR test results to the hospital within 7 days.

We performed elution of DNA fragments from the agarose gel using a QIAquick gel extraction kit (QIAGEN). We then sequenced the purified DNA fragments in both directions using a BigDye Terminator v3.1 Cycle Sequencing Kit and an ABI 3500XL genetic analyzer (Thermo Fisher Scientific, <https://www.thermofisher.com>).

Individual gene sequences underwent multiple sequence alignment using the MUSCLE algorithm in the MEGA X program (22). We constructed a maximum-likelihood phylogenetic tree using *Rickettsia* species from various phylogenetic groups as references. We used nonparametric bootstrapping with 500 replicates to calculate confidence estimates.

Indirect Immunofluorescence Antibody Assay for Serum Samples

We tested serum samples using an indirect immunofluorescence antibody assay (IFA). We prepared multiantigen slides with strains of *R. raoultii*, *R. sibirica*, and *R. slovaca*. We used serum containing human IgG to spotted fever group *Rickettsia* as positive controls. Slides and positive controls were provided by the Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA). For determination of acute case status, positive samples were titrated to determine endpoint titer and to examine 4-fold titer increases. Comparison of titers provided presumptive etiology for this study (23). We defined in IFA the species of *Rickettsia* implicated in the disease for patients with paired or single serum samples as the species with the highest difference in titers or the highest titer. In case of equivalent titers for >1 species, we considered the results unidentified species of SFGR. We considered a single serum IgG titer of 1/64 a minimum positive titer, which supports *Rickettsiosis* for patients for surveillance purposes at CDC with a clinically compatible acute illness and with an epidemiologic history of the disease (5). However, to increase the specificity for identification of clinical cases, we included only patients with 1 serum sample with titers $\geq 1:128$ (probable cases in our study) in the data analysis (24).

Sampling and Data Collection of Ticks

Two groups collected ticks for 4 days (May 13–16) in 2 districts of Pavlodar region. Using a map and a hand-held global positioning system tracker, we selected 4 ordinal points within each designated village for tick collection. Groups conducted sampling for ≥ 3

person-hours at each location within a suitable habitat (i.e., woods, grassland, and trail margins) (25).

We collected ticks using the dragging method, in which light-colored flannel material is dragged behind the collector by a rope handle attached to a wooden base. We used a flagging method in areas with high vegetation density in which the drag cloth could not be pulled between plants. For the flagging method, we used the material attached to 1 end of a wooden rod like a flag to sweep it through vegetation (26). We collected ticks in the second week of May 2019. This window coincided with peak activity for *Dermacentor* ticks, which are known to carry *Rickettsia* species (27–29).

We identified tick species, development stage, and sex morphologically using a stereomicroscope according to published tick specification guidelines (30,31). We then placed adult ticks individually into tubes containing 70% ethanol for storage before sending them to the Central Reference Laboratory for testing.

Laboratory Testing of Ticks

We homogenized each tick specimen individually using the TissueLyser II (QIAGEN). We extracted total nucleic acid using the PrepMan Ultra Sample Preparation Reagent (ThermoFisher Scientific) according to the manufacturer's instructions. We used *Rickettsia* genus-specific (pan-*Rickettsia*) real-time PCR for testing each tick individually. We tested positive samples using a seminested PCR for amplification and sequencing targeting *rOmpA* gene to determine species, as described previously for human samples.

Key Definitions

We defined a suspected case of rickettsiosis as illness in a person hospitalized with fever (temperature $\geq 38^{\circ}\text{C}$) and ≥ 1 of the following clinical signs or symptoms: rash, eschar, headache, myalgia, anemia, thrombocytopenia, hepatic transaminase elevation (16), or suspicion of rickettsiosis by the clinician. We defined a probable case of rickettsiosis as illness in a person meeting the definition of a suspected case of rickettsiosis with only 1 serum sample available, with IFA *Rickettsia* IgG titers $\geq 1:128$ (24). We defined a laboratory-confirmed case of rickettsiosis as illness in a person meeting the definition of a suspected case of rickettsiosis with a positive PCR result for blood, eschar, or skin, or a 4-fold increase in IFA *Rickettsia* IgG titers (*R. raoultii*, *R. sibirica*, or *R. slovaca*) from a sample taken upon admission and a convalescent sample (16). Severe disease was defined by clinicians as having any of the following: acute respiratory

distress syndrome (shortness of breath [respiration rate >25 breaths/min], cyanosis, bilateral infiltrates on the lung radiograph, progressive hypoxemia [$\text{SpO}_2 \leq 90\%$]); disseminated intravascular coagulation (blood clotting time >12 s, plasma D-dimer $\geq 250 \mu\text{g/L}$, platelet count $<120 \times 10^9/\text{L}$, plasma fibrinogen $<1.5 \text{ g/L}$, prothrombin time (PT) >16 s); or renal failure (oliguria up to anuria and accompanied by proteinuria of $\geq 0.5 \text{ g/L}$, serum creatinine $>117 \text{ mkmol/L}$, blood serum urea $>8 \text{ mmol/L}$).

Data Analysis

Our data analysis only included laboratory-confirmed and probable cases. Electronic questionnaire development, data entry, data cleaning, and analysis were done in EpiInfo 7.2. We used the χ^2 or Fisher exact tests to assess the differences in clinical symptoms by *Rickettsia* species. We considered a p value of <0.05 significant.

Ethics Statement

This study was approved by the Ethical Committee of the Kazakh Medical University ("The Highest School of Public Health") (protocol no. IRB-A096, April 2019) and extended (protocol no. IRB-A12, September 2020). This activity was reviewed by CDC, deemed not research, and was conducted consistent with applicable federal law and CDC policy (e.g., 45 C.F.R. part 46, 21 C.F.R. part 56; 42 U.S.C. §241(d); 5 U.S.C. §552a; 44 U.S.C. §3501 et seq.).

Results

Characteristics of Participants

We obtained consent from and enrolled 105 persons hospitalized with suspected rickettsiosis; among those, 62 (59%) were determined to be laboratory-confirmed or probable rickettsiosis. Among participants with rickettsiosis, 20 (32%) were confirmed by IFA in paired samples (a 4-fold increase of IgG titers to 1 of 3 investigated *Rickettsia* species), 35 (56%) by PCR, and 7 (11%) as probable cases in a single available acute sample with a titer of $\geq 1:128$ by IFA. We did not include 10 patients with a titer of 1:64 with a single available IFA in the analysis. We identified 3 *Rickettsia* species among the participants: *R. sibirica* (47%), *R. raoultii* (31%), and *R. slovaca* (6%). No *Rickettsia*-specific species were identified in 16% of participants. (Appendix Table 1, <https://wwwnc.cdc.gov/EID/article/31/10/25-0037-App1.pdf>).

Of the 62 participants with acute rickettsiosis, 85% were adults (≥ 18 years of age), 48% were male, 66% lived in rural areas or suburbs, 55% owned a cat

or dog, and 23% owned livestock (Table 1). In the 2 weeks before disease onset, 53% had worked in a garden or orchard, 48% had noted a tick bite, and 31% had been in an open countryside area. Fifty-eight percent of participants reported usually removing ticks with their bare hands. To protect themselves against tick bites, 32% reported taking some protective measures, such as wearing long-sleeved clothing or gloves or using repellents; 68% reported taking no measures (Table 1).

We detected rickettsiosis cases in all sentinel sites. Eleven percent of cases were primarily referrals from nonsentinel sites to the regional hospitals in Pavlodar City. Based on the place of residence, we identified rickettsiosis cases in 85% (11/13) of the administrative territories of Pavlodar region.

Rickettsiosis cases peaked in May (53% of cases). Although *R. sibirica* cases occurred throughout the endemic season, *R. raoultii* cases occurred predominantly in the summer months (Appendix Figure).

Clinical Features

The most common signs and symptoms among those with rickettsiosis were fever (100%), headache (90%), rash (79%), myalgia (61%), and eschar (48%). More than half (53%) had thrombocytopenia, and 39% had elevated aspartate aminotransferase. Fifteen percent had severe disease; no deaths occurred (Table 2).

Rash, eschar, or swollen lymph nodes were present in 87% and absent in 13% of patients. Eschar was the most common among participants with *R. sibirica* and least common among those with *R. raoultii* with a difference in prevalence of 7.5 times (crude prevalence ratio 7.5 [95% CI -2.0 to 28.3]; $p<0.001$). Severe disease was also most common among participants with *R. sibirica* (21%) and least common among those with *R. raoultii* (5%) or *R. slovaca* (0%). (Table 2).

Rickettsia Species in *D. marginatus* and *D. reticulatus* Ticks

We collected 959 ticks; 64% were identified as *D. reticulatus* ticks and 36% were *D. marginatus* ticks. *R. raoultii* was detected in 46 (5%), SFG rickettsiae in 37 (4%), and *R. sibirica* in only 2 ticks. In both tick species, *R. raoultii* was in almost equivalent proportions of 4% (13) in *D. marginatus* ticks to 5% (33) in *D. reticulatus* ticks (Appendix Table 2).

Discussion

Rickettsioses were detected in ticks or humans in 85% of districts in the Pavlodar region. By establishing sentinel sites and enhanced laboratory testing for surveillance of rickettsioses, we observed a 27% increase

in the number of detected cases compared with the mean of the previous 3 years (62 vs. 49). *R. sibirica* and *R. raoultii* were the most frequent etiologic agents identified among cases. Those findings highlight missed opportunities for diagnosis and treatment of rickettsioses.

Our observation of a 27% increase in the number of detected cases compared with the mean of the previous 3 years after we established sentinel sites for surveillance is concerning because of potential gaps in medical interventions for persons with rickettsioses. Although most persons had mild illness, 15% had severe disease. Empirical treatment is most effective at preventing death and severe illness when started within the first 5 days after symptom onset. Although our findings do not enable us to evaluate missed opportunities for early treatment, the substantial under-detection of cases in an endemic region suggests there could be infected persons who develop severe disease or are at risk for developing severe disease who do not receive the appropriate diagnosis and early treatment by healthcare providers (32,33).

Consistent with known clinical manifestations of SFGR, fever, headache, and myalgia were the most common symptoms among case-patients (5). Cutaneous findings occurred frequently, but 13% of case-patients had none. Cutaneous signs can differ widely by species. The presence of eschar has been reported to be 7 times less common among persons with *R. raoultii* than *R. sibirica* (5,34,35), and we found that 26% of participants with *R. raoultii* did not have cutaneous signs. Because of the lack of a standard case definition and clinical protocol for rickettsioses in Kazakhstan, patients without cutaneous signs might not receive appropriate diagnostics or treatment for rickettsioses. The inclusion of headache, fever, or myalgia in the clinical case definition for suspected rickettsioses could contribute to improved recognition of the disease and faster initiation of appropriate treatment.

D. marginatus and *D. reticulatus* ticks were the only species identified in our study. The detection of *R. raoultii* and *R. sibirica* in those ticks suggests that they might serve as vectors of SFGR in the Pavlodar region. However, given that only 3%–5% of the tested ticks were positive for any *Rickettsia* species, this association should be interpreted with caution. The vector capacity of *D. marginatus* and *D. reticulatus* ticks in transmitting *R. raoultii* has been demonstrated in previous studies (11). Transmission of *R. sibirica* is generally associated with *Haemaphysalis concinna*, *Dermacentor* spp., and *Hyalomma* spp. ticks (8,13,36). *H. concinna* ticks have been found in Pavlodar region by a previous study (37). Therefore, tick species other

than those identified in this study might also contribute to the transmission of *Rickettsia* in the region. This information can assist in prompting clinicians to suspect rickettsioses among persons who are bitten by ticks of those species. In addition, persons bitten by those ticks can be given information about the need to self-monitor for fever, headache, myalgia, eschar, or rash and to seek medical care if those signs develop.

We also identified gaps in the use of protective clothing and tick removal practices. Regular use of repellents and protective clothing that covers the skin are recommended to decrease the risk for tick bites when spending time outdoors in tick environments in rickettsioses-endemic regions (38,39). Public health risk communication about preventing tick exposure can help increase awareness of tickborne diseases and ways to reduce the risk for tick bites.

The first limitation of our study is that fever was a required symptom for participation, so cases of asymptomatic SFGR could have been missed. We also did not survey all hospitals in the region. We

Table 1. Demographic and tick-related behavior characteristics of 62 patients in study of spotted fever group rickettsioses among hospitalized patients and circulation of *Rickettsia* in ticks, Kazakhstan, 2019

Characteristic	No. (%)
Age group, y	
≥18	53 (85)
<18	9 (15)
Sex	
M	30 (48)
F	32 (52)
Place of residence	
Countryside/suburb*	41 (66)
City	21 (34)
Owned a summer cottage	9 (15)
Owned pets (cats, dogs)	34 (55)
Owned livestock	14 (23)
During the previous 2 weeks	
Worked in a garden or orchard	33 (53)
Noticed a tick bite	30 (48)
Went out to the open countryside	19 (31)
Usual method of tick removal	
Removed ticks with bare hands	36 (58)
Removed ticks at the hospital or polyclinic	7 (11)
Using tweezers to remove tick	2 (3)
Using gloves when removing	1 (2)
Other methods of removing	3 (5)
Never removed ticks	13 (21)
Methods used to protect yourself from ticks	
None	42 (68)
Long clothes in several layers, gloves†	14 (23)
Repellents only	5 (8)
Avoided bushes	1 (2)
Methods used to protect your pets, n = 34‡	
None	30 (88)
Insecticides	4 (12)

*If a patient lived in a dacha (summer cottage) for ≥1 month, the place of residence was considered a suburb.

†This number includes 4 patients who used the listed items and repellents.

‡Protection of pets was calculated for patients who had pets.

Table 2. Characteristics of 62 human rickettsiosis cases by *Rickettsia* species in study of spotted fever group rickettsioses among hospitalized patients and circulation of *Rickettsia* in ticks, Kazakhstan, 2019*

Characteristic	Total	<i>R. sibirica</i>	<i>R. raoultii</i>	SFGR	<i>R. slovaca</i>
No. patients	62 (100)	29 (100)	19 (100)	10 (100)	4 (100)
Symptoms and signs					
Fever ≥38°C	62 (100)	29 (100)	19 (100)	10 (100)	4 (100)
Headache	56 (90)	27 (93)	17 (89)	9 (90)	3 (75)
Rash	49 (79)	26 (90)	12 (63)	8 (80)	3 (75)
Myalgia	38 (61)	21 (72)	11 (58)	5 (50)	1 (25)
Primary skin lesion: eschar	30 (48)	23 (79)	2 (11)	4 (40)	1 (25)
Nausea	18 (29)	8 (28)	7 (37)	3 (30)	0
Vomiting	11 (18)	5 (17)	4 (21)	2 (20)	0
Swollen lymph nodes	3 (5)	1 (4)	1 (5)	0	1 (25)
Any rash, eschar, or swollen lymph nodes†	54 (87)	28 (97)	14 (74)	9 (90)	3 (75)
Severe disease‡					
Had severe disease	9 (15)	6 (21)	1 (5)	2 (20)	0
Had acute respiratory distress syndrome	6 (10)	5 (17)	1 (5)	0	0
Had disseminated intravascular coagulation	4 (6)	2 (7)	0	2 (20)	0
Had renal failure	2 (3)	2 (7)	0	0	0
Blood abnormalities					
Thrombocytopenia	33 (53)	18 (62)	12 (63)	3 (30)	0
AST elevation§	23 (39)	12 (46)	8 (44)	2 (20)	1 (25)
ALT elevation§	11 (19)	6 (23)	1 (6)	1 (10)	3 (75)
Anemia	6 (10)	3 (10)	1 (5)	2 (20)	0

*Values are no. (%). The 62 patients include 55 confirmed (35 by PCR and 20 by indirect immunofluorescence assay with 4-fold seroconversion) and 7 probable cases (IgG titers ≥1:128). Probable cases with the only available acute serum had IgG titers of 1:128. The species of *Rickettsia* implicated in the disease for patients with paired or single serum samples were defined in indirect immunofluorescence assay as the species with the highest difference in titers or the highest titer. Bold indicates cutaneous signs and lymphadenopathy (swollen lymph nodes). ALT, alanine aminotransferase; AST, aspartate aminotransferase; SFGR, spotted fever group rickettsia.

†All patients with swollen lymph nodes had a rash or eschar.

‡Severe disease was diagnosed on the basis of the following criteria: 1) renal failure (oliguria up to anuria and accompanied by proteinuria of ≥0.5 g/L, serum creatinine >117 μmol/L, blood serum urea >8 mmol/L); 2) acute respiratory distress syndrome (shortness of breath (respiration rate >25 breaths/min), cyanosis, bilateral infiltrates on the lung radiograph, progressive hypoxemia (SpO₂ ≤90%); 3) disseminated intravascular coagulation (blood clotting time (>12 s), plasma D-dimer (≥250 μg/l), platelet count (<120×10⁹/l), plasma fibrinogen (<1.5 g/L), prothrombin time (PT) (>16 s). Blood abnormalities assessment was done by clinicians using the following levels: thrombocytopenia, platelet (<150 × 10⁹/L); leukopenia, leukocytes <4 × 10⁹ cells/L; anemia, hemoglobin <130 g/L for men, <120 g/L for women, <115 g/L for children; AST elevation, >40 U/L for men, 31 U/L for women, >43 U/L for children; ALT elevation, >40 U/L for men, 33 U/L for women, >36 U/L for children).

§Three *R. sibirica* cases had no data on AST or ALT.

included the reference hospital where the most severe cases are transferred. Those limitations might have resulted in an underestimation of the total number of cases in the region but might also have resulted in an overestimation of cases of severe disease among persons with SFGR. PCR confirmation of the rickettsia species in our study was obtained on the basis of a short sequence of 1 gene (rOmpA), which is commonly used in *Rickettsia* diagnostics (19). However, relying on a single, short amplicon limits the confidence with which one can make a precise species diagnosis because of the high genetic similarity and conserved nature of *Rickettsia* genomes. We could not identify species of *Rickettsia* for 6 rickettsioses and 37 ticks (SFGR without species identification), which might have been related to the sequencing methodology of only using the short sequence of 1 gene. The methods used could possibly have contributed to misclassification or missed diagnoses of species. Sequencing of 2 or 3 genes would have improved the accuracy and reliability of species differentiation. Last, we collected ticks only from 2 districts, which could have resulted in incomplete identification of ticks and *Rickettsia* species diversity (40).

In conclusion, we identified rickettsia-positive humans in at least 85% of the Pavlodar region districts and positive ticks in all 2 investigated districts. The predominant species identified among persons hospitalized with rickettsioses were *R. sibirica* and *R. raoultii*. Among the collected ticks, *Rickettsia* prevalence was ≈9%, and 5% carried *R. raoultii*. Almost 80% of persons with *Rickettsia* had a rash, half had an eschar, and 13% had no SFGR cutaneous signs or lymphadenopathy. More than half of participants removed ticks with bare hands, and almost 70% reported not using any protection against tick bites. Enhanced routine testing in the region is key to improving clinical management and disease surveillance. Increased awareness among clinicians and the general population about rickettsial symptoms and risk for tick bites could help reduce disease transmission and improve medical care.

Acknowledgments

We thank Toni Whistler for her support as a scientific advisor during the protocol writing and clearance. We thank the participating investigator team: Umirbek Usenov; Yuriy Skiba and laboratory team; Oljas

Yeskhodzhayev and laboratory team; Kazira Toktaganova and laboratory and parasitology teams; epidemiology team from Pavlodar Regional/District Departments of the Committee of Sanitary and Epidemiological Control; clinician team from Pavlodar oblast hospital named after Sultanov, Pavlodar oblast children hospital, and district hospitals (Aksu, Akkuli, Trernkol, Zhelezin) under the Regional Healthcare Department.

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Multidrug-Resistant pESI-Harboring *Salmonella* *enterica* Serovar Muenchen Sequence Type 82 in Poultry and Humans, Israel, 2020–2023

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Salmonella enterica serovar Muenchen emerged in Israel in 2018 and became a major public health threat. We aimed to determine the role of poultry in rising human cases, transmission routes within the broiler industry, and genetic similarity to *Salmonella* Muenchen found globally. We used whole-genome sequencing to compare *Salmonella* Muenchen isolates from poultry, food, and humans collected in Israel (2020–2023; n = 109) and globally (n = 125). *Salmonella* Muenchen sequence type 82 isolates from Israel harbored pESI plasmid, exhibited high genetic similarity between human and poultry sources, and closely resembled international pESI-positive strains; we found quinolone-resistance determinants in 58.6% of isolates. *Salmonella* Muenchen prevalence in commercial broiler flocks was 61.5% (95% CI 51.5%–71.5%); strains could not be traced to breeder flocks, but on-farm persistence existed. The clonal spread of *Salmonella* Muenchen in poultry contributes to increased incidence in humans. Horizontal transmission in broilers requires control measures to protect public health.

Nontyphoidal *Salmonella* (NTS) is a major cause of foodborne illness worldwide (1); poultry serve as a reservoir and a source of infection to humans for multiple serotypes (2). Although most serotypes cause little harm to the infected bird (3),

controlling NTS infection in poultry is essential for the protection of public health (2). Given the pyramidal structure of the poultry industry (a large number of production flocks [broilers and layers] are descendants of a small number of parent and grandparent breeding flocks), control measures for NTS spread, such as vaccination against specific serotypes (2), are often focused on the parent and grandparent flocks and not on the high number of short-lived production birds.

In Israel, NTS surveillance in humans relies on passive reporting of clinical cases to the national reference laboratory of the Ministry of Health (4), similar to the approach used in Europe (5). In the poultry sector, the Egg and Poultry Board and the Israeli Veterinary Services conduct an active surveillance program for *Salmonella* only in breeding flocks and layer hen farms, in accordance with international regulations (European Regulation no. 2160/2003).

Since 2018, *Salmonella* Muenchen and *Salmonella* Virginia, formerly misclassified as separate serotypes (6) (both referred to as *Salmonella* Muenchen hereafter), emerged as the dominant serotypes in clinical cases in humans in Israel. These serotypes accounted for 2.0% (101/5,072) of all *Salmonella* cases in 2017 and 35.4% (1,124/3,171) in 2022 (7). A similar trend was found in *Salmonella* isolates from poultry: from 3.8% (63/1,672) of the animal and poultry sources in 2017 to 23.6% (202/857) in 2021, and from 4.9% (12/245) of the food industry and ready-to-eat products (most of which are from poultry slaughterhouses) in 2017 to 20.0% (43/215) in 2021 (7).

Another study in Israel (8) reported that *Salmonella* Muenchen had acquired pESI, a plasmid first identified in *Salmonella* Infantis (9). pESI is described

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DOI: <https://doi.org/10.3201/eid3110.250191>

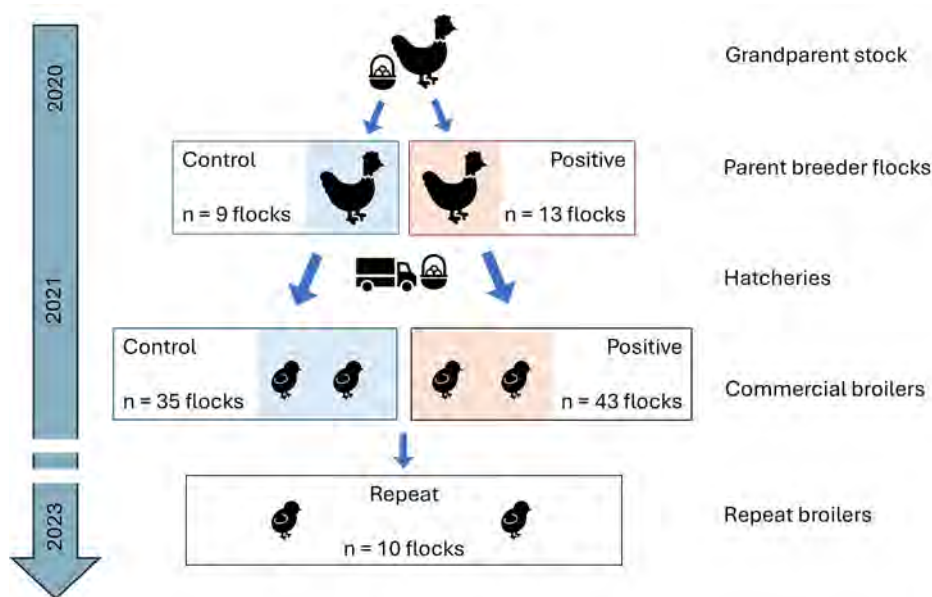


Figure 1. Timeline showing the selection of the positive and control breeder flocks for study of *Salmonella enterica* serovar Muenchen in poultry and humans, Israel, 2020–2023. We sampled commercial broiler flocks that were direct descendants of the positive and control breeder flocks during 2020–2021 and detected *Salmonella* Muenchen infection. We resampled a subset of the barns housing new broiler flocks during 2023 (repeat broilers).

as a mega-plasmid that contributed to stress tolerance and increased pathogenicity that enabled the rapid spread of *Salmonella* Infantis in humans and poultry in Israel since 2007 and later in Italy (10) and other locations worldwide (11). The spread in Israel was ultimately controlled by the introduction of mandatory vaccination against *Salmonella* Infantis in breeder flocks in 2011 (4). However, despite a rapid reduction in the prevalence of *Salmonella* Infantis in breeder flocks, the success of the vaccination program to reduce the prevalence in broiler flocks was only evident several years after its initiation (A. Wiseman, pers. comm., telephone, 2020 Jul 1). Therefore, we suspected that the persistence of *Salmonella* Infantis in the farm environment and horizontal transmission between flocks, rather than vertical transmission from parent flocks, played a substantial role in its spread.

We used a large collection of *Salmonella* Muenchen isolates recovered from broiler flocks in Israel to determine the genetic relatedness among isolates from broilers, humans, and other poultry sources and the presence of relevant genetic determinants such as pESI and antimicrobial resistance (AMR) genes. We also determined the prevalence of *Salmonella* Muenchen in commercial broiler flocks and the potential transmission routes between different growth stages to observe whether vertical or horizontal transmission play a major role in bacterial spread. Finally, we studied genetic similarities between the emerging *Salmonella* Muenchen in Israel and global strains to reflect on the potential for its global spread and the risk posed to public health globally.

Materials and Methods

Study Population and Sample Collection

We conducted 3 analyses of *Salmonella* Muenchen isolates (Appendix 1, <https://wwwnc.cdc.gov/EID/article/31/10/25-0191-App1.pdf>). We chose study populations and collected samples in accordance with the intention of each analysis. For analysis A, we identified *Salmonella* Muenchen isolates collected in Israel during June 2020–January 2022 from a broad geographic distribution from broilers ($n = 60$; see analysis B) and layers ($n = 9$), poultry slaughterhouses ($n = 9$) (Appendix 1 Figure 1), and human clinical cases ($n = 21$). For analysis B, we used isolates from heavy breeders, grandparent flocks and hatcheries that were collected as part of the national *Salmonella* active surveillance program and were tested for *Salmonella* in accordance with International Standards Organization protocol ISO-6579-1 (12). Subsequently, we screened all serogroup C isolates for *Salmonella* Muenchen by multiplex PCR (13). We selected 13 heavy-breeder flocks that tested positive for *Salmonella* Muenchen on ≥ 2 successive collections as the positive group and 9 flocks with ≥ 3 successive negative results for *Salmonella* as the control group. We identified newly hatched broiler flocks ($n = 78$) and purposely sampled them for detection of *Salmonella* Muenchen: of those, 43 flocks were descendent from the positive group, and 35 flocks were descendent from the control group (Figure 1). We used those samples to estimate prevalence and assess risk factors for positivity to *Salmonella* Muenchen in broilers. In addition, 15–20 months after the initial broiler

sampling, we resampled 10 new flocks in barns where *Salmonella* Muenchen was previously detected (hereafter, repeat broilers) (Figure 1). We obtained samples from broiler farms by using environmental drag swabs either inside the barn (interior swabs) or around the exterior of the barn (exterior swabs), or by using chick sampling and testing, conducted independently of this study (Appendix 2 Table 1, <https://wwwnc.cdc.gov/EID/article/31/10/25-0191-App2.xlsx>). We conducted environmental sampling of the barns just before marketing, during marketing time (i.e., when transferred to the slaughterhouse at age 35–42 days), or after marketing when the barn was emptied but before cleaning.

We selected 70 of the *Salmonella* Muenchen isolates, representing a broad geographic distribution and representative of the different production stages, to undergo whole-genome sampling. That group included isolates from commercial broilers, subdivided into those descendent from the positive ($n = 20$) and control ($n = 18$) groups, hatcheries ($n = 3$), breeder flocks ($n = 10$), breeder pullets ($n = 6$), grandparent flocks ($n = 3$), and repeat broilers ($n = 10$) (Appendix 2 Table 3).

For analysis C, we chose 11 isolates that had undergone whole-genome sequencing and genetic analysis (from analysis A) to represent the different sources and genetic diversity of *Salmonella* Muenchen in Israel. In addition, we downloaded paired-end reads of *Salmonella* Muenchen global isolates ($n = 125$; i.e., strains collected during 2018–2021 from various sources in other countries) and strains known to contain pESI (8) from the National Center for Biotechnology Information (NCBI; <https://www.ncbi.nlm.nih.gov>) (Appendix 2 Table 4).

Genetic Analysis

We generated paired-end reads for all isolates using the NextSeq platform (Illumina, <https://www.illumina.com>). In addition, we subjected isolate Br19 to long-read sequencing using the Oxford Nanopore platform (Nanopore Technologies, <https://nanoporetech.com>). We used its hybrid assembly, built using Unicycler version 0.5.0 (github.com/rrwick/Unicycler) (14), as a genetically relevant reference genome. We aligned Illumina reads to the reference genome using Snippy version 4.6.0 (<https://github.com/tseemann/snippy>). We created maximum-likelihood trees using RAxML version 8.2.12 (<https://github.com/amkozlov/raxml-ng>) (15). We assessed branch support for the trees using 10,000 bootstrap replicates. We conducted de novo genome assemblies using SPAdes version 3.12.0 (16). We used contigs as an input for detection of AMR genes, known point

mutations, and genes associated with virulence and resistance; detection of plasmid replicons; detection of a pESI target sequence (8); in silico serotyping; and multilocus sequence typing.

For analyses A and B, we rooted the maximum-likelihood trees to a *Salmonella* Muenchen sequence type (ST)112 outgroup strain (NCBI Sequence Read Archive accession no. SRR6222324). For analysis C, we rooted the tree to a *Salmonella* Braenderup strain (accession no. SRR5874793), a closely related yet genetically distinct serotype (17).

Phenotypic Analysis

We assessed antimicrobial resistance of a selection of broiler isolates ($n = 15$), representing the diversity of genetic elements present that might confer resistance, using Sensititre susceptibility plates (Thermo Fisher Scientific, <https://www.thermofisher.com>). We used human clinical breakpoints for MIC value interpretation.

Criteria for the Presence of the pESI Plasmid

We determined that the pESI plasmid was present if the plasmid replicon IncFIB (pN55391) or hyp_pESI, a 559bp target sequence (8), were present, together with ≥ 1 of 3 groups of genes previously described in pESI (8,18). Those gene groups included 4 AMR genes, *sul1*, *tetA*, *aadA1*, and *qacEdelta1*; yersiniabactin genes *ybtP* and *ybtQ*; or genes associated with the mer operon, *merC*, *merP*, *merT*, and *merR*.

Statistical Analysis

For statistical analysis, we used WINPEPI version 11.65 (19). We calculated Pearson χ^2 , Fisher exact test, and odds ratios (ORs) with 95% CIs, as appropriate.

Ethics Statement

Isolates used for the purpose of this research were collected as part of mandatory routine surveillance programs that were conducted according to the ethical standards and in compliance with Israel law. Clinical isolates from humans were obtained anonymously from the Ministry of Health, Public Health Laboratories, Jerusalem, Israel. Additional sampling was limited to environmental sampling of poultry farms.

Results

Analysis A—Genetic Variability of *Salmonella* Muenchen in Poultry and Humans

We identified *Salmonella* Muenchen from all sources ($n = 99$) as ST82. Clustering according to source was not evident in the reconstructed maximum-likelihood

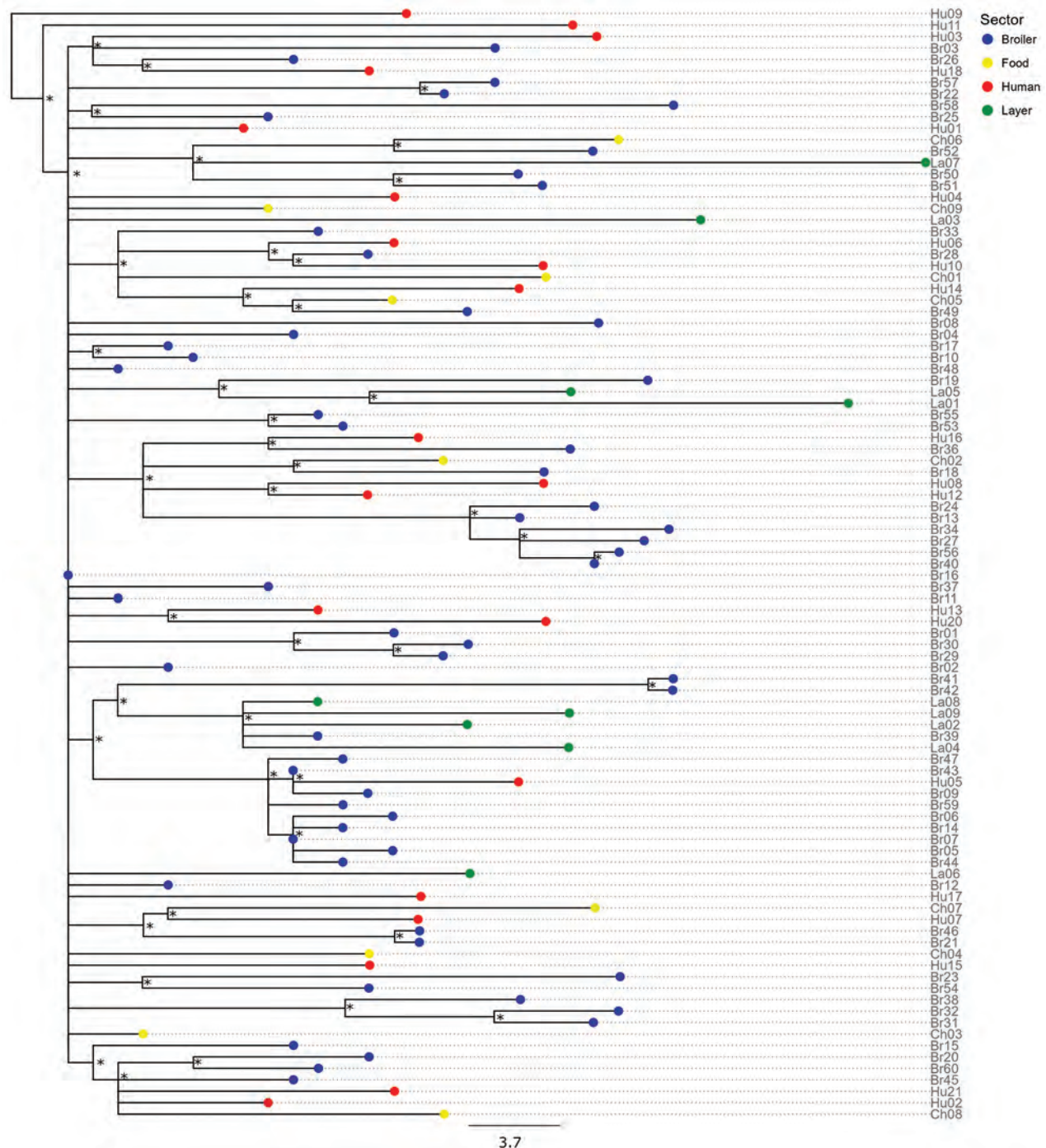


Figure 2. Maximum-likelihood tree reconstructed using whole-genome sequences of *Salmonella enterica* serovar Muenchen isolates collected from broilers (n = 60), layers (n = 9), food products of poultry origin (whole chicken carcasses sampled in poultry slaughterhouses; n = 9), and humans (n = 21) in study of *Salmonella* Muenchen in poultry and humans, Israel, June 2020–January 2022. We used a hybrid assembly of *Salmonella* Muenchen sequence type 82 (Br19) as a reference genome and used *Salmonella* Muenchen sequence type 112 (National Center for Biotechnology Information Sequence Read Archive accession no. SRR6222324) to root the tree (not shown). The analysis included 1,227 single-nucleotide polymorphisms (SNPs) in the alignment (0–69 pairwise SNPs, median 31). Because isolates Hu19 and Br35 were duplicates of isolate Br16, we removed them from the analysis. Tip colors indicate sector. Asterisks (*) indicate nodes with >70% bootstrap support. Additional data (Appendix 1 Figure 2, <https://wwwnc.cdc.gov/EID/article/31/10/25-0191-App1.pdf>) include a heatmap and show the presence of acquired antimicrobial resistance genes and point mutations indicate the isolates in which antimicrobial sensitivity was tested in vitro and the presence of a pESI genetic marker, hyp_pESI, and plasmid replicons. Scale bar represents SNP difference.

Table 1. Acquired antimicrobial resistance genes detected in strains of *Salmonella enterica* serovar Muenchen, by source, in study of *Salmonella* Muenchen in poultry and humans, Israel, 2020–2023

Source	Total	No. (%) isolates with resistance genes							
		Aminoglycoside	β -lactam	Florfenicol	Sulfonamide	Tetracycline	Trimethoprim	Quinolone	Quaternary disinfectant
Poultry									
Broiler	60	58 (96.7)	12 (20)	7 (11.7)	58 (96.7)	59 (98.3)	54 (90)	35 (58.3)	58 (96.7)
Food	9	9 (100)	2 (22.2)	2 (22.2)	8 (88.9)	9 (100)	8 (88.9)	5 (55.6)	8 (88.9)
Layer	9	9 (100)	0 (0)	0 (0)	9 (100)	9 (100)	9 (100)	3 (33.3)	9 (100)
Human	21	18 (85.7)	3 (14.3)	2 (9.5)	18 (85.7)	19 (90.5)	15 (71.4)	10 (47.6)	18 (85.7)
Total	99	94 (94.9)	17 (17.2)	11 (11.1)	93 (93.9)	96 (97)	86 (86.9)	53 (53.5)	93 (93.9)

tree (Figure 2). We identified 19 acquired AMR genes. We found the combination of *aadA1*, *sul1*, *tetA*, and *qacE* genes, associated with pESI and conferring resistance to streptomycin, sulfonamides, tetracyclines, and quaternary ammonium, in 92/99 (92.9%) isolates. We found that 86/99 (86.9%) isolates harbored the *dfrA14* gene, conferring resistance to trimethoprim, and 16/99 (16.2%) isolates harbored the *bla_{TEM}* gene, conferring resistance to β -lactams. In addition, 1 isolate from a chicken carcass harbored the *bla_{OXA-808}* gene. Genetic determinants associated with quinolone resistance were present in 58/99 (58.6%) isolates. We found the *qnrB19* gene, associated with the presence of the Col (pHAD28) plasmid replicon, in 40/99 (40.4%) isolates, and the *qnrS1* gene in 13/99 (13.1%) isolates. We found the S83A or S83F mutation in the

DNA gyrase A (*gyrA*) gene in 6.1% (6/99) of isolates (Appendix 1 Figure 2; Appendix 2 Table 5). We further verified our findings using the Br19 hybrid assembly (Appendix 1). We found no significant difference in the presence of AMR genes across the different sources (Table 1). We identified the plasmid replicon IncFIB (pN55391), *hyp*_pESI, and the yersiniabactin genes *ybtP* and *ybtQ*, indicating the presence of pESI plasmid, in all but 1 strain, a human clinical isolate (Appendix 2 Table 5).

Phenotypic analysis of broiler isolates ($n = 15$) showed that all were resistant to tetracycline and sulfisoxazole (Appendix 2 Table 2). Despite a few minor exceptions, we observed an overall alignment between phenotype and the genotype (Table 2). The presence of either the *qnrB19* or *qnrS1* gene or *gyrA*

Table 2. Phenotypic resistance and presence of acquired antimicrobial resistance genes known to confer resistance in *Salmonella enterica* serovar Muenchen in study of *Salmonella* Muenchen in poultry and humans, Israel, 2020–2023*

Antimicrobial class and drug	MIC cutoff, $\mu\text{g/mL}$	No. infections/ no. tested (%)	Relevant AMR genes and point mutations present in tested isolates (no.)
Tetracyclines			
Tetracycline	16	15/15 (100)†	<i>tetA</i> (13), <i>tetA</i> + <i>tetM</i> (1)
Aminoglycosides			
Streptomycin	32	14/15 (93.3)	<i>aadA1</i> (13), <i>aadA1</i> + <i>aadA2</i> (1)
Gentamicin	16	0/15 (0)	None
Phenicol			
Chloramphenicol	32	6/15 (40.0)†	<i>floR</i> (4), <i>floR</i> + <i>cmlA1</i> (1)
Penicillins			
Ampicillin	32	7/15 (46.7)†	<i>bla_{TEM-1}</i> (5), <i>bla_{TEM-176}</i> (1)
Amoxicillin/clavulanic acid, 2:1 ratio	32/16	0/15 (0)	None
Cephalosporins			
Cefoxitin	32	0/15 (0)	None
Ceftriaxone	4	0/15 (0)	None
Folate pathway antagonists			
Sulfisoxazole	256	15/15† (100)	<i>sul1</i> (13), <i>sul2</i> (1)
Trimethoprim/sulfamethoxazole	4/76	14/15† (93.3)	<i>dfrA14</i> + <i>sul1</i> (10), <i>dfrA14</i> (1), <i>dfrA12</i> + <i>dfrA14</i> + <i>sul2</i> (1)
Quinolones			
Ciprofloxacin	0.12‡	11/15 (73.3)	<i>qnrB19</i> (7§), <i>qnrS1</i> (3), <i>gyrA</i> S83F (1), <i>qnrS1</i> + <i>gyrA</i> S83F (1)
Nalidixic acid	32	4/15 (26.7)	<i>qnrB19</i> (1), <i>qnrS1</i> (1), <i>gyrA</i> S83F (1), <i>qnrS1</i> + <i>gyrA</i> S83F (1)
Macrolides			
Azithromycin	32	0/15 (0)	None
Carbapenems			
Meropenem	4	0/15 (0)	None

*AMR, antimicrobial resistance; +, genes found simultaneously on the same isolate.

† ≥ 1 isolates exhibited phenotypic resistance without the identification of a relevant AMR gene

‡MIC cutoff for decreased susceptibility as a marker for emerging fluoroquinolone resistance (20).

§One isolate (Br04) harbored the *qnrB19* gene but was sensitive to ciprofloxacin (MIC = 0.03 $\mu\text{g/mL}$)

Table 3. Risk factors for positivity to *Salmonella enterica* serovar Muenchen in broiler flocks by chick sampling, in study of *Salmonella* Muenchen in poultry and humans, Israel, 2020–2023

Risk factor	No. positive/no. tested (%)		Odds ratio (95% CI)	p value*
	First risk group	Second risk group		
Breeder group, positive vs. control	9/42 (21.4)	9/35 (25.7)	0.79 (0.24–2.61)	0.658
Integration member,† yes vs. no	10/52 (19.2)	8/25 (32)	0.51 (0.15–1.76)	0.215
Chick age, ≥2 weeks vs. <2 weeks	4/6 (66.7)	14/71 (19.7)	8.14 (1.35–49.03)	0.024
Hatchery, positive or mixed vs. negative	14/63 (22.2)	4/14 (28.6)	0.71 (0.17–3.62)	0.728

*Calculated using Pearson χ^2 test; if values to compare were <5, Fisher exact test was used. A significance level of $\alpha = 0.05$ was applied.

†Broiler farms contracted by a company that owns breeder farms, hatcheries, feed mills, and transportation.

S83F mutation alone conferred decreased susceptibility to ciprofloxacin (MICs ≥ 0.12 and < 1 $\mu\text{g/mL}$) in 9/11 (81.8%) and clinical resistance (MIC = 1 $\mu\text{g/mL}$) in 1/11 of the tested isolates. The *gyrA* S83F mutation was associated with resistance to nalidixic acid; the presence of the *gyrA* mutation together with the *qnrS1* gene ($n = 1$) conferred clinical resistance to ciprofloxacin (MIC = 1 $\mu\text{g/mL}$).

Analysis B—*Salmonella* Muenchen Transmission in the Broiler Production Industry

We determined *Salmonella* Muenchen prevalence of 64.0% (412/644) in group C samples collected as part of the active surveillance program from heavy-breeder flocks in 2021. We estimated *Salmonella* Muenchen prevalence in broiler flocks at 61.5% (95% CI 51.5%–71.5%; 48/78 isolates). We found no significant difference ($p = 0.83$) in prevalence between the broilers descendent from positive (26/43) and control (22/35) breeding groups. Moreover, we recovered *Salmonella* Muenchen isolates in 8/10 (80.0%) of the broiler barns in which it was previously detected, 15–20 months after the initial sampling; we referred to the poultry in those barns as repeat broilers.

We found no significant association between positivity in the breeder farm and detection of *Salmonella* Muenchen in chick samples (Table 3) or environmental swab samples collected in the barn (Table 4) and around the barn (Table 5). Positivity in the chicks was significantly associated with older age of chicks (OR = 8.1; $p < 0.05$). Positivity in the interior swab samples was associated with positivity in the exterior swabs collected at the same time (OR = 123; $p < 0.01$). In addition, positivity in the exterior swab samples

was associated with sampling time, sampling after versus before marketing (OR = 8.25; $p < 0.05$).

Clustering of strains collected from parent breeding flocks and the descendant broiler flocks was not evident in the reconstructed phylogeny tree (Figure 3). In addition, we observed no genetic similarity between grandparents, pullets, hatcheries, and the breeder flocks, except for an instance of an isolate from a pullet flock (Br46) persisting (difference of 2 single-nucleotide polymorphisms [SNPs]) in the same flock when we sampled it 10 months later as a heavy-breeder flock (Br21). We observed evidence of clustering of samples collected by various sampling techniques (chicks, interior swabs, and exterior swabs) from the same barn in 7/13 barns (Figure 3). Repeat sampling of the broiler barns produced isolates that were closely related in 6/10 (60.0%) of the cases; 4 of those were highly genetically similar (4–8 SNPs) to the previous isolates from the same farm (Figure 3).

Although the samples we collected in various production stages were genetically heterogeneous, we found the frequencies of AMR gene presence in different production stages to be similar, based on a limited number of samples compared between sources (Table 6). We found similar frequencies of AMR genes in the positive and control groups ($p > 0.05$) (Table 7).

Analysis C—Global Distribution of *Salmonella* Muenchen Harboring pESI

We reconstructed a maximum-likelihood phylogenetic tree from 11 isolates chosen from our study and 125 isolates from elsewhere globally (Appendix 1 Figure 3). We noted that *Salmonella* Muenchen was

Table 4. Risk factors for positivity to *Salmonella enterica* serovar Muenchen in broiler flocks by drag swab sampling taken from inside broiler barns in study of *Salmonella* Muenchen in poultry and humans, Israel, 2020–2023

Risk factor	No. positive/no. tested (%)		Odds ratio (95% CI)	p value*
	First risk group	Second risk group		
Breeder group, positive vs. control	18/25 (72)	18/24 (75)	0.86 (0.20–3.67)	0.812
Integration member, yes vs. no	26/36 (72.2)	9/12 (75)	0.87 (0.13–4.53)	1
Time of sampling				
During evacuation vs. before	17/23 (73.9)	8/10 (80)	0.71 (0.06–5.31)	1
After evacuation vs. before	11/16 (68.8)	8/10 (80)	0.55 (0.04–4.64)	0.668
Exterior swab, positive vs. negative	20/20 (100)	6/25 (24)	∞ (10.45– ∞)	<0.001

*Calculated using Pearson χ^2 test; if values to compare were <5, Fisher exact test was used. A significance level of $\alpha = 0.05$ was applied.

Table 5. Risk factors for positivity to *Salmonella enterica* serovar Muenchen in broiler flocks by drag swab sampling taken from the exterior environment of broiler barns in study of *Salmonella* Muenchen in poultry and humans, Israel, 2020–2023

Risk factor	No. positive/no. tested (%)		Odds ratio (95% CI)	p value*
	First risk group	Second risk group		
Breeder group, positive vs. control	14/25 (56)	12/21 (57.1)	0.95 (0.25–3.58)	0.938
Integration member, yes vs. no	18/35 (51.4)	8/11 (72.7)	0.40 (0.06–2.06)	0.302
Time of sampling				
During evacuation vs. before	12/22 (54.6)	2/8 (25)	3.60 (0.47–42.40)	0.226
After evacuation vs. before	11/15 (73.3)	2/8 (25)	8.25 (0.87–105.55)	0.039
Interior swab, positive vs. negative	20/26 (76.9)	0/19 (0)	∞ (10.45–∞)	<0.001

*Calculated using Pearson χ^2 test; if values to compare were <5, Fisher exact test was used. A significance level of $\alpha = 0.05$ was applied.

genetically diverse globally, represented by 11 different STs in our analysis. We distinguished 2 main genetic subpopulations; 1 genetic subpopulation consisted of ST83 ($n = 38$), and the other contained predominantly ST112 ($n = 25$) and ST82 ($n = 49$). ST83 was detected in the United States, Canada, and the United Kingdom from both human and food-animal sources. ST112 had a broader distribution, including strains from the United States, Canada, Mexico, United Kingdom, and Australia. We identified pESI exclusively in ST82 isolates. ST82, which also included isolates without pESI, was found in most of the countries represented. The pESI-positive ST82 isolates formed a clade and included all the isolates from our study and an additional 18 strains from other countries including the United States ($n = 12$), United Kingdom ($n = 5$), and South Africa ($n = 1$). Apart from the isolates collected in Israel representing both human and poultry sources, pESI-positive isolates were from human samples ($n = 15$) or from isolates from the NCBI database that lacked detailed information about source ($n = 3$) (Appendix 2 Tables 4, 6).

Discussion

Salmonella Muenchen has become the dominant serovar in Israel; a surge in human clinical cases was associated with an increased prevalence in the poultry industry (7). We demonstrated genetic similarity, including the presence of the pESI megaplasmid, in isolates collected from poultry and chicken meat and from humans. Furthermore, we estimated a high prevalence (61.5% [95% CI 51.5%–71.5%]) of the emerging *Salmonella* Muenchen in broilers, and by tracing transmission pathways within the broiler industry we demonstrated that horizontal transmission plays a major role in its spread. Finally, we found that the emerging multidrug-resistant *Salmonella* Muenchen strain in Israel closely resembles pESI-positive *Salmonella* Muenchen isolates from other countries, underscoring the potential for clonal expansion and the risk to public health.

Various sources, such as food (21) and animal hosts (22), are associated with NTS infections, and

circulation between animal hosts, which could potentially form additional transmission pathways to humans, have been described previously (23). *Salmonella* Muenchen has been detected in a wide range of foods of animal origin, including beef, seafood, and pork (24), and in plant-based foods (25). Chicken meat has rarely been documented as a source of infection; the last reported outbreak in the United States occurred in 2015 (26). However, our findings strongly suggest that poultry broilers are an important source of human infections caused by *Salmonella* Muenchen in Israel. The high genetic similarity between strains collected from broiler flocks and layer flocks may indicate that eggs serve as an additional source for human infections, however their role as a route of transmission to humans needs to be further explored.

Controlling *Salmonella* Muenchen in the poultry industry in Israel will be crucial to curb the incidence of infections in the human population. Vaccinating breeder flocks can be effective (2), assuming that the transmission of the pathogen is mainly vertical from the parent to the production flocks. A previous study (27) examining the transmission of *Salmonella* within vertically integrated poultry operations suggested, on the basis of serologic and molecular methods, that transmission pathways are varied and might depend on the serotype; certain serotypes, including *Salmonella* Muenchen, transmitted horizontally to broiler flocks after hatching. Our findings, supported by advanced large-scale genetic analysis, further support that route of transmission in the emerging *Salmonella* Muenchen spread. Our conclusion is based on the lack of clustering of isolates from broiler flocks with their parent breeding flocks and lack of significant risk for *Salmonella* Muenchen positivity in broiler flocks descending from *Salmonella* Muenchen-positive breeder flocks. Additional findings supported on-site infection and horizontal transmission, such as the significantly increased risk for *Salmonella* Muenchen positivity in older chicks, genetic clustering of isolates within and between broiler farms, and evidence of strains persisting in broiler barns over time. Therefore, we suspect that the initiation of mandatory vaccination of breeder

flocks in Israel against *Salmonella* Muenchen as of March 2024 (Bulletin notice, Ministry of Agriculture, 2023 Aug) may result in a limited reduction in the prevalence of *Salmonella* Muenchen, because *Salmonella* persists in the farm soil or barn litter (28). Other

possible measures that can be employed on the farms or along the production chain (e.g., during transport) to decrease the prevalence of *Salmonella* Muenchen include increasing biosecurity by different measures, such as controlled farm access and thorough cleaning

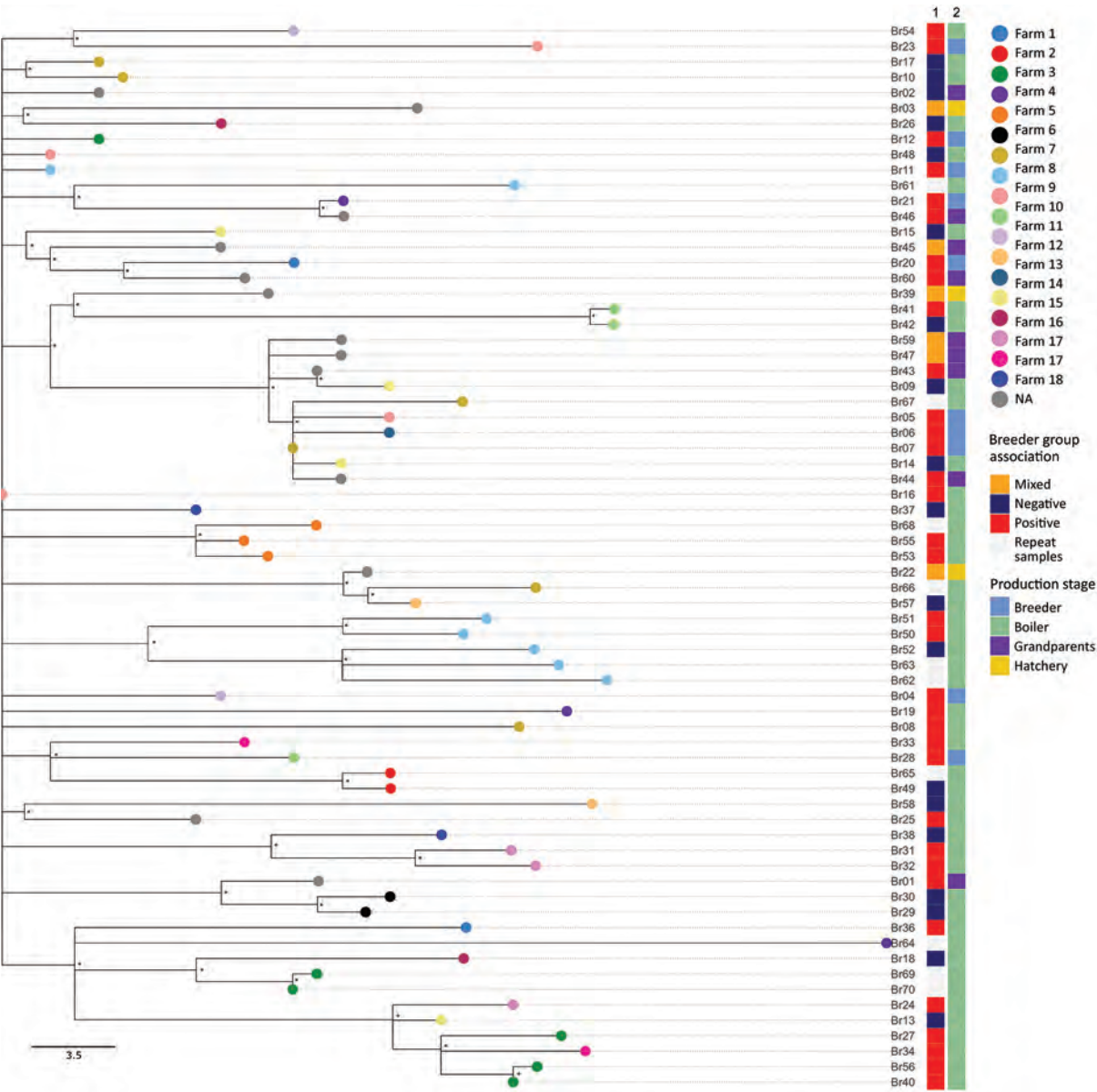


Figure 3. Maximum-likelihood tree reconstructed using whole-genome sequences of *Salmonella enterica* serovar Muenchen isolates collected from the various stages of the broiler production industry in study of *Salmonella* Muenchen in poultry and humans, Israel, June 2020–June 2023. We used a hybrid assembly of *Salmonella* Muenchen sequence type 82 (Br19) strain as a reference genome and used *Salmonella* Muenchen sequence type 112 (National Center for Biotechnology Information Sequence Read Archive accession no. SRR6222324) to root the tree (not shown). The analysis included 874 single-nucleotide polymorphisms (SNPs) in the alignment (0–61 pairwise SNPs, median 30). Tip colors indicate the commercial broiler farm either where barns were tested (in the case of broiler samples), or the commercial broiler farm to which the breeder flock supplied (breeder samples). Asterisks (*) indicate nodes with >70% bootstrap support. Column 1 indicates data for the breeder group to which the broiler flocks were related (positive or control/negative). We labeled hatcheries and grandparent flocks as mixed if they received/supplied chicks from/to both positive and control heavy breeder farms. Column 2 indicates data for the production stage from which the sample was collected. Scale bar represents SNP difference.

Table 6. Acquired antimicrobial resistance genes detected in strains of *Salmonella enterica* serovar Muenchen collected in the broiler industry, by production stage, in study of *Salmonella* Muenchen in poultry and humans, Israel, 2020–2023

Production stage	No. (%) isolates with resistance genes								
	No.	Aminoglycoside	β -lactam	Florfenicol	Sulfonamide	Tetracycline	Trimethoprim	Quinolones	Quaternary disinfectant
Grandparent and pullets	9	8 (88.9)	1 (11.1)	0	8 (88.9)	9 (100)	8 (88.9)	3 (33.3)	8 (88.9)
Breeder	10	10 (100)	1 (10)	0	10 (100)	10 (100)	8 (80)	6 (60)	10 (100)
Hatchery	3	3 (100)	0	0	3 (100)	3 (100)	3 (100)	0	3 (100)
Broiler	38	37 (97.4)	10 (26.3)	7 (18.4)	37 (97.4)	37 (97.4)	35 (92.1)	26 (68.4)	37 (97.4)
Broiler repeat	10	9 (90)	3 (30)	3 (30)	9 (90)	10 (100)	10 (100)	7 (70)	9 (90)
Total	70	67 (95.7)	15 (21.4)	10 (14.3)	67 (95.7)	69 (98.6)	64 (91.4)	42 (60)	67 (95.7)

and disinfection between flocks. Sources for *Salmonella* contamination, including poultry feed and pests (29–31), may also be controlled. In addition, techniques such as fecal microbiota transplants to chicks to prevent colonization of *Salmonella* spp. via competitive exclusion and the use of probiotics are potential methods to reduce gastrointestinal colonization (31,32). Increased surveillance and control of this serotype along the production chain, such as during transport, in the slaughterhouse, and during processing, might also reduce transmission to the human consumer.

The public health risk associated with transmission of multidrug-resistant *Salmonella* is considered one of the biggest threats to global health (33). Surveillance data from the Ministry of Health reported that 30/77 (39.0%) of *Salmonella* Muenchen isolates collected in 2021 from humans and 21/41 (51.2%) from nonhuman sources had reduced susceptibility to ciprofloxacin (7), which is critical for treatment of invasive salmonellosis in humans (34). In our study, 11/21 (52.4%) of human and 47/78 (60.3%) of poultry isolates contained chromosomal point mutations on the *gyrA* gene, or harbored *qnr* genes known to be involved in conferring resistance to quinolones (35). Phenotypic testing revealed that all isolates exhibiting decreased susceptibility or resistance to ciprofloxacin had ≥ 1 of those resistance determinants present. The presence of the transmissible *bla*_{OXA-808} gene in an isolate recovered from poultry meat was unusual. That gene, belonging to the *bla*_{OXA-23} family, a group of carbapenem-hydrolyzing β -lactamases, was previously reported in *Acinetobacter* species (36). Although *Salmonella* has been known to harbor *bla*_{OXA} genes (37), this specific variant had not been previously reported. Potential carbapenem resistance is an alarm-

ing finding because carbapenem is a broad-spectrum antimicrobial drug reserved as a last-resort treatment for bacterial infections in humans (34).

Phenotypic resistance aligned with genotype with a few exceptions: tetracycline, chloramphenicol, ampicillin, sulfisoxazole, and trimethoprim/sulfamethoxazole. We observed 3 MDR isolates exhibiting resistance to ≥ 1 of those antimicrobial drugs with no known AMR gene present. Such discordances may be the result of various potential mechanisms, such as AMR determinants yet to be described (38).

The surge in prevalence of *Salmonella* Muenchen in poultry since 2018 correlated with a marked increase in the frequency of pESI-positive isolates: from 146 *Salmonella* Muenchen isolates collected by the EPB during 2009–2021, pESI was detected in only 3/50 (6.0%) of isolates before 2018 but in 85/96 (88.5%) of isolates since January 2018 (E. Elnkave, unpub. data). In this study, we demonstrated the continuation of the trend; 100.0% (88/88) of poultry isolates harbored the plasmid. The pESI plasmid provides fitness advantages to the bacterial host, such as increased tolerance to mercury and oxidative stress, and AMR (9). Those advantages might contribute to the high prevalence and spread of the pESI-harboring *Salmonella* Muenchen; further studies are required to determine which of the specific advantages and under what conditions enable *Salmonella* Muenchen propagation in the broiler industry in Israel.

We found that the Israel *Salmonella* Muenchen strains were genetically similar to strains collected in the United Kingdom, South Africa, and the United States. All belonged to ST82 and harbored pESI. Such clonality might suggest travel-associated transmission of *Salmonella* Muenchen or might be a result of a

Table 7. Acquired antimicrobial resistance genes detected in strains of *Salmonella enterica* serovar Muenchen collected in broiler flocks, by parent breeder flock status, in study of *Salmonella* Muenchen in poultry and humans, Israel, 2020–2023

Broiler group	No. (%) isolates with resistance genes								
	No.	Aminoglycoside	β -lactam	Florfenicol	Sulfonamide	Tetracycline	Trimethoprim	Quinolones	Quaternary disinfectant
Positive	20	19 (95)	8 (40)	4 (20)	19 (95)	19 (95)	19 (95)	16 (80)	19 (95)
Control	18	18 (100)	2 (11.1)	3 (16.7)	18 (100)	18 (100)	16 (88.9)	10 (55.6)	18 (100)
Total	38	37 (97.4)	10 (26.3)	7 (18.4)	37 (97.4)	37 (97.4)	35 (92.1)	26 (68.4)	37 (97.4)

common origin and global dissemination of contaminated poultry breeding stock, as suggested previously with this serotype (8) and others (39). In addition, the strain was recently reported from the West Bank (40), which suggests regional spread. The relatively rare identification of pESI in strains other than *Salmonella* Infantis ST32 and *Salmonella* Muenchen ST82 (18) might indicate an adaptation mechanism to the plasmid that has not been determined. Given the vast global spread of *Salmonella* Infantis harboring pESI (11) since its detection (9), it is highly possible that a pESI-harboring *Salmonella* Muenchen ST82 will be the next *Salmonella* to emerge globally.

Acknowledgments

We thank the poultry farm owners and poultry veterinarians for their collaboration.

The Hebrew University of Jerusalem internal fund supported this work in a seed fund to E.E. The Israeli Ministry of Agriculture supported the work (grant no. 12-11-0001 to E.E., A.W. and A.R.).

Raw reads from isolates sequenced in this study (n = 109) are available in the Sequence Read Archive database of NCBI under Bioproject PRJNA1192251, accession nos. SRR31558526–SRR31558634 (short reads) and SRR31558786 (long reads). Metadata and accession numbers are provided in Appendix 2 Table 4.

Author contributions: E.E. and A.W. initiated and designed the study. A.W., C.S., Y.P., G.M., I.M., E.Y., and A.R. contributed samples and data. K.A., T.R., and J.P. performed laboratory work and data analysis. T.R. and J.P. collected samples. J.P. drafted the manuscript. All authors contributed to the scientific discussion and have read and approved the final manuscript.

About the Author

Dr. Perry is a PhD student specializing in molecular epidemiology, focusing on the epidemiology and genetic characterization of emerging zoonotic pathogens. Her research on *Salmonella* serotypes contributes to preventing future infections, improving public and animal health and welfare.

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Bat-Associated Hemotropic Mycoplasmas in Immunosuppressed Children, Spain, 2024

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We report the detection of hemotropic mycoplasmas in 4 immunosuppressed pediatric patients in Spain: 2 solid organ transplant recipients, 1 hematopoietic stem cell transplant recipient, and 1 cancer patient. Sequences were 100% identical to a strain previously identified in *Miniopterus schreibersii* bats, which raises concerns about unnoticed zoonotic transmission.

Hemotropic mycoplasmas (hemoplasmas) are small, cell-wall-deficient bacteria that attach to erythrocyte surfaces and can induce hemolytic anemia in mammals, particularly in immunocompromised hosts (1). Species such as *Mycoplasma hemofelis* in cats and *M. ovis* in sheep are well documented in veterinary medicine (1). Human cases, although rare, have been reported in association with immunosuppression or zoonotic exposure (2–4). Molecular evidence has revealed human infections with multiple hemoplasma species, including *Candidatus Mycoplasma hematominutum* and *M. ovis* (4). *Candidatus Mycoplasma hematomominis*, a likely bat-origin species, has been reported in human patients from New Caledonia (5), Japan (6), France (7), and the United Kingdom (8); the cases in France and the United Kingdom likely originated in Australia. *Candidatus M. hematomominis*, which causes an illness called flying fox hemolytic fever, is present in *Pteropus* bats;

reported overall prevalence is 40% (5). Clinical signs in humans typically were anemia, fever, asthenia, abdominal pain, and weight loss (6–9).

In a previous study (10), zoonotic pathogens were investigated in immunosuppressed children and their companion animals in Spain. That cross-sectional study was performed at a national reference center for pediatric transplantation and immunocompromised children. In this study, we included patients <20 years of age in Spain who lived in a house that owned dogs or cats if they had received a solid organ or a hematopoietic stem cell transplantation or if they had an inborn error of immunity or an oncologic or rheumatologic disease for which they were undergoing immunosuppressive treatments or chemotherapy. We contacted patients and families who fulfilled the inclusion criteria by telephone or in person during a visit to the hospital. We used stored blood samples to investigate the presence of hemotropic mycoplasmas by molecular methods. The local Clinical Research Ethics Committee of La Paz University Hospital (PI-4770) approved the study, and all participants or legal guardians provided informed consent.

The Study

We analyzed 69 EDTA-anticoagulated blood samples collected in 2024 from immunocompromised pediatric patients using real-time PCR targeting a 366-bp fragment of the hemoplasma 16S rRNA gene (11). We used sterile deionized water as a negative control; we included an internal positive control to *M. hemocanis*, obtained from a blood sample previously tested and sequenced, in each run. We Sanger sequenced positive samples using both PCR primers and analyzed the sequences by using BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). We constructed a maximum-likelihood phylogenetic tree using MEGA version 12 (<https://www.megasoftware.net>). The analysis was based on a 329-bp fragment of the 16S rRNA gene (primers

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DOI: <https://doi.org/10.3201/eid3110.250862>

excluded), with 500 bootstrap replicates to assess branch support. We selected the Tamura-Nei model with gamma distribution and a proportion of invariant sites as the best-fit nucleotide substitution model in accordance with the Bayesian Information Criterion.

Four samples (5.8% [95% CI 0.3%–11.3%]) tested positive. All 4 were from immunosuppressed children; 1 had chemotherapy-induced bone marrow aplasia (Table). Of the 4 patients, 3 had a history of multiple blood transfusions during their treatment: patient 2 received 3 red blood cell transfusions in 2022; patient 3 received 6 red blood cell and 9 plasma transfusions in 2019; and patient 4 received 30 red blood cell transfusions, 37 platelet pools, and 1 plasma transfusion during 2019–2020. Patient 1 did not receive any blood transfusions. All 4 patients had dogs as companion animals.

The sequences from the 4 children's samples were 100% identical and matched a previously reported strain from Schreiber's bats (*Miniopterus schreibersii*) in Spain (12). We deposited the sequence we isolated into GenBank (accession no. PV698513). BLAST analysis showed 97.5% identity with *Candidatus* *M. hemohominis*. Phylogenetic analysis clustered those sequences within a clade of bat- and human-associated hemoplasmas (Figure).

Although all 4 patients lived with household pets, we found no evidence that their animals were the source of infection. The main hemoplasma species in dogs is *M. hemocanis*, genetically distinct from the hemoplasmas we detected (4). In contrast, the sequences we detected are related to *Candidatus* *M. hemato-hominis*, previously associated with bats and humans (5–9).

All other related sequences were from bat hosts. Of note, the observed 97.5% sequence identity is below the levels typically reported among members of the same hemoplasma species, which suggests the existence of a distinct taxon. Longer fragments of the 16S rRNA gene or additional targets such as the *rpoB* gene would improve taxonomic resolution. In our study, the high cycle threshold values (>32) of the positive samples hindered the successful amplification of the full-length gene. Nevertheless, a previous study (12) reported consistent classification outcomes when comparing short- and full-length 16S rRNA gene sequences, supporting the hypothesis that the hemoplasmas we detected might represent a distinct species within the broader *M. hemato-hominis*-like clade. The zoonotic potential of this group, including genetically divergent but closely related variants, warrants further investigation.

Unlike previously reported cases that were typically associated with wildlife exposure or rural environments, 2 of our patients lived in urban areas. Moreover, none of the patients had known contact with wildlife or recent travel history, and they lived up to 500 km apart from each other, which suggests not only the possibility of domestic or peridomestic reservoirs or vectors but also that these hemotropic mycoplasmas are widely distributed in the country. Schreiber's bats are widespread in Spain; they roost primarily in caves, although they are also known to use abandoned buildings, tunnels, and cellars as shelters, which could cause indirect human exposure (12). Seasonal movements of Schreiber's bats can span 300 km. The prevalence of hemoplasmas in this species has been

Table. Characteristics of mycoplasma-positive patients in study of bat-associated hemotropic mycoplasmas in immunosuppressed children, Spain, 2024*

Characteristic	Patient no.			
	1	2	3	4
Age, y/sex	17/F	2/F	4/M	13/M
Region of origin (type)	Zaragoza (urban)	Orense (rural)	Estremera (rural)	Madrid (urban)
Underlying pathology	Papillorenal syndrome, renal transplantation	Rhabdomyosarcoma, chemotherapy	Alagille syndrome, liver transplantation	Acute myeloid leukemia, hematopoietic stem cell transplantation
Mycoplasma detection, Ct	33.95	32.47	33.44	34.17
Blood parameters				
Anemia diagnosis†	No	Yes	No	No
Type of anemia	None	Aplastic anemia‡	None	None
Hb, g/dL	11.8	8.5	12.7	14.9
Hct, %	39.4	26.7	40.8	44.8
Hb after 4 mo, g/dL	11.9	9.3	12.8	14.9
Hct after 4 mo, %	37.4	29	42.2	44
Leukocytes <5,000	No	No	830/mm ³	No
Splenomegaly†	No	No	No	No
Biochemical parameters				
LDH elevation†	No	No	No	No
Bilirubin elevation†	No	No	No	No

*Ct, cycle threshold; Hb, hemoglobin; Hct, hematocrit; LDH, lactate dehydrogenase.

†Hematologic data correspond to the day the PCR-positive blood sample was collected.

‡Secondary effect of chemotherapy.

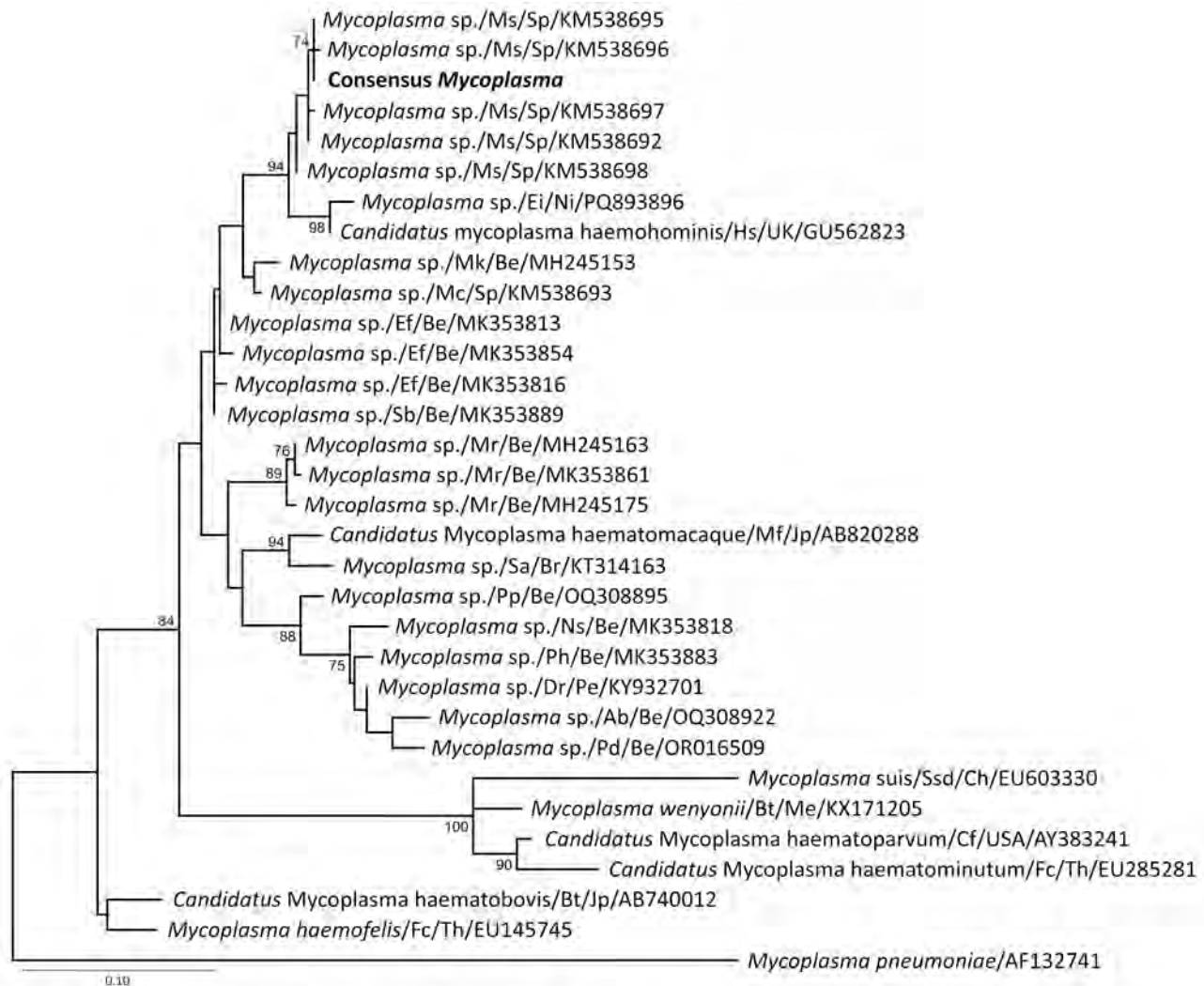


Figure. Phylogenetic analysis of sequences from samples collected in 2024 from mycoplasma-positive pediatric patients in study of bat-associated hemotropic mycoplasmas in immunosuppressed children, Spain, 2024. Bold indicates sequence from this study. Numbers at the nodes represent bootstrap support values; values >75% are not shown. GenBank accession numbers are indicated for reference sequences. Scale bar indicates nucleotide substitutions per site. Hosts are indicated as follows: Ab, *Artibeus phaeotis* (pygmy fruit-eating bat); Bt, *Bos taurus* (cattle); Cf, *Canis familiaris* (dog); Dr, *Desmodus rotundus* (common vampire bat); Ef, *Eptesicus furinalis* (Argentine brown bat); Ei, *Eidolon* sp. (palm bat); Fc, *Felis catus* (cat); Hs, *Homo sapiens* (human); Mc, *Myotis capaccinii* (long-fingered bat); Mf, *Macaca fuscata* (Japanese macaque); Mk, *Myotis keaysi* (hairy-legged myotis bat); Mr, *Molossus rufus* (black mastiff bat); Ms, *Miniopterus schreibersii* (bent-wing bat); Ns, *Natalus stramineus mexicanus* (Mexican funnel-eared bat); Pd, *Phyllostomus discolor* (pale spear-nosed bat); Pp, *Pteronotus parnellii* (Parnell's mustached bat); Ph, *Platyrrhinus helleri* (Heller's broad-nosed bat); Sa, *Sapajus apella* (tufted capuchin); Sb, *Saccopteryx bilineata* (greater sac-winged bat); Ssd, *Sus scrofa domesticus* (domestic pig). Countries are indicated as follows: Be, Belize; Br, Brazil; C, China; Jp, Japan; Me, Mexico; Ni, Nigeria; Pe, Peru; Sp, Spain; Th, Thailand; UK, United Kingdom.

reported as high as 97% (12). Those factors could explain the detection of the hemoplasma in children living 500 km apart. Because studies have detected 16S rRNA gene sequences with high (>99.5%) nucleotide identity to known hemoplasmas in ectoparasites such as ticks and sand flies collected from bats (9,13), vectorborne transmission of hemoplasmas has been hypothesized; however, our patients had no reported arthropod contact. Previous cases of *M. ovis* and *Candidatus M.*

haematoparvum infection in humans also lacked known arthropod exposure (14), indicating potentially complex transmission routes.

All hemoplasma-positive cases in our study were in severely immunocompromised children, a group particularly vulnerable to emerging infections. Although direct clinical consequences were unclear, 1 patient experienced bone marrow aplasia, likely attributable to chemotherapy.

Conclusions

Hemoplasmas are known to persist in the bloodstream for extended periods (15). Although we observed no direct clinical effect in this study, the unexpected detection of bat-related hemoplasmas in immunosuppressed patients raises questions about their potential for transfusion-associated or vectorborne transmission. We emphasize that PCR detection alone does not indicate viability. In addition, the 4 positive samples showed high cycle threshold values, suggesting low DNA copy numbers compatible with trace levels or nonviable infections. Because we did not test organ, hematopoietic stem cell, or blood donors related to the patients, we cannot assess the possibility of transfusion- or transplant-associated transmission. Further investigation could determine whether these organisms can withstand blood processing and storage, and whether they might pose any risk for transmission under specific conditions.

In summary, our study presented molecular evidence of bat-related hemotropic mycoplasmas in immunosuppressed children from Spain who have pets in a setting without reported wildlife contact or travel history. Our findings highlight the need for increased surveillance of emerging zoonotic infections, especially in high-risk populations.

This study was supported by the Health Institute Carlos III, Fondo de Investigaciones Sanitarias-Spanish Health Research Fund (grant no. PI23CIII/00917).

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Detection of Monkeypox Virus Clade Ib DNA in Wastewater Solids at Wastewater Treatment Plants, United States

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We used a sensitive and specific PCR to detect monkeypox virus clade Ib DNA from >3,000 wastewater samples collected prospectively across the United States. Monkeypox virus clade Ib DNA was detected in 1 sample from a location with a confirmed case; it was not detected in locations with no confirmed cases.

Monkeypox virus (MPXV), which causes mpox, is divided into 2 clades, I and II (1). MPXV clade I (now Ia) is endemic in Central Africa and causes mpox outbreaks primarily in children, (1) but in 2024, a new subclade, clade Ib, was identified in the Democratic Republic of the Congo (1). Clade Ib has spread rapidly since its emergence, primarily among adults through sexual contact (1). As of January 1, 2024, nearly 36,000 mpox clade Ib cases and >100 deaths have been confirmed in Central and Eastern Africa (2). Since February 12, 2025, at least 4 travel-associated mpox clade Ib cases have been confirmed in the United States, 1 each in California (3), Georgia (2), New Hampshire (4), and New York (5). Outbreaks of clade I mpox have previously been associated with a higher case-fatality rate than for clade II, but data to establish the severity of clade Ib during the ongoing outbreak are limited (1).

MPXV clade II DNA can be detected in wastewater solids, and its concentrations correlate to community incident cases of infections (6). Because MPXV clade II DNA is shed in saliva, feces, urine, and semen

and from skin lesions of infected persons (7), it might end up in wastewater (8).

We have been prospectively measuring MPXV clade II DNA across the United States for use by public health organizations to identify outbreaks (9). Recent work has established that MPXV clade I DNA is shed via excretions that enter wastewater (10). We aimed to test if MPXV clade Ib DNA can be detected in wastewater solids to provide information on potential spread.

The Study

We used a previously published and validated MPXV clade Ib hydrolysis-probe PCR (11) (Table) in a drop-let digital PCR format. We confirmed the specificity and sensitivity of the assay in vitro and in silico (Appendix, <http://wwwnc.cdc.gov/EID/article/31/10/25-0270-App1.pdf>).

We retrospectively applied the assay to 10 wastewater samples (Appendix). The 10 samples were collected from a wastewater treatment plant (WWTP) serving ≈750,000 people in San Francisco, California, USA. MPXV clade II DNA was previously detected at both high and low concentrations in those samples, and the samples were collected at a time when MPXV clade Ib was not known to be circulating in the United States (Appendix). Samples were stored at –80°C as purified nucleic acids for 1–3 years before analysis. We obtained the nucleic acids by using previously published methods (9) (Appendix). We confirmed limited degradation of nucleic acids during storage by also measuring SARS-CoV-2 RNA concentration and comparing the measurements to those obtained from the same samples before storage (Appendix). MPXV clade Ib DNA concentrations were nondetectable in the 10 samples, supporting the specificity of the molecular assay.

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DOI: <https://doi.org/10.3201/eid3110.250270>

We began prospective measurements of MPXV clade Ib DNA in samples from 147 WWTPs located in 40 states (Figure). Samples were collected during November 22, 2025–January 31, 2025, typically 3 times/week. Full descriptions of WWTP locations and the number of samples and the time during which they were collected are provided (Appendix). Per WWTP, 11–71 samples were tested (median 21 samples/WWTP).

We conducted sampling as previously described (9) (Appendix). After collection, we stored samples at 4°C, shipped them to the laboratory on ice, and processed to completion within 48 hours of receipt at the laboratory. We do not expect the time between sample collection and analysis to affect target quantification because our work and previously published studies (12–14) show limited decay of the short length nucleic-acid targets over multiple weeks at 4°C. We collected and analyzed a total of 3,292 samples.

We processed samples and extracted and purified their nucleic acids from wastewater solids using previously described methods (9) (Appendix). We measured concentrations of MPXV clade Ib DNA by using droplet digital PCR on 6–10 replicates for each

Table. Monkeypox virus clade Ib probe and primers used to detect mpox in wastewater solids at wastewater treatment plants, United States

Probe or primer	Sequence, 5'→3'
Probe	ATATTCAGGCGCATATCCACCCACGT
Forward primer	AAGACTTCCAACTTAATCACTCCT
Reverse primer	CGTTTGATATAGGATGTGGACATT

sample. We ran extraction and PCR positive and negative controls on each 96-well plate. We merged results from replicate wells for analysis. Concentrations are presented as copies per gram of dry weight. For a sample to be scored as a positive, there had to be ≥ 3 positive droplets. The lowest measurable concentration was $\approx 1,000$ copies/g (3 positive droplets). We reported errors as SDs of the measurements obtained from QX Manager software version 2.0 (Bio-Rad Laboratories, <https://www.bio-rad.com>). Data are available through the Stanford Digital Repository (<https://doi.org/10.25740/vk458br0887>).

Only 1 sample of 3,292 tested positive. In the positive sample, the concentration of MPXV clade Ib DNA was 1,174 copies/g (68% CI 674–1,875 copies/g; 4 positive droplets). The positive sample was obtained from a sewershed in California, USA, serving

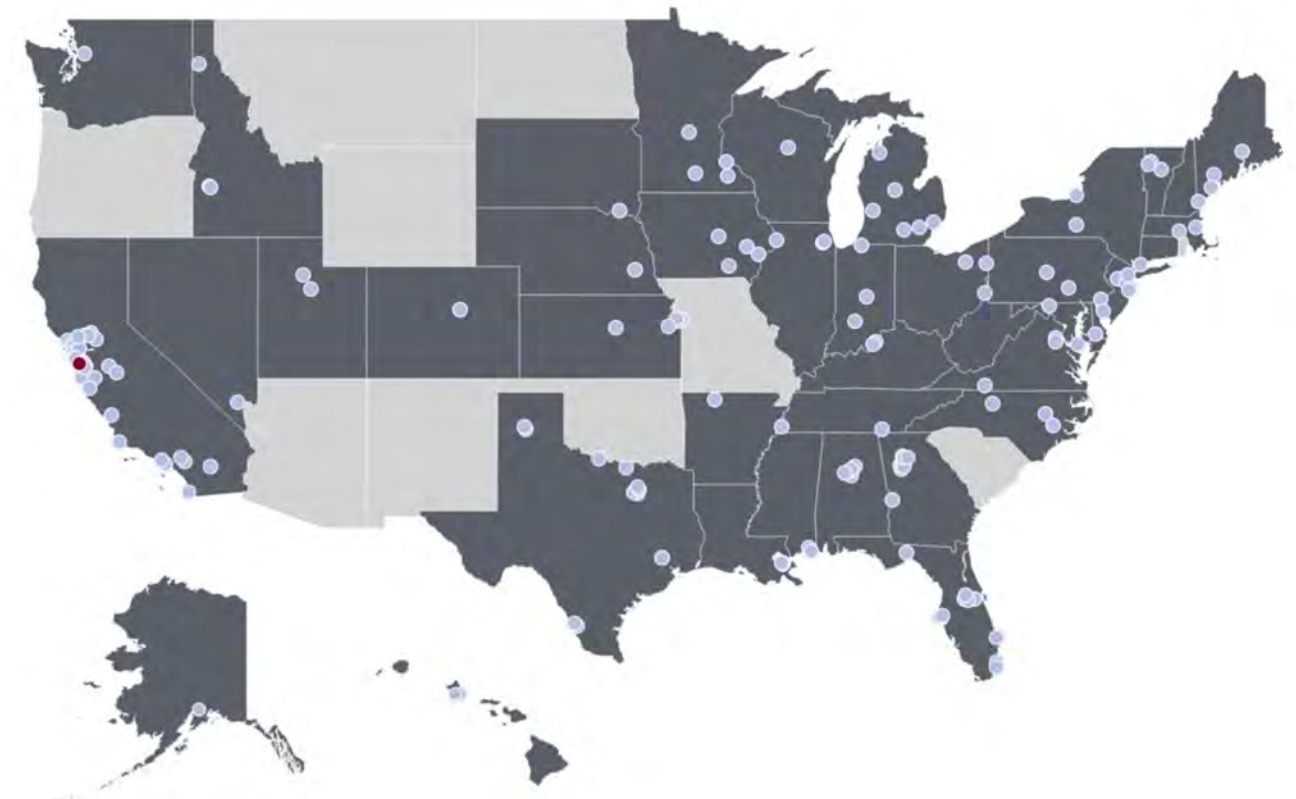


Figure. Locations of wastewater treatment plants (WWTPs) enrolled in study to detect monkeypox virus (MPXV) clade Ib in wastewater solids at WWTPs, United States. Dark gray shading indicates states with enrolled sites. The red dot represents the location of the WWTP where there was a positive detection of MPXV clade Ib ($n = 1$), and the light blue dots represent participating WWTPs where no samples were positive for MPXV ($n = 146$).

≈199,000 persons, including a person with confirmed clade Ib mpox (3). The positive sample was collected on December 16, 2024, twenty-nine days after a news story broke of a travel-associated infection in the community. Sewer transit times are <1 day. MPXV clade I DNA shedding from infected patients via skin, feces, saliva, and mucus can persist for >20 days after symptom onset (10). It is therefore possible that DNA from excretions of the infected person led to the positive detection.

A total of 3,291 samples were negative for MPXV clade Ib DNA, consistent with a lack of known cases across the United States. A negative sample is defined as a sample with <3 positive droplets across all generated droplets for that sample. Negative controls and positive controls in this study performed as expected (Appendix). There have also been confirmed cases of MPXV clade Ib infections in Georgia, New Hampshire, and New York. However, according to news reports, those persons were not expected to be within a WWTP sewershed included in this study, and the New Hampshire and New York cases were first reported after the last sample in this study was collected.

Conclusions

In this study, only 1 wastewater sample was positive for MPXV clade Ib DNA, and that sample was from a location where there was a known, travel-associated case. Samples from the same location before and after the positive sample were negative, and no secondary cases were reported. Variability in concentrations of rare nucleic acid targets in wastewater should be expected and is caused by many factors. Wastewater MPXV DNA concentrations might vary temporally because of variability in shedding by infected persons, movement of people into and out of the sewersheds, or deliveries of septic wastes to the system. In addition, spatial heterogeneities in MPXV DNA within the solid matrix might exist. To address variability, we processed high frequency samples and ran 6–10 replicates and numerous controls to ensure high quality measurements.

This study establishes methods for measuring MPXV clade Ib DNA in wastewater solids for surveillance of MPXV clade Ib infections. Results from our analysis of wastewater solids samples are consistent with information on the presence and absence of MPXV clade Ib infections in the included WWTP sewersheds during the study, and the results suggest wastewater monitoring for MPXV clade Ib might be useful to monitor disease outbreaks. The methods used to measure MPXV clade Ib could be used to monitor MPXV clade Ia as well.

Acknowledgments.

We thank the participating wastewater treatment plants for their samples for the project.

This work was supported by a gift to A.B.B. from the Sergey Brin Family Foundation.

B.H. and D.D. are employees of Verily Life Sciences, LLC. B.J.W. is an employee of Google LLC and was an employee of Verily Life Sciences, LLC, when this work was conducted.

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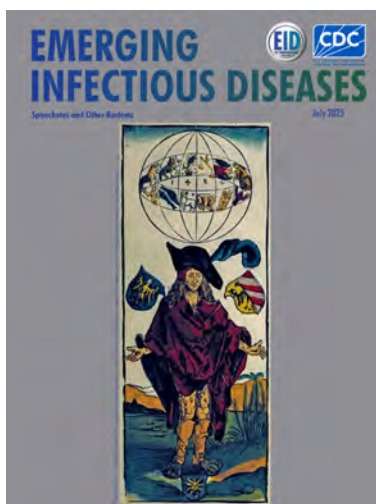
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July 2025

Spirochetes and Other Bacteria

- Human *Streptococcus suis* Infections, South America, 1995–2024
- Systematic Review of Contact Investigation Costs for Tuberculosis, United States
- Assessing Readiness of International Investigations into Alleged Biological Weapons Use
- Community Outbreak of OXA-48–Producing *Escherichia coli* Linked to Food Premises, New Zealand, 2018–2022
- Multicenter Case–Control Study of Behavioral, Environmental, and Geographic Risk Factors for Talaromycosis, Vietnam
- Persistence of SARS-CoV-2 Alpha Variant in White-Tailed Deer, Ohio, USA
- Transmission Dynamics and Parameters for Pertussis during School-Based Outbreak, South Korea, 2024
- Emergence and Prevalence of *Vibrio cholerae* O1 Sequence Type 75 Clonal Complex, Fujian Province, China, 2009–2023



- Estimation of Incubation Period for Oropouche Virus Disease among Travel-Associated Cases, 2024–2025
- Spatiotemporal Distribution and Clinical Characteristics of Zoonotic Tuberculosis, Spain, 2018–2022
- Emergence of Flucytosine-Resistant *Candida tropicalis* Clade, the Netherlands
- *Borrelia* Lineages Adjacent to Zoonotic Clades in Black Flying Foxes

- *Peromyscus* spp. Deer Mice as Rodent Model of Acute Leptospirosis
- Disseminated Histoplasmosis in Persons Living with HIV, France and Overseas Territories, 1992–2021
- Emergence of Distinct *Salmonella enterica* Serovar Enteritidis Lineage since 2020, South Korea
- Epidemiologic and Genomic Investigation of Sexually Transmitted *Shigella sonnei*, England
- Role of Nonpharmaceutical Interventions during 1918–1920 Influenza Pandemic, Alaska, USA
- Evidence of Viremia in Dairy Cows Naturally Infected with Influenza A Virus, California, USA
- Multisystemic Disease and Septicemia Caused by Presumptive *Burkholderia pseudomallei* in American Quarter Horse, Florida, USA
- Environmental Exposures Relative to Locally Acquired Hansen Disease, United States
- Community Infections Linked with Parvovirus B19 Genomic DNA in Wastewater, Texas, USA, 2023–2024

**EMERGING
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Seoul Virus Infection Acquired at Private Pet Rat Breeding Facility, Germany, 2024

Fabian Baalman,¹ Mario Hönemann,¹ Stephan Drewes, Martin Eiden, Calvin Mehl, Dennis Tappe, Birte Pantenburg, Steffi Bellmann, Ingrid Möller, Melanie Maier, Corinna Pietsch, Rainer G. Ulrich, Johannes Münch

We report a case of severe acute kidney injury in a patient in Germany infected with Seoul virus. Clinical and laboratory analysis linked the infection to a pet rat breeding facility in central Germany. Increased surveillance and a One Health approach are needed, given the rising popularity of pet rats.

Hantaviruses, a diverse group of zoonotic pathogens carried primarily by rodents, can cause hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (1). In Europe, Puumala virus and Dobrava-Belgrade virus are the most prevalent human pathogenic hantaviruses (2). Seoul virus (SEOV) (species *Orthohantavirus seoulense*) is the only hantavirus with a worldwide distribution. Norway rats (*Rattus norvegicus*), black rats (*R. rattus*), and related species represent the main natural reservoir; however, pet rats recently have emerged as an important source of SEOV infections (3). The clinical spectrum of SEOV infection in humans ranges from mild febrile illness to HFRS-like disease that can include fever, hepatitis, and gastroenteritis, and, less commonly, kidney involvement (4,5). In contrast to its worldwide distribution, autochthonous SEOV infections in Germany remain rare; only a few cases have been reported to date (4,6).

The Study

In March 2024, a previously healthy 44-year-old woman without any underlying conditions sought

care at the nephrology clinic at University Hospital Leipzig (Leipzig, Germany) with a 6-day history of fever, fatigue, diarrhea, and hypotension. Five weeks before symptom onset, the patient visited a private pet rat breeding facility and subsequently acquired rats for her children. Initial laboratory workup revealed thrombocytopenia, mildly elevated transaminases, and acute kidney injury (Appendix Figure 1, panel A; <https://wwwnc.cdc.gov/EID/article/31/10/25-0362-App1.pdf>). Urinalysis revealed hematuria and nephrotic-range proteinuria (urine protein-creatinine ratio >6 g/g [reference range <0.07 g/g]). Renal ultrasound showed enlarged, edematous kidneys (Appendix Figure 1, panel B), mild ascites, and splenomegaly. We observed no sonographic sign of chronic kidney disease or liver pathology. Given the deteriorating kidney function (peak serum creatinine was 14.3 mg/dL [reference range 0.67–1.18 mg/dL]), we initiated acute hemodialysis and performed a kidney biopsy.

Histopathologic examination revealed severe acute tubular injury with interstitial hemorrhage, edema, and inflammation (Figure 1, panel A). Only 2 of 21 glomeruli were sclerotic, and we observed minimal interstitial fibrosis with tubular atrophy. Specific immunohistochemical staining for hantavirus antigen was positive within the tubular cells and the tubulointerstitial space (Figure 1, panel B). Concurrently, lineblot assays performed by using re-

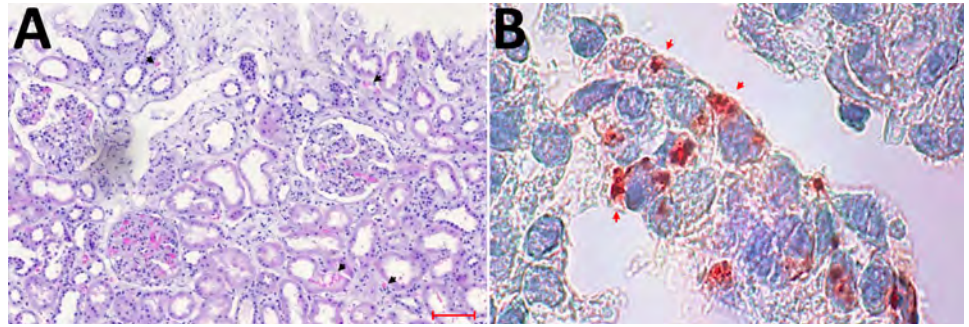
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DOI: <https://doi.org/10.3201/eid3110.250362>

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Figure 1. Light microscopic analysis and immunohistochemical staining results of index patient's kidney biopsy indicating severe Seoul virus infection with acute kidney injury, Germany, March 2024. A) Light microscopy of the hematoxylin- and eosin-stained biopsy demonstrating severe acute tubular injury with interstitial and tubular bleeding (black arrow heads), tissue edema, and interstitial inflammation. Scale bar indicates 100 μ m. B) Immunohistochemical staining (red) using mouse monoclonal antihantavirus antibody (Progen Biotechnik, <https://www.progen.com>) and the DCS-AEC chromogen kit (Agilent-DAKO, <https://www.agilent.com>) showing hantavirus antigen in renal tubular cells (red arrow heads). Original magnification $\times 100$.



comLine HantaPlus (Mikrogen Diagnostics, <https://www.mikrogen.de>) of admission serum samples demonstrated positivity for hantavirus IgG and IgM, supporting the diagnosis of hantavirus-associated nephritis. Reactive bands included Hantaan virus antigen for IgG and Puumala, Hantaan, Dobrava-Belgrade, and Seoul virus antigens for IgM (Appendix Table 1). To further delineate the infecting hantavirus, we used a panhantavirus reverse transcription PCR targeting a 412-nt region of the viral large segment (7) on blood and urine samples. The subsequent sequence analysis and comparison to GenBank entries confirmed an infection with SEOV.

After 3 hemodialysis sessions (hospital days 5–7), the patient's clinical condition improved; we observed normalization of platelet counts by day 3, transaminase activities by day 6, and serum creatinine within 3 weeks. At 2- and 8-month follow-ups, serum creatinine had returned to normal (0.75 mg/dL), and proteinuria had resolved. Of note, the patient's 2 daughters and husband were serologically negative and did not show any symptoms related to a hantavirus infection throughout the initial treatment and follow-up period. We performed those serologic tests 2 weeks after the initial diagnosis of the patient for the 2 daughters and 2 months later for the husband.

Given the unexpected finding of SEOV as causative agent, we initiated an epidemiologic investigation in collaboration with the local public health and veterinary department, which led to the identification of the pet rat breeder. The breeding facility (≈ 30 m³ in size) was located inside a private apartment, and the rats were bred with the intention of being pet animals. During the epidemiologic investigation (and 3 weeks after the patient tested positive for hantavirus IgG), the breeder, her husband, and her daughter also tested positive for hantavirus IgG but could not recall having symptoms suggestive of a hantavirus infection.

To assess the source of infection, we also analyzed samples from the index patient's rats and from the breeding facility. For all 4 of the patient's rats, we could not determine evidence of SEOV infection by serologic and molecular assays. In contrast, among the 6 pet rats obtained from the breeding facility, all yielded positive results across ≥ 1 assays (Appendix Table 2). One rat (source of sample KS24/530) was seropositive but negative by molecular testing, possibly reflecting a low viral load or virus clearance. Another rat (source of sample KS24/528) was SEOV RNA-positive but seronegative, suggesting an acute phase of infection. In general, results were concordant across 4 different assays performed on liver and lung tissues. In addition, we performed a phylogenetic analysis of a 731-nt long region of the viral small segment of the strains obtained from the breeder rats and publicly available SEOV sequences. All strains clustered closely together and shared a relatedness to sequences from other SEOV outbreaks in Germany but also to breeder rats or pet rats from other countries in Europe and the United States (Figure 2). Together with the high sequence similarity of the large segment sequences of 291 nt between the virus detected in the patient and those from the rats of the breeding facility (Appendix Figure 2), those findings strongly suggest the breeding facility as the source of SEOV infection. Given that the pet rats owned by the patient had no evidence of SEOV, the infection probably was acquired through aerosols during a visit to the breeding facility before purchase of the pet rats, followed by a 5-week incubation period until onset of disease symptoms.

Conclusions

This report underscores several important observations. First, SEOV infection can lead to major clinical disease, including severe acute kidney injury

requiring hemodialysis, even in the absence of hemorrhagic fever. Second, the proportion of asymptomatic infection in exposed persons suggests that SEOV infection might be underdiagnosed. Moreover, with the increasing popularity of pet rats, breeding facilities might serve as critical sources for SEOV, warranting enhanced surveillance. Proactive measures, such as routine screening of rat colonies and comprehensive public education, are warranted.

In Germany, practical implementation of a One Health approach for SEOV must consider the current lack of regulation for private breeders and the effect of animal welfare constraints, which preclude routine invasive testing of asymptomatic animals. Instead, feasible measures should focus on

strict hygiene protocols, appropriate ventilation of animal housing, quarantine of newly acquired rats, and thorough documentation of animal origin and health status to facilitate traceability in case of outbreaks (9). Given that SEOV is primarily transmitted through inhalation of virus-contaminated aerosols from rodent excreta, risk reduction strategies should include avoiding dry cleaning methods, using damp cleaning techniques, and wearing protective masks during cage maintenance. Immunocompromised persons and other vulnerable groups should be explicitly advised against keeping pet rats. In addition, raising public awareness of zoonotic disease risks through veterinarians, breeders, pet stores, and public health agencies remains essential.

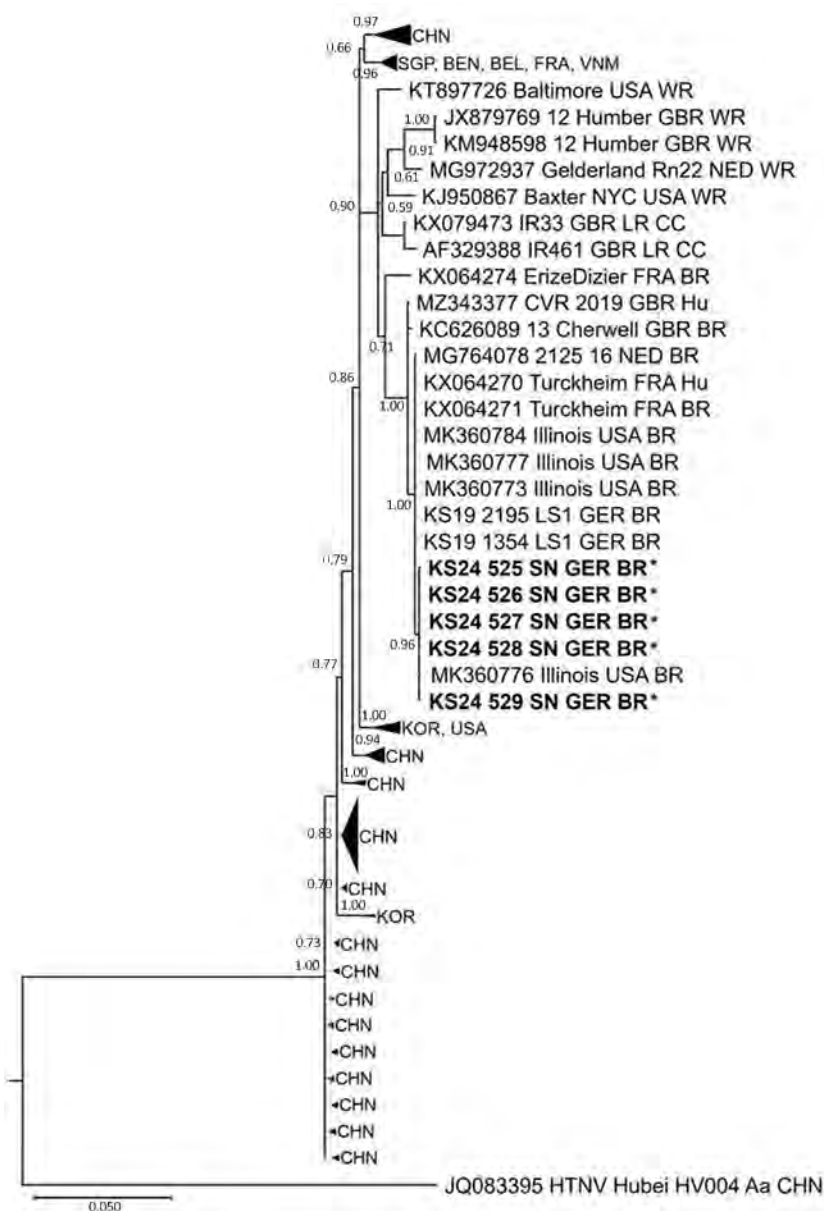


Figure 2. Phylogenetic reconstruction of a partial small segment (731 nt) of Seoul virus isolates (obtained in Saxony state, Germany) in 2024 from rat breeder's rats implicated in case of severe Seoul virus infection with acute kidney injury, Germany, March 2024. Phylogeny indicates high phylogenetic relatedness among the isolates from the breeder's rats (bold and asterisks) and other publicly available Seoul virus outbreak strains (4,8) (Appendix, <https://wwwnc.cdc.gov/EID/article/31/10/25-0362-App1.pdf>). Scale bar indicates the number of substitutions per site. BEL, Belgium; BEN, Benin; BR, breeder rat; CC, cell culture isolate; CHN, China; FRA, France; GBR, Great Britain; GER, Germany; HU, human; KOR, Korea; NED, the Netherlands; SGP, Singapore; USA, United States; VNM, Vietnam; WR, wild rat.

Clinicians should routinely inquire about rodent exposures when evaluating patients with unexplained febrile illnesses, particularly when acute kidney injury has occurred (9).

Looking ahead, the development and provision of easy-to-use, noninvasive, and animal welfare-compatible diagnostic tools, such as point-of-care tests for SEOV RNA detection from environmental, oral, or fecal swabs, would offer veterinarians and breeders a practical means of monitoring colonies without causing undue stress to the animals. Such approaches could contribute substantially to early detection and prevention efforts, thereby strengthening One Health strategies in this context.

Acknowledgments

We kindly acknowledge the technical assistance of Dörte Kaufmann, Birke Böttcher, and Sandra Bergs.

This study was funded by the German Centre for Infection Research (DZIF) Thematic Translational Unit “Emerging Infections” Commensal Rodent Pathogens (grant no. 01.80) awarded to R.G.U.

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Antimicrobial-Resistant Clonal Complex 11 *Neisseria meningitidis*–Associated Urethritis Cluster, Thailand

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Sexually transmitted infections clinics in Bangkok, Thailand, reported increasing numbers of men with *Neisseria meningitidis*–associated urethritis during 2017–2023. Genomic analysis indicated global expansion of the nongroupable clonal complex 11 *N. meningitidis* urethritis clade. Continued global surveillance is needed to monitor the spread of antimicrobial-resistant *N. meningitidis* with urethral adaptability.

Neisseria meningitidis is known to cause severe invasive infections, such as meningitis, but asymptomatic *N. meningitidis* nasopharyngeal colonization occurs in ≈10% of the human population (1). *N. meningitidis*–associated urethritis cases also have been reported, including a cluster caused by nongroupable clonal complex 11 (CC11) *N. meningitidis* detected in the United States in 2015 (2,3). That US clade has since expanded globally, and *N. meningitidis* urethritis cases have been found in the United Kingdom, Japan, and Vietnam (4–6). Phylogenetic analysis revealed that the globally expanding clade has formed a distinct branch within the CC11 lineage, designated as the *N. meningitidis* urethritis clade (NmUC) (3,6,7). Further genomic characterization of NmUC revealed integration of multiple *Neisseria gonorrhoeae* genomic

regions into genomes of *N. meningitidis* isolates via recombination, and those recombinations are thought to increase the ability of *N. meningitidis* to colonize the urethra (3,6,7).

Antimicrobial resistance (AMR) is not yet well established in NmUC (3), but recombination between *N. meningitidis* and *N. gonorrhoeae* is a concern because AMR is prevalent in *N. gonorrhoeae* (5,8). In a 2019–2020 outbreak of *N. meningitidis*–associated urethritis in Vietnam, isolates from NmUC had elevated MICs to ciprofloxacin (5). The isolates harbored common mutations found in *N. gonorrhoeae* that confer resistance to ciprofloxacin (9), which is concerning because ciprofloxacin is commonly used for prophylaxis against invasive meningococcal diseases (8). We analyzed isolates collected from a cluster of *N. meningitidis*–associated urethritis among men in Thailand to assess AMR and urethral adaptation.

The Study

In 2015, Thailand began surveillance for *N. gonorrhoeae* urethritis as part of the World Health Organization Enhanced Gonococcal Antimicrobial Surveillance Programme (EGASP; <https://www.who.int/initiatives/gonococcal-antimicrobial-surveillance->

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DOI: <https://doi.org/10.3201/eid3110.250464>

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programme). Through EGASP surveillance, 31 urethritis-causing *N. meningitidis* isolates were collected from men in Thailand during 2017–2023 (10). All cases were successfully treated with 250 mg or 500 mg intramuscular ceftriaxone in accordance with national guidelines for male patients with urethral symptoms and gram-negative intracellular diplococci. The national reference laboratory (accredited according to ISO 15189:2012) confirmed *N. meningitidis* in isolates by using culture and biochemical characteristics of *Neisseria* spp. bacteria.

We selected 16 isolates for further investigation, ensuring representation from the earliest detected case to the most recent case (Appendix, <https://wwwnc.cdc.gov/EID/article/31/10/25-0464-App1.xlsx>). We combined whole-genome long-read sequencing using a PromethION P2i with an R10.4.1 flow cell (both Oxford Nanopore Technologies, <https://nanoporetech.com>) and short-read sequencing using NovaSeq 6000 (Illumina Inc., <https://www.illumina.com>) and 150-bp paired-end reads for hybrid genome assembly according to standard protocols (11). We used PubMLST (<https://pubmlst.org>) to determine the specific gene alleles and genotype of the isolates (12).

Multilocus sequence typing showed that 15 of 16 isolates belonged to CC11 and 1 to sequence type 35 clonal complex (CC35). Within the CC11 isolates, 3 isolates (NM13, NM14, and NM15) collected during April–June 2022 from men who had sex with women were separated by an average of 13 pairwise single-nucleotide polymorphisms (SNPs), representing a potential transmission cluster or locally circulating strain.

To clarify the global placement of the CC11 urethral *N. meningitidis* isolates from Thailand, we performed core-genome SNP phylogenetic analysis on the 15 CC11 isolates from Thailand and 241 previously reported global *NmUC* isolates (Figure 1). Most (14/15) isolates from Thailand formed a monophyletic clade with the newly emerging *NmUC-B* subclade (6). *NmUC-B* is diverging from the original US *NmUC*, and isolates from *NmUC-B* were collected in Europe and Asia during 2019–2023. Of note, we found the first *N. meningitidis* isolate (NM1) collected from Thailand's EGASP activities in 2017 formed an outgroup for the entire *NmUC* CC11 and is distantly related to the rest of the isolates from Thailand that clustered in the *NmUC-B* subclade.

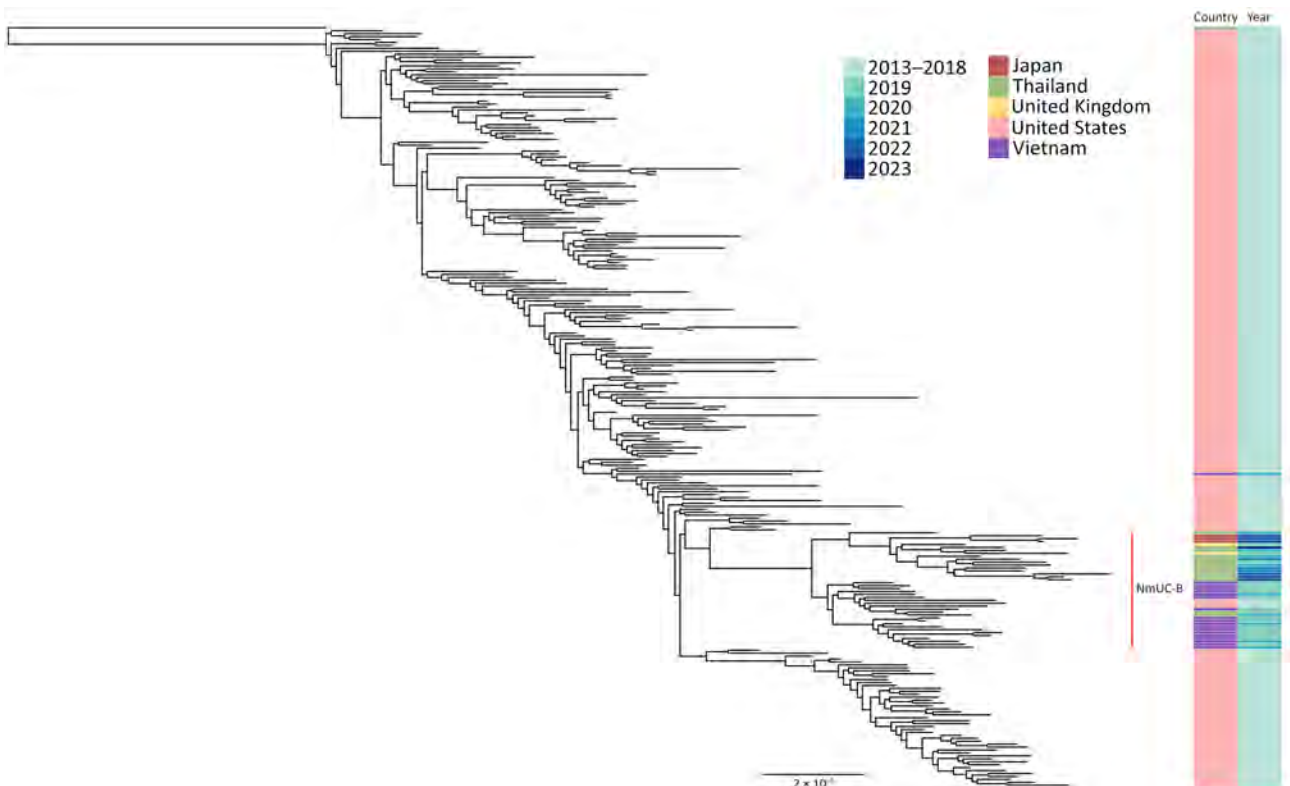


Figure 1. Maximum-likelihood phylogenetic tree from investigation of antimicrobial-resistant clonal complex 11 *Neisseria meningitidis*–associated urethritis cluster, Thailand. The tree is based on core-genome single-nucleotide polymorphisms of 259 clonal complex 11 *N. meningitidis* isolates collected from symptomatic male urethritis patients, representing the *N. meningitidis* urethritis clade. Scale bar indicates nucleotide substitutions per site. *NmUC-B*, *N. meningitidis* urethritis clade–subclade B.

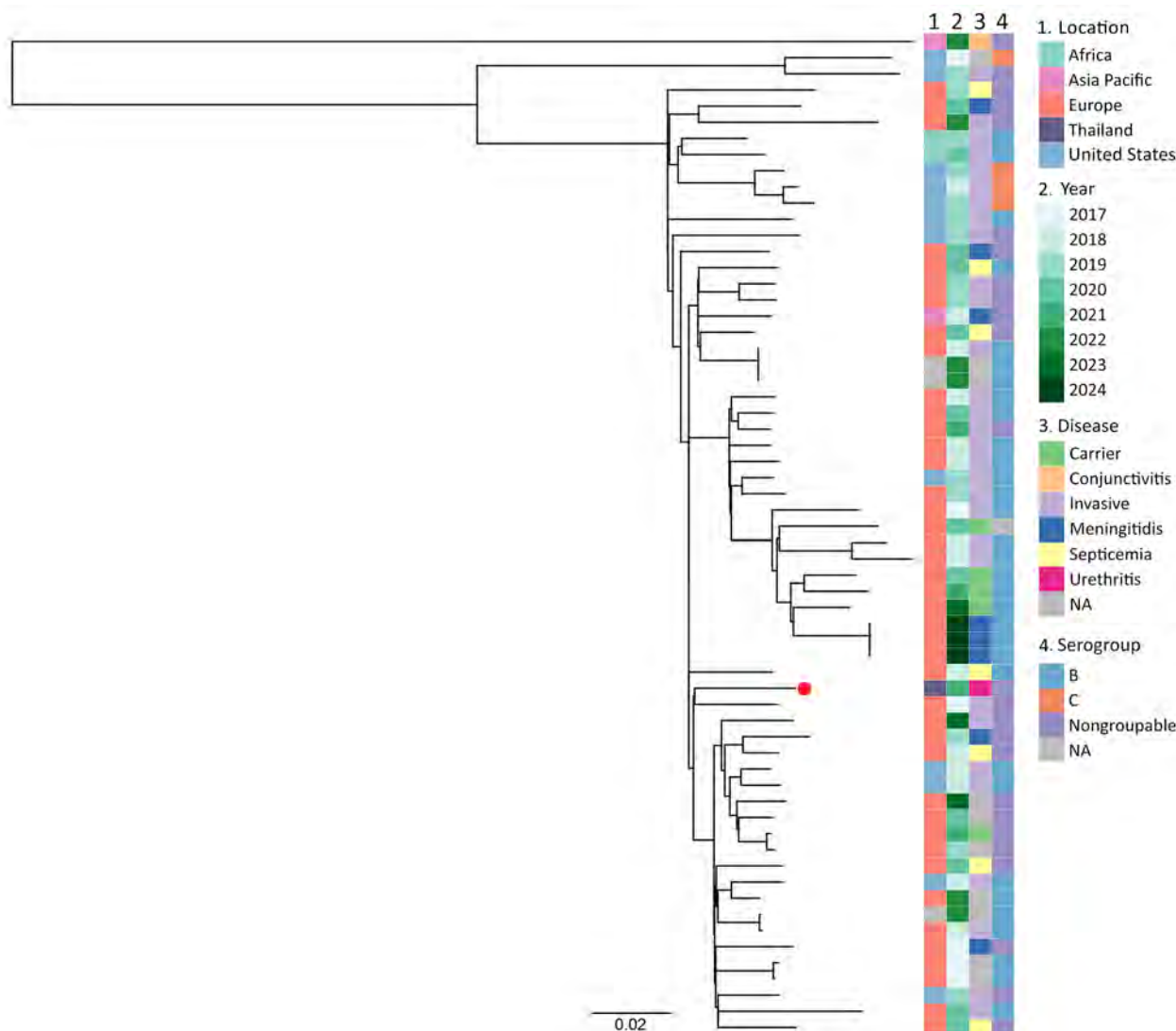


Figure 2. Maximum-likelihood phylogenetic tree of clonal complex 35 *Neisseria meningitidis* isolates used in investigation of antimicrobial-resistant clonal complex 11 *N. meningitidis*-associated urethritis cluster, Thailand. The tree is based on 65 core-genome single-nucleotide polymorphisms of isolates collected from multiple disease types. Red dot indicates the single *N. meningitidis*-associated urethritis isolate (NM4) from this study. Scale bar indicates nucleotide substitutions per site. NA, not available.

We genotyped 1 isolate (NM4) as CC35 and found it was distantly related to the CC11 isolates. We conducted a phylogenetic analysis of that CC35 isolate and 63 CC35 isolates collected during 2017–2024 from Africa, Asia Pacific, Europe, and the United States (Figure 2). The other 63 isolates were collected from multiple disease types, including conjunctivitis, invasive infections, meningitis, and septicemia, and from asymptomatic carriers. Isolate NM4 was the only isolate from a urethritis case and was most closely related (141 SNPs difference) to an isolate collected in Germany in 2017 from an invasive infection case.

We conducted antimicrobial susceptibility testing for azithromycin, cefixime, ceftriaxone,

ciprofloxacin, and gentamicin on the 16 selected isolates by using Etest Antibiotic Susceptibility Testing Reagent Strips (bioMérieux, <https://www.biomerieux.com>). We determined categorical MICs for *N. meningitidis* by following published guidelines (13). Most (15/16) isolates were susceptible to all antimicrobial drugs (Table). Three isolates from the NmUC-B subclade had an elevated MIC or resistance to ciprofloxacin: MIC for isolate NM8 was 0.125 µg/mL, for NM10 was 1.5 µg/mL, and for NM16 was 0.38 µg/mL. Upon further genomic analysis into AMR marker determinants, NM10 carried dual mutations, T91F and D95A, in the *gyrA* allele, and NM16 carried the *gyrA* T91I mutation.

Table. MICs of 16 isolates from investigation of antimicrobial-resistant clonal complex 11 *Neisseria meningitidis*–associated urethritis cluster, Thailand*

Isolate no.	Azithromycin MIC	Cefixime MIC	Ceftriaxone†		Ciprofloxacin‡		Gentamicin MIC
			MIC	CLSI category	MIC	CLSI category	
NM1	0.75	<0.016	<0.002	Susceptible	0.002	Susceptible	1.5
NM2	1	<0.016	0.002	Susceptible	0.003	Susceptible	3
NM3	0.75	<0.016	<0.002	Susceptible	0.003	Susceptible	3
NM4	0.5	<0.016	<0.002	Susceptible	0.002	Susceptible	3
NM5	0.75	<0.016	<0.002	Susceptible	0.003	Susceptible	3
NM6	1	0.016	0.003	Susceptible	0.004	Susceptible	2
NM7	0.25	0.75	0.38	NA	0.006	Susceptible	2
NM8	1	<0.016	<0.002	Susceptible	0.125	Resistant	3
NM9	1	<0.016	<0.002	Susceptible	0.006	Susceptible	3
NM10	0.38	<0.016	<0.002	Susceptible	1.5	Resistant	3
NM11	0.38	<0.016	<0.002	Susceptible	0.003	Susceptible	2
NM12	0.75	<0.016	<0.002	Susceptible	0.003	Susceptible	3
NM13	1	<0.016	<0.002	Susceptible	<0.002	Susceptible	3
NM14	0.38	<0.016	<0.002	Susceptible	0.004	Susceptible	2
NM15	0.5	<0.016	<0.002	Susceptible	0.003	Susceptible	3
NM16	0.25	<0.016	0.002	Susceptible	0.38	Resistant	1.5

*MIC values are µg/mL. Antimicrobial susceptibility testing was conducted by gradient strips. Categorical designations are shown for antimicrobial drugs with official clinical breakpoints for *N. meningitidis* based on CLSI guidelines (13). CLSI, Clinical and Laboratory Standards Institute (Wayne, Pennsylvania, USA); NA, not available.

†*N. meningitidis* is considered susceptible to ceftriaxone when the MIC values are ≤0.12 µg/mL. CLSI does not define a resistant category for ceftriaxone for *N. meningitidis* because a resistant breakpoint has not yet been established.

‡*N. meningitidis* is considered resistant to ciprofloxacin when the MIC values are ≥0.12 µg/mL.

Those *gryA* variants have been shown to reduce susceptibility to ciprofloxacin in both *N. gonorrhoeae* and *N. meningitidis* (5,9).

Isolate NM7, which had elevated MICs to both ceftriaxone and cefixime, harbored the *penA*-2840 allele type. That allele is closely related (93.6% sequence similarity) to the mosaic *penA*-60 allele, which can cause elevated MICs to ceftriaxone and cefixime in *N. gonorrhoeae* (14,15). Ceftriaxone is the recommended first-line treatment for *N. gonorrhoeae* and *N. meningitidis* infections. NM7 was also part of the *NmUC*-B subclade.

We also analyzed genomic features associated with *N. meningitidis* urethral colonization. All 16 isolates were nongroupable, and in silico prediction showed they had lost the *N. meningitidis* capsule. Eleven of the 16 isolates carried the insertion sequence element IS1301 in the capsule polysaccharide (*cps*) locus, disrupting capsule biosynthesis (Appendix Table 2). We determined that 14 of the 15 CC11 isolates carried the *aniA/norB* denitrification cassette associated with microaerobic and anaerobic growth, as well as urethral colonization (Appendix Table 5). Those 14 isolates were all part of the *NmUC*-B subclade and also harbored the ≈3-kb gonococcal partial operon NEIS1446–NEIS1442 and the gonococcal *argB* (NEIS1038) alleles. The single CC35 isolate did not contain the hallmark genomic features associated with urethral colonization commonly found in the *NmUC*. However, we found that isolate had lost its capsule and therefore was nongroupable via in silico prediction.

Conclusions

Our study indicates that the ongoing, global *NmUC* clade expanded into Thailand as early as 2017. That clade, which originated from a 2015 outbreak in the United States, has not only continued to spread globally but also continued to evolve to create the *NmUC*-B subclade of isolates that have increased urethral adaptability because of more homologous recombination events with gonococcal DNA (6). That increased urethral adaptability could have contributed to the observed increase in the local prevalence of *N. meningitidis*–associated urethritis in Thailand.

We also identified concerning AMR genomic markers with corresponding elevated MICs to both ciprofloxacin and extended-spectrum cephalosporins in the *NmUC*-B isolates from Thailand. That finding is a public health concern because ciprofloxacin is used as prophylaxis for invasive *N. meningitidis* infections, and extended-spectrum cephalosporins are used to treat *N. gonorrhoeae*–associated urethritis. One isolate had a mosaic *penA* allele that has been associated with decreased susceptibility to ceftriaxone in *N. meningitidis* and cefixime in *N. gonorrhoeae*, demonstrating that *N. meningitidis* could be a reservoir for AMR variants and contribute to the spread of AMR among *Neisseria* spp. bacteria. Those findings raise concerns for both gonococcal and meningococcal disease control.

In summary, we found increased urethral adaptability and AMR markers among *N. meningitidis* isolates from Thailand. Continued global

surveillance is needed to monitor the spread of urethral *N. meningitidis* and the possibility of further AMR in both *N. meningitidis* and *N. gonorrhoeae*.

Acknowledgments

We thank the Silom Community Clinic (SCC) and the Bangrak STI Center (BSC) patients for contributing their samples and information and the SCC and BSC clinical teams for their efforts in sample and data collection. We also thank Pattaraporn Nimsamer and Worarat Kruasuwan for performing DNA QC management, library preparation for sequencing, nanopore long-read sequencing at Siriraj Long-read Lab (Si-LoL), Division of Medical Bioinformatics, Research and Innovation Department, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, and sending to Novogene, China, for Novaseq short-read sequencing. We also thank Eileen Dunne for the initiative and encouragement of the *N. meningitidis* isolate collection.

This activity was reviewed by CDC, deemed research not involving human subjects, and was conducted consistent with applicable federal law and CDC policy. The Thailand Ministry of Public Health reviewed and approved the EGASP protocol as a routine disease surveillance activity. The activity was considered a public health practice and routine surveillance. No personal identifier information was collected in the analysis database.

All fastq files can be found in the National Center for Biotechnology Information (BioProject no. PRJNA1237777). Accession numbers are provided (Appendix Table 1).

This work was supported by the National Biobank of Thailand, National Science and Technology Development Agency (NSTDA), Thailand and Siriraj Research Development Fund (project no. (OI) R016436003), Faculty of Medicine Siriraj Hospital, Mahidol University, and Siriraj Foundation fund (no. D003474). Additional support was provided by the US Centers for Disease Control and Prevention's Antimicrobial Resistance Solutions Initiative through the Global Antimicrobial Resistance Laboratory and Response Network (cooperative agreement no. 1 NU3HCK000017-01-00).

At the time of this work, T.W. and I.M. were staff members of the World Health Organization. The authors alone are responsible for the views expressed in this article, and they do not necessarily represent the decisions, policy, or views of the World Health Organization. Use of trade names are for identification only and do not imply endorsement by any of the groups named above. This manuscript and the work described within were completed on December 5, 2024.

About the Author

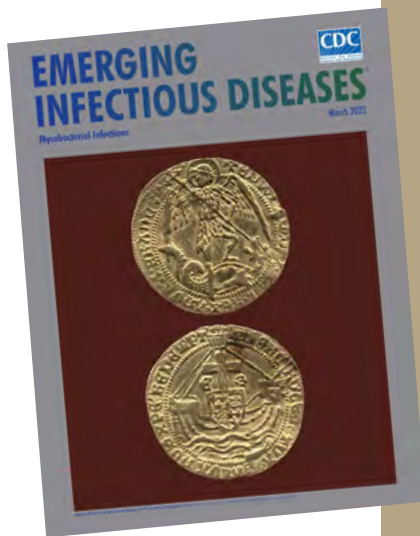
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Originally published
in March 2022

https://wwwnc.cdc.gov/eid/article/28/3/et-2803_article

etymologia revisited

Schizophyllum commune

[skiz-of'-ī-ləm kom'-yoon]

Schizophyllum commune, or split-gill mushroom, is an environmental, wood-rotting basidiomycetous fungus. *Schizophyllum* is derived from “*Schíza*” meaning split because of the appearance of radial, centrally split, gill like folds; “*commune*” means common or shared ownership or ubiquitous. Swedish mycologist Elias Magnus Fries (1794–1878), the Linnaeus of Mycology, assigned the scientific name in 1815. German mycologist Hans Kniep in 1930 discovered its sexual reproduction by consorting and recombining genomes with any one of numerous compatible mates (currently >2,800).

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Emergence and Polyclonal Dissemination of *bla*_{NDM-7}-Carrying IncX3 Plasmid in *Enterobacter cloacae* Complex, France, 2021–2023

Ines Rezzoug, Delphine Girlich, Aurelien Birer, Richard Bonnet, Josephine Poiraud, Pierre Bogaerts, Cécile Emerald, Laurent Dortet

Among 3,367 New Delhi metallo-β-lactamase–producing Enterobacterales isolates collected in France during 2021–2023, we found the *bla*_{NDM-7} gene systematically localized on 2 closely related IncX3 plasmids known to harbor antimicrobial resistance and virulence factors. Enhanced surveillance to monitor spread of antimicrobial resistance is needed among New Delhi metallo-β-lactamase–producing Enterobacterales.

Carbapenems are among the last-resort antimicrobial agents available to treat infections caused by multidrug-resistant gram-negative bacteria. Extensive use of carbapenems has led to emergence of carbapenem-hydrolyzing enzymes, known as carbapenemases, that hamper last-resort antimicrobial drug therapies (1). Carbapenemase-encoding genes are mostly carried on plasmids, which enable rapid dissemination of those genes among gram-negative bacteria.

In France, OXA-48-like enzymes are the most prevalent carbapenemases in Enterobacterales (2). However, in recent years, the prevalence of metallo-β-lactamases, particularly New Delhi metallo-β-lactamase (NDM), increased exponentially (3), and 86 NDM variants had been identified by April 2025 (<http://www.bldb.eu>). Diverse plasmid types, such

as IncF, IncA/C, IncL/M, IncH, IncN, and IncX3, carry *bla*_{NDM} genes (3,4). We characterized NDM-producing Enterobacterales circulating in France during 2021–2023 to decipher the prevalence of NDM variants among Enterobacterales species.

The Study

Among 11,825 carbapenemase-producing Enterobacterales (CPE) isolates received at the French National Reference Center for Antimicrobial Resistance (F-NRC) during January 1, 2021–December 31, 2023, we recovered and genetically characterized 3,367 NDM-producing isolates (Appendix 1, <https://wwwnc.cdc.gov/EID/article/31/10/25-0830-App1.pdf>). We found that the number of NDM producers increased from 22.5% (592/2,582) of all CPE isolates in 2021 to 27.1% (1,135/4,187) in 2022 and 32.4% (1,640/5,056) in 2023 (Figure 1, panel A; Appendix 2, <https://wwwnc.cdc.gov/EID/article/31/10/25-0830-App2.xlsx>). Among isolates, NDM-1, NDM-5, NDM-7, and NDM-14 represented 98% of all NDM enzymes (Figure 1, panel B).

We identified NDM-type carbapenemases in 10 different genera (Appendix 1), but the distribution of NDM variants varied drastically within Enterobacterales species (Figure 1, panel B). We identified NDM-1

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DOI: <https://doi.org/10.3201/eid3110.250830>

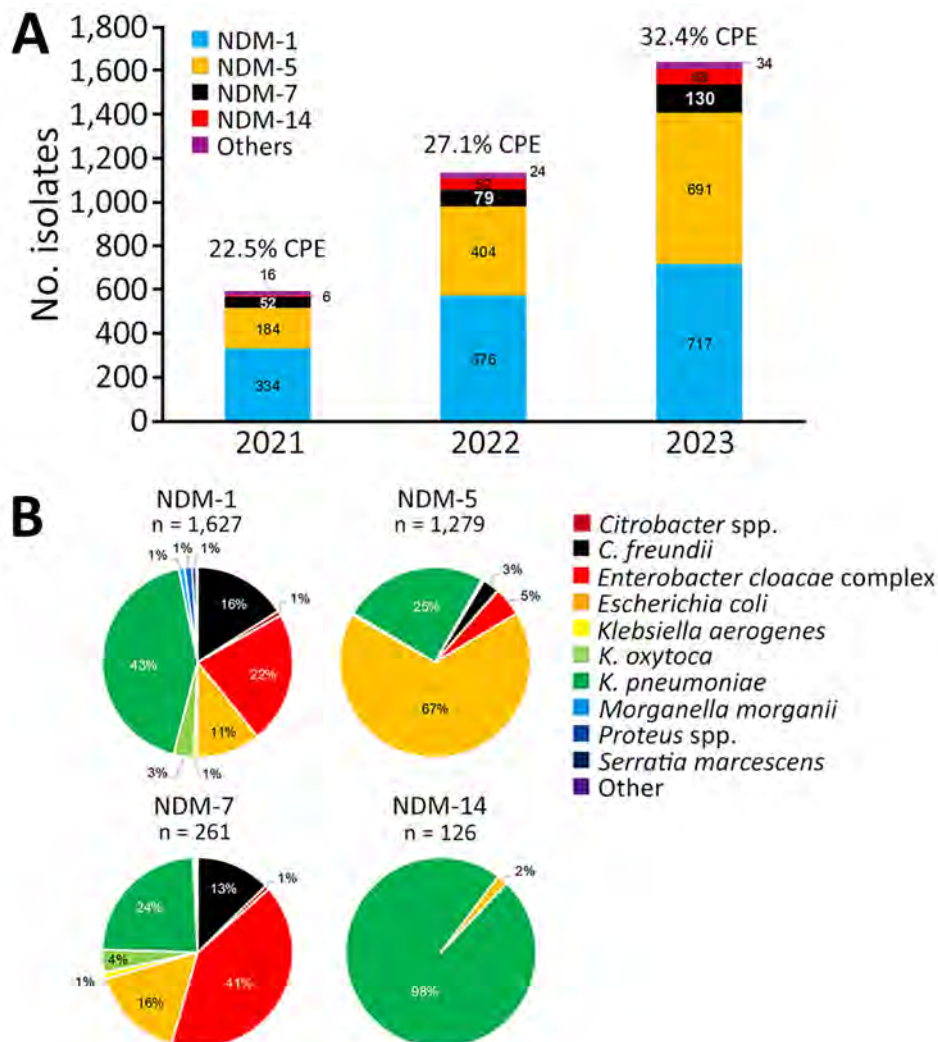


Figure 1. Variants and species involved in emergence and polyclonal dissemination of bla_{NDM-7}-carrying IncX3 plasmid in *Enterobacter cloacae* complex, France, 2021–2023. A) Distribution of NDM variants among CPE. B) Enterobacterales species distribution for the 4 most prevalent NDM variants detected. CPE, carbapenemase-producing Enterobacterales; NDM, New Delhi metallo-β-lactamase.

mainly in *Klebsiella pneumoniae* (43%) and NDM-5 predominantly in *Escherichia coli* (67%), and we found that NDM-14 was nearly exclusively associated with *K. pneumoniae* (98%). Unexpectedly, *Enterobacter cloacae* complex (ECC) accounted for 41% (107/261) of the NDM-7 producers.

Among the 107 NDM-7-producing ECC isolates, we identified 32 different sequence types (STs), the most prevalent of which were ST873 (22.4%), ST135 (10.2%), ST145 (10.2%), ST683 (9.3%), ST252 (6.5%), and ST32 (5.6%) (Figure 2). Those STs corresponded to 8 different *Enterobacter* species, including 46.7% of *E. hormaechei* subspecies *hoffmanii* (mostly ST135, ST145, and ST683), 22.4% *E. quasihormaechei* (all ST873), 7.4% *E. hormaechei* subsp. *steigerwaltii*, 6.5% (7/107) *E. xianfangensis*, 6.5% *E. asburiae*, 5.6% *E. kobei* (all ST32), 2.8% *E. cloacae*, 0.9% *E. hormaechei* subsp. *oharae*, and 0.9% *E. hormaechei* subsp. *hormaechei*. Phylogenetic analysis confirmed the polyclonal dissemination of NDM-7-

producing ECC, including in the 4 major STs (ST873, ST145, ST135, and ST683) comprising several isolates (Appendix 1 Figure 1).

Among the 107 NDM-7-producing ECC isolates, short-read sequencing enabled us to identify 12 different plasmid replicases (Appendix 2). Long-read sequencing performed on 30 representative NDM-7-producing ECC showed that bla_{NDM-7} was carried on an IncX3-type plasmid in all isolates (Appendix 1). Of note, 93.3% (28/30) of the IncX3 plasmids were 46,161-bp long, and 6.7% (2/30) were 49,830-bp long (Appendix 1 Figure 2). The size difference corresponded to the Tn5403 transposon. Those 2 plasmids shared 99.9% nucleotide identity with the 46,161-bp bla_{NDM-5}-carrying plasmid pEC21Z078-46K (GenBank accession no. CP10126), previously described in an *E. coli* isolate from China (5).

To compare the bla_{NDM-7}-carrying plasmids with those harboring bla_{NDM-5} in *Enterobacter* spp., we

performed long-read sequencing on 19 additional NDM-5-ECC isolates (Appendix 2). We found that 96% of those isolates also harbored an IncX3 plasmid. Among them, 84.2% (16/19) harbored an IncX3 plasmid almost identical to one of the *bla*_{NDM-7}-carrying plasmids (except the 2 single-point mutations between *bla*_{NDM-5} and *bla*_{NDM-7}); 12 harbored the 46,161-bp plasmid and 4 harbored the 49,830-bp plasmid. Among the other 3 NDM-5-producing ECC isolates, we localized *bla*_{NDM-5} on different IncFII-type plasmids: a 177,571-bp and a 96,517-bp plasmid similar to pABC143C-NDM (GenBank accession no. KY130431.1), and a 187,303-bp plasmid similar to p60214CZ (GenBank accession no. CP085746.1).

To investigate the origin of the *bla*_{NDM-7} IncX3 plasmid, we further explored 79 full-length sequences of NDM-1-producing ECC for IncX3 plasmids. Among the 11 different replicases identified, IncX3 was in only 14% of NDM-1-producing ECC. Long-read

whole-genome sequencing of those 11 IncX3-positive NDM-1-producing ECC identified the *bla*_{NDM-1} gene on IncX3 plasmids of 39,582-bp (n = 7) and 44,682-bp (n = 2) length (Appendix 1 Figure 2). Both of those plasmids were close to *bla*_{NDM-5}- and *bla*_{NDM-7}-carrying IncX3 plasmids but showed notable differences in the genetic region surrounding the *bla*_{NDM} gene (Appendix 1 Figure 2). Despite detection of an IncX3 plasmid, the other 2 strains harbored *bla*_{NDM-1}, either directly localized on the chromosome or on a 51,088-bp IncFII plasmid that had no similarity to plasmids available in GenBank.

The close genetic context of *bla*_{NDM} genes localized on IncX3 plasmids showed that both *bla*_{NDM-7} and *bla*_{NDM-5} were flanked upstream by Δ Tn2- Δ IS3000-ISAb₁₂₅-IS5 and downstream by genes encoding *ble*_{MBL}, PAI, *ccdA*, IS26, UmuD protein, ISKox3, a resolvase encoding gene, and Tn5403 (Appendix 1 Figure 2). Several elements were truncated or absent

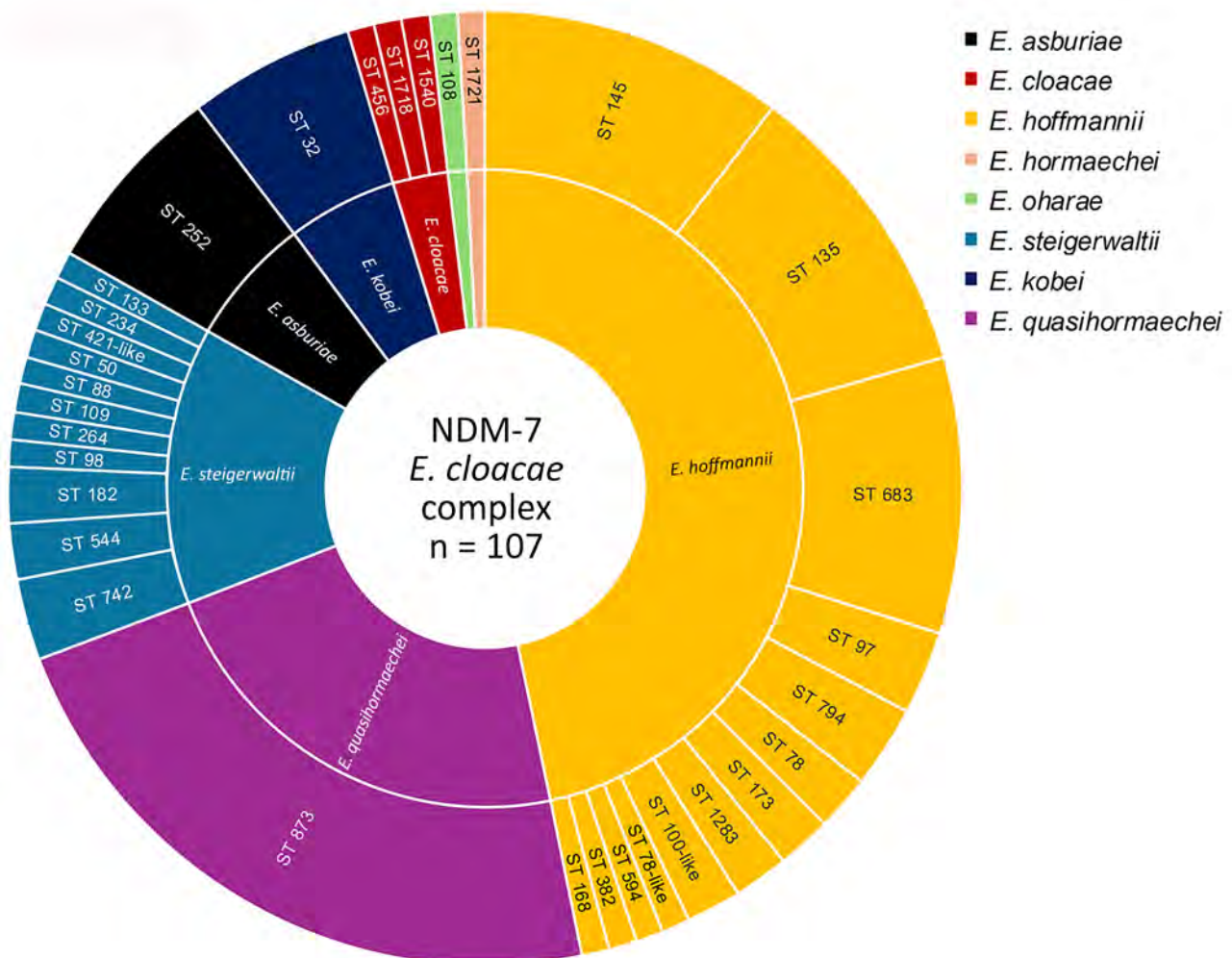


Figure 2. Distribution of STs among 107 *Enterobacter cloacae* complex isolates used in a study of emergence and polyclonal dissemination of *bla*_{NDM-7}-carrying IncX3 plasmid, France, 2021–2023. ST, sequence type.

in the bla_{NDM-1} genetic environment compared with bla_{NDM-5} and bla_{NDM-7}, including truncation of ISAba125 and deletions of IS5, ISKox3, and Tn5403.

Finally, to decipher whether the IncX3 plasmids were more prevalent in ECC, we looked for IncX3 replicase in 20,028 multidrug-resistant Enterobacterales genomes in the F-NRC database collected since 2022. Of those, 11.9% (n = 2,393) of isolates carried the IncX3-encoding replicase gene, 27.5% (n = 658) of which were *Klebsiella* spp., 27.3% (n = 653) were *Citrobacter* spp., 26.8% (n = 642) were *E. coli*, and 17.7% (n = 423) were ECC (Appendix 1 Figure 3).

Conclusions

During 2021–2023, F-NRC received increasing numbers of carbapenem-resistant Enterobacterales isolates: 2,582 in 2021, 4,187 in 2022, and 5,056 in 2023. In addition, the number of NDM-producing isolates more than tripled during that timeframe (Figure 1).

Although >86 NDM variants have been reported globally in the Beta-Lactamase DataBase (<http://blddb.eu>), 4 main variants are dominant in Europe: NDM-1, NDM-5, NDM-7, and NDM-14. In France, our results demonstrated that NDM-1 remains predominantly associated to *K. pneumoniae*, among which ST147 has been described as highly prevalent (48%) (6,7). The bla_{NDM-1} gene is carried on various plasmid types, including IncX3 (8), IncFIB, IncHI1B, and IncL, that harbor both resistance and virulence factors (9). In France, NDM-1 producers have been progressively replaced by NDM-5 producers since 2023. NDM-5 and NDM-7 differ from NDM-1 by substitutions responsible for an enhanced carbapenemase activity (7). In Europe, dissemination of NDM-5 is mostly caused by *E. coli* (10), which represented 70% of NDM-5 producers in our study. As previously described (11), our results confirmed that bla_{NDM-5} gene dissemination is mainly mediated by IncX3 plasmids. Our results confirmed that NDM-14-producing *K. pneumoniae* ST147 became established after it emerged in France in 2022 (12).

Our results highlight the emergence of NDM-7 in ECC, as noted in 41% of the isolates in our study. However, we did not identify any exclusive or particularly dominant clone involved in the dissemination of NDM-7 ECC, except ST873, which was slightly overrepresented. Of note, ECC isolates have been reported to trigger dissemination of Verona-integron-encoded metallo-β-lactamases in France, of which ST893 is the most prevalent ST (13). Our results also raise concerns about *E. quasihormaechei* ST873 as a high-risk clone for acquiring multidrug resistance, particularly carbapenemase resistance.

In summary, we demonstrated that the IncX3 bla_{NDM-7}-carrying plasmids most probably derived of IncX3 bla_{NDM-5}-carrying plasmids that were derived from IncX3 bla_{NDM-1}-carrying plasmids and contain several additional features, including IS5, ISKox3, and Tn5403. Despite the high transfer frequency (14) and low fitness cost (15) of IncX3 plasmids, their implication in the dissemination of bla_{NDM} remain unequal between Enterobacterales species. The strong association between IncX3 bla_{NDM-7}-carrying plasmids and ECC underscores the need for enhanced surveillance to monitor spread of antimicrobial resistance.

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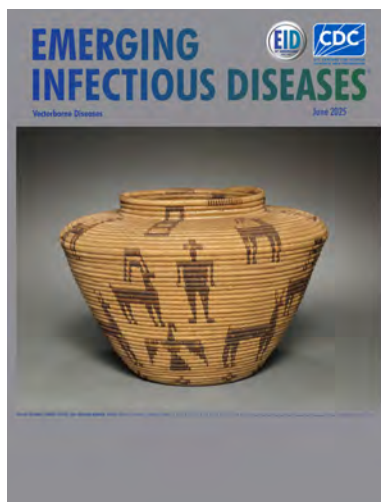
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June 2025

Vectorborne Diseases

- Clinical Manifestations, Risk Factors, and Disease Burden of Rickettsiosis, Cambodia, 2007–2020
- Multicenter Retrospective Study of *Spiroplasma ixodetis* Infantile Cataract in 8 Countries in Europe
- Genesis and Spread of Novel Highly Pathogenic Avian Influenza A(H5N1) Clade 2.3.4.4b Virus Genotype EA-2023-DG Reassortant, Western Europe
- Characterization of Adult and Pediatric Healthcare-Associated and Community-Associated *Clostridioides difficile* Infections, Canada, 2015–2022
- Prospective Multicenter Surveillance of Non-*H. pylori Helicobacter* Infections during Medical Checkups, Japan
- Safety and Immunogenicity of Poultry Vaccine for Protecting Critically Endangered Avian Species against Highly Pathogenic Avian Influenza Virus, United States
- Diagnostic Accuracy of 3 Mpox Lateral Flow Assays for Antigen Detection, Democratic Republic of the Congo and United Kingdom



- Force of Infection Model for Estimating Time to Dengue Virus Seropositivity among Expatriate Populations, Thailand
- Long-Term Clinical Outcomes of Adults Hospitalized for COVID-19 Pneumonia
- High Genetic Diversity of Histoplasma in the Amazon Basin, 2006–2017
- Emergence of Oropouche Virus in Espírito Santo State, Brazil, 2024
- Three Cases of Human Babesiosis, Italy, 2017–2020
- Cadaveric Human Growth Hormone–Associated Creutzfeldt-Jakob Disease with Long Latency Period, United States
- Oral Flea Preventive to Control *Rickettsia typhi*-Infected Fleas on Reservoir Opossums, Galveston, Texas, USA, 2023–2024
- OXA-204 Carbapenemase in Clinical Isolate of *Pseudomonas guariconensis*, Tunisia
- Investigation of Influenza A(H5N1) Virus Neutralization by Quadrivalent Seasonal Vaccines, United Kingdom, 2021–2024
- Prevalence of Nitroimidazole-Refractory Giardiasis Acquired in Different World Regions, Sweden, 2008–2020
- Genomic Surveillance of Climate-Amplified Cholera Outbreak, Malawi, 2022–2023
- *Francisella tularensis* Subspecies holarctica in Stranded Beluga Whales, Cook Inlet, Alaska, USA
- Skin Infections Caused by Panton-Valentine Leukocidin and Methicillin-Susceptible *Staphylococcus aureus* in Child, Japan

**EMERGING
INFECTIOUS DISEASES**

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Genomic Investigation of Disseminated Gonococcal Infections, Minnesota, USA, 2024

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This article summarizes a genomic investigation of a 4-fold increase in disseminated gonococcal infections in Minnesota, USA, in 2024. We detected the emergence of a *Neisseria gonorrhoeae* strain of a rarely observed sequence type, which carries a *porB1a* allele previously associated with disseminated disease and lacks a gonococcal genetic island.

Although most *Neisseria gonorrhoeae* infections remain localized to the urogenital tract of infected patients, this sexually transmitted pathogen can spread to other body sites and cause systemic infections (1). Disseminated gonococcal infections (DGIs) are thought to occur in <3% of infections, often cause severe illness, and are poorly understood in their pathogenesis (1,2).

In Minnesota, USA, the Minnesota Department of Health (MDH) performs routine surveillance of urogenital and disseminated *N. gonorrhoeae* infections, according to state reporting rules that include submission of isolates or other clinical materials for gonococcal infections in normally sterile sites. In this article, we report an increase in DGI cases in Minnesota in 2024. We used whole-genome sequencing (WGS) technology to investigate that increase. Our objectives were to assess the relatedness of DGI-causing strains and to identify potential genetic factors that contributed to dissemination.

The Study

In 2024, a total of 27 culture-confirmed *N. gonorrhoeae* infections from normally sterile sites were reported to MDH, a nearly 4-fold increase over the average

yearly number of cases reported since surveillance began in 2020. Because further investigation of those cases was conducted as public health surveillance subject to state reporting rules, institutional review board approval was not necessary. We confirmed the species of 23 isolates from 20 DGI cases ($n = 2$ cases with multiple isolates collected) by using Biotyper matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker, <https://www.bruker.com>) with an internally validated research use only database. We prepared WGS libraries from those isolates by using the Illumina DNA Prep kit (Illumina, <https://www.illumina.com>) and sequenced genomes on the Illumina MiSeq platform with reagent kit v2 (500 cycles) or the Illumina NextSeq 2000 platform with P1 reagents (300 cycles). We assembled and phylogenetically compared genomes by using the Spriggin v1.3.0 (3) and Dryad v3.0.0 (4) bacterial bioinformatics pipelines (Appendix, <http://wwwnc.cdc.gov/EID/article/31/10/25-0785-App1.pdf>). To classify assembled genomes by sequence type (ST) and resolve gonococcal genetic island sequences, we queried assembled genomes against the PubMLST database (5,6). Then, we performed *N. gonorrhoeae* multilocus sequence typing (MLST) by antimicrobial resistance (NG-STAR) by using the NG-STAR v2.0 database and identified *porB* allele types (Appendix Table 1) (7).

Of the 20 DGI cases with available WGS data, we classified 14 (70.0%) isolates as both ST11184 and NG-STAR profile 394. We sequenced 17 genomes of that type, which included 3 repeat isolates from 2 patients (Figure 1). One genome did not identically match any documented MLST profile or NG-STAR profile for *N. gonorrhoeae*. The ST11184 genomes ranged in genetic similarity from 0–207 pairwise single-nucleotide polymorphisms (SNPs) by the reference-based

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DOI: <https://doi.org/10.3201/eid3110.250785>

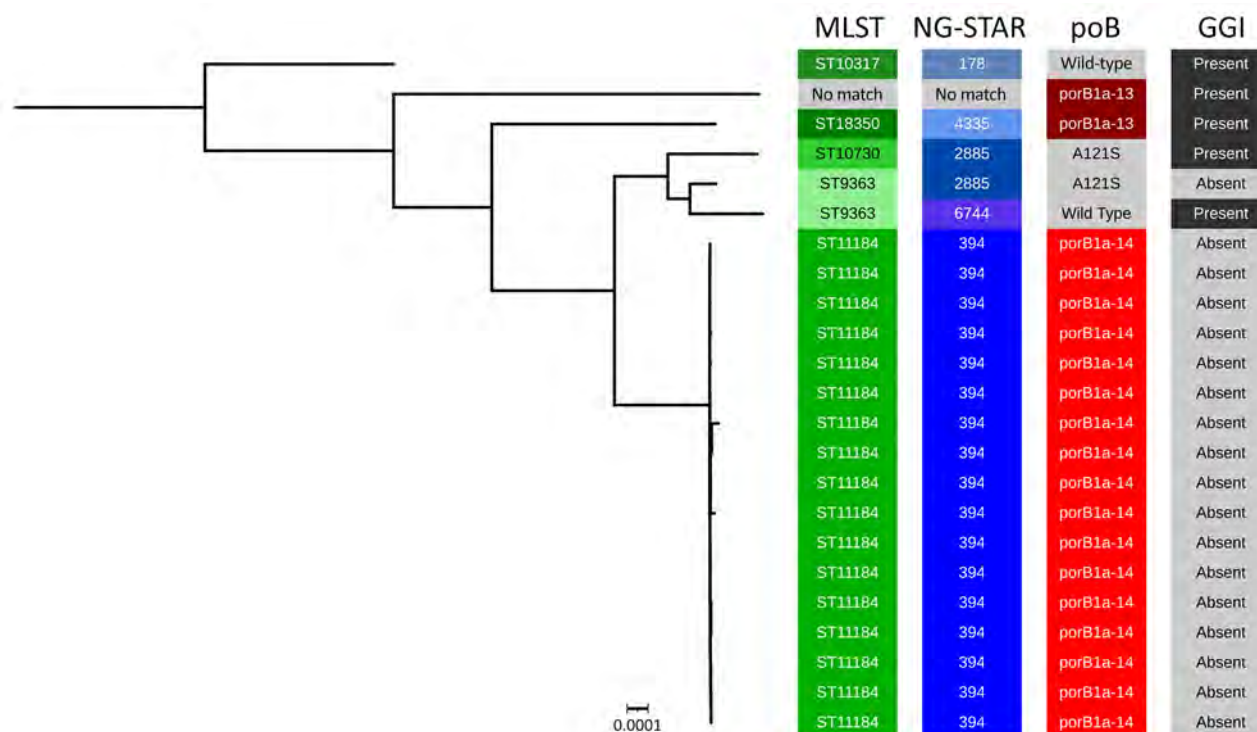


Figure 1. Maximum-likelihood, distance-scaled, core gene phylogenetic tree of *Neisseria gonorrhoeae* genomes from disseminated gonococcal infection cases in Minnesota, 2024. We constructed this tree by using the Dryad v3.0 pipeline (Appendix, <http://www.ncdc.gov/EID/article/31/10/25-0785-App1.pdf>) (4). GGI, presence or absence of a gonococcal genetic island sequence; MLST, multilocus sequence typing; NG-STAR, *N. gonorrhoeae* sequence type by antimicrobial resistance; porB, porin B allele type within the NG-STAR classification scheme; ST, sequence type.

approach in the Dryad pipeline (Figure 2) (4). Repeating that analysis by using Bakta v1.9.4, Panaroo v1.5.0, and snp-dists v0.8.2 in a reference-free, core genome alignment-based approach yielded a range of 4–168 pairwise SNPs (Appendix Figure 1) (8–12).

All genomes belonging to this closely related ST11184 strain encoded a *porB1a* allele sequence (NG-STAR *porB* type 14). Two other genomes, including the genome without an MLST match, carried a *porB1a* allele that matched NG-STAR *porB* type 13. Previous studies have associated strains encoding those *porB1a* alleles with an increased likelihood of causing disseminated infection (13). PubMLST queries also revealed that the ST11184 genomes did not carry a gonococcal genetic island, which functions in horizontal transfer of antimicrobial resistance and virulence genes are well documented (14).

To contextualize the genomes of our DGI-causing strains, we used PubMLST and the National Center for Biotechnology Information Pathogen Detection Browser (PDB; <https://www.ncbi.nlm.nih.gov/pathogens/isolates>) tool to search for publicly available genomes that were closely related to those we sequenced. After performing comparisons with >60,000

N. gonorrhoeae genomes that were publicly available as of May 2025, metadata from which indicated that most were from isolates collected during 2015–2024, the PDB tool grouped all 17 Minnesota ST1184 genomes into their own cluster (cluster identification no. PDS000214546.1). When those genomes were first processed by PDB, the cluster included only 1 other genome belonging to an isolate collected from a urogenital infection in Minnesota in September 2024 and sequenced through the Centers for Disease Control and Prevention’s Gonococcal Isolate Surveillance Project, and no other genomes in the database (15). All other DGI genomes were grouped into other PDB clusters, except for the genome that did not match any MLST or NG-STAR profile (Appendix Table 1).

To estimate when the DGI-causing ST11184 strain might have emerged, we generated a core-genome alignment and phylogenetic tree of the Minnesota and publicly available ST11184 genomes by using Bakta, Panaroo, and IQTree2 v2.3.6 (8–11). We then used TimeTree v0.11.4 to perform 16 time-scaled phylodynamic analyses with iteratively varied inputs (11) (Appendix). The dataset used for this analysis consisted of 41 genomes, including all 17 ST11184

genomes from Minnesota, the previously published genome from the same PDB cluster, all publicly accessible genomes documented on PubMLST as belonging to that MLST ($n = 10$), and all genomes shown by the National Center for Biotechnology Information PDB tool to be closely related to the PubMLST genomes ($n = 13$). Of the 24 publicly available genomes from other sources, only 5 (20.8%) genomes were assigned to NG-STAR profile 394. We used the distance-scaled tree as an input for phylodynamic analysis, which grouped all 18 Minnesota ST1184 genomes from the same PDB cluster into their own subclade (Appendix Figure 2). Across all 16 iterations, TreeTime calculated estimated times of most recent common ancestors of that clade during December 2022–June 2023, and 90% confidence intervals all intersected during March–April 2023 (Figure 3; Appendix Table 2).

Epidemiologists at MDH completed investigations of all DGI infections with the ST1184 strain ($n = 14$). Among those cases, the median patient age was 40.5 years (range 28–60 years). Eight (57.1%) case-patients had *N. gonorrhoeae* isolated from joint or synovial fluid and 6 (42.9%) from blood samples.

Thirteen (92.9%) case-patients were hospitalized, with a mean length of stay of 4.7 days (range 2–15 days), and no patients died. Six (42.9%) case-patients reported underlying conditions, including diabetes ($n = 2$ [14.3%]), concomitant sexually transmitted infection ($n = 2$ [14.3%]), immunosuppressive therapy ($n = 1$ [7.1%]), and history of intravenous drug use within the previous 12 months ($n = 1$ [7.1%]). Three (21.4%) case-patients reported being on HIV preexposure prophylaxis medications. Case-patients predominantly resided in Hennepin and Ramsey counties, within the Minneapolis-St. Paul metropolitan area ($n = 13$ [92.9%]). We did not identify any direct epidemiologic links between cases from those investigations.

Conclusions

The genomic data from this study of DGI cases in Minnesota, USA, yielded several conclusions and opened further lines of investigation. The similarity among the genomes of the ST1184 *N. gonorrhoeae* strain indicated that it emerged in Minnesota recently, and its substantial difference from other previously documented genomes from global surveillance suggests it

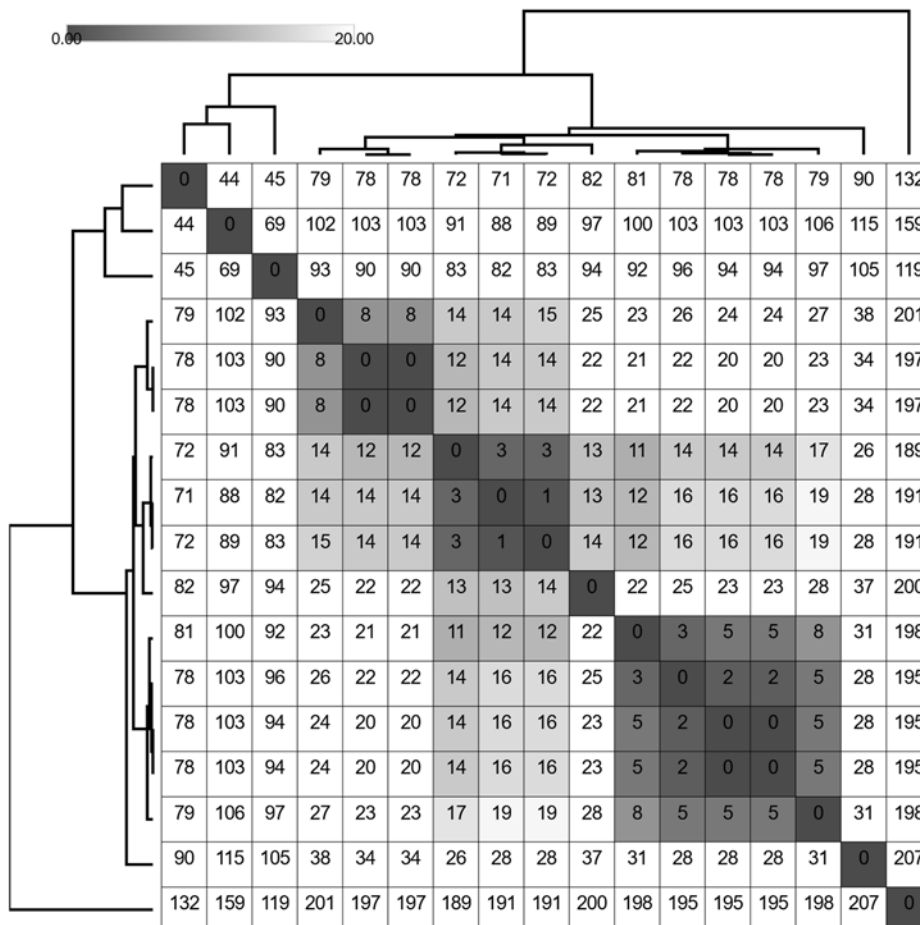


Figure 2. Pairwise single-nucleotide polymorphism (SNP) matrix of *Neisseria gonorrhoeae* sequence type (ST) 1184 genomes from disseminated gonococcal infection cases, Minnesota, USA. We clustered and visualized the matrix by using Morpheus (<https://morpheussoftware.net>) from SNPs identified within a reference-based genome alignment we generated by using the Dryad v3.0 pipeline (4), with an internal reference genome. The grayscale shading represents SNP values of 0–20. The branches represent hierarchical clustering of rows and columns of pairwise SNP calls.

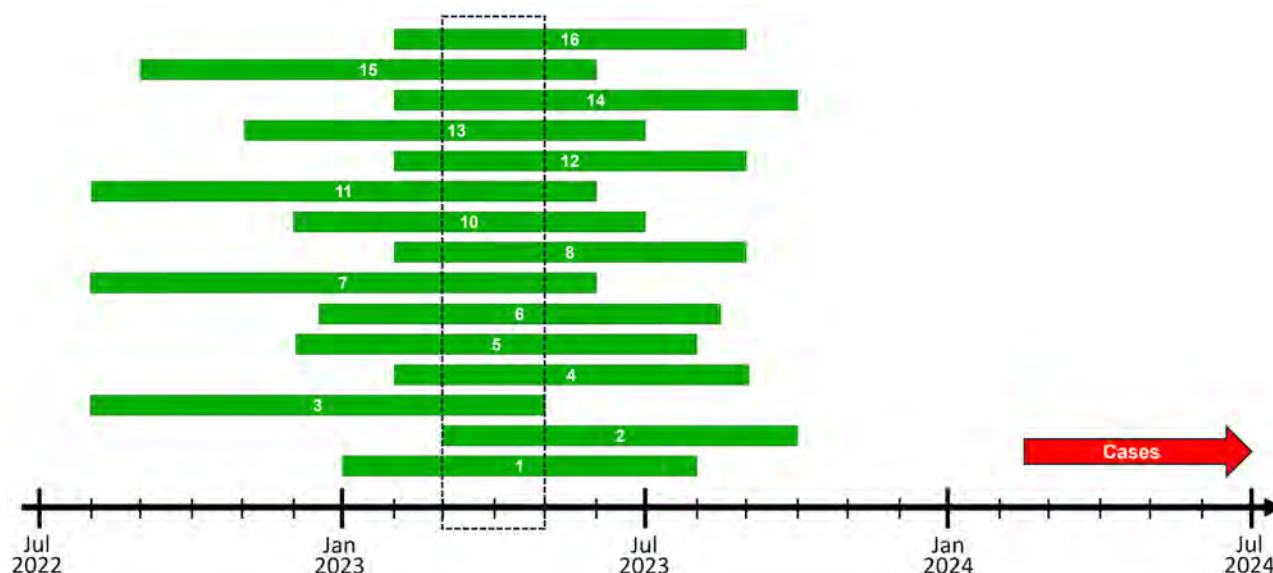


Figure 3. Phylodynamic inference of the emergence of *Neisseria gonorrhoeae* sequence type (ST) 11184 genomes that infected disseminated gonococcal infection cases, Minnesota, USA. The green bars denote the 90% CIs of the estimated time to most recent common ancestor (tMRCAs) of a clade of 18 ST11184 genomes from Minnesota, rounded to the nearest month. We calculated tMRCAs by refining a maximum-likelihood, distance-scaled, core gene phylogenetic tree of publicly available ST11184 genomes into a time-scaled tree by using TreeTime v0.11.4 software (Appendix, <http://wwwnc.cdc.gov/EID/article/31/10/25-0785-App1.pdf>) (10). The dashed box denotes the period in which the tMRCAs 90% CIs of all 16 iterations of time-scaled refinement overlapped. The red arrow denotes the time after the earliest collection of an isolate from a 2024 disseminated gonococcal infection case in Minnesota.

might not have been observed elsewhere. The presence of 2 *porB1a* alleles among DGI-causing isolates also reinforces previous studies that associated the allele with disseminated infections (2,13). Conversely, the breadth of genetic distance between the genomes of the emerging strain contraindicates potential direct transmission among all infected cases and raises questions about the incidence of unobserved transmission through urogenital infections. Future genomic surveillance of DGI is critical to expand comparisons of strains causing disseminated versus urogenital infections, identify other genetic determinants of dissemination, retrospectively sequence other known DGI-causing isolates from previous years, and further evaluate the utility of phylodynamic methods in tracking gonococcal outbreaks.

Acknowledgments

We thank John C. Cartee and Sandeep J. Joseph for their efforts to generate whole-genome sequencing data for *Neisseria gonorrhoeae* infections that were used in this study.

This project was financially supported by the following grants from the Department of Health and Human Services: Emerging Infections Program (grant no. NU50CK000648), Epidemiology and Laboratory Capacity (grant nos. NU50CK000508 and NU51CK000361),

and Pathogen Genomics Centers of Excellence (grant no. NU50CK000628).

All sequencing reads from Minnesota, USA, disseminated gonococcal infections isolate genomes generated in this investigation are publicly available on National Center for Biotechnology Information database (BioProject no. PRJNA1204341).

About the Author

Mr. Evans is a genomic epidemiologist with the Minnesota Department of Health. His work focuses on developing, implementing, and optimizing the use of microbial genomics for pathogen surveillance and outbreak intervention.

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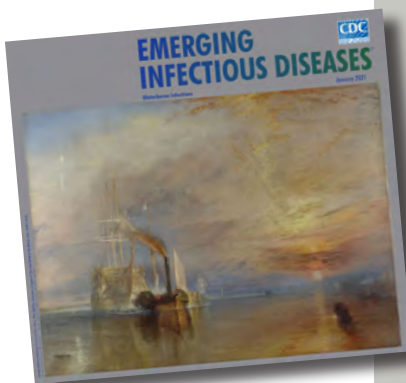
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etymologia revisited

Petri Dish

[pe'tre 'dish]



Originally published
in January 2021

The Petri dish is named after the German inventor and bacteriologist Julius Richard Petri (1852–1921). In 1887, as an assistant to fellow German physician and pioneering microbiologist Robert Koch (1843–1910), Petri published a paper titled “A minor modification of the plating technique of Koch.” This seemingly modest improvement (a slightly larger glass lid), Petri explained, reduced contamination from airborne germs in comparison with Koch’s bell jar.

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Fatal Pneumocephalus Caused by Hypervirulent *Klebsiella pneumoniae*, Germany

Nico Gläser,¹ Nina Schwab,¹ Yvonne Pfeifer, Anika Wahl, Martin A. Fischer, Anja Osterloh, Thomas F.E. Barth, Armin Imhof, Jochen Breitruck, Andreas Essig, Jürgen Benjamin Hagemann

We report a fatal case of pneumocephalus in Germany caused by hypervirulent *Klebsiella pneumoniae* sequence type 23, confirmed by using clinical, histopathologic, and genomic analyses. The patient reported no travel history, suggesting local emergence. This unusual case reveals an unclear pathogen prevalence and demonstrates the need for increased awareness of global spread.

The global spread of hypervirulent *Klebsiella pneumoniae* from Asia poses a major risk to human health that requires constant surveillance (1). Those strains are characterized by severe clinical manifestations in previously healthy patients or the presence of convergence plasmids conferring virulence and multidrug resistance that causes difficult-to-treat infections. In this article, we describe a fatal hypervirulent *K. pneumoniae* infection in Germany.

The Case

A 71-year-old patient was hospitalized with signs of septic shock. Thoracic and abdominal computed tomography (CT) imaging revealed pneumonia and liver and prostate abscesses. We initiated intravenous treatment with piperacillin/tazobactam and clarithromycin. Cranial CT revealed extensive pneumocephalus. We conducted CT by using the SOMATOM Force multidetector (Siemens, <https://www.siemens.com>) with secondary multiplanar reconstructions and maximum intensity projections. We conducted brain angiography natively and in the arterial phase

after contrast medium application, and we conducted thoracic and abdominal imaging in the venous phase.

Three days after hospitalization, the patient died of septic shock with central hypoxia. We isolated hypermucoviscous *K. pneumoniae* from the patient's blood cultures and postmortem brain samples. The fulminant clinical course justified extensive molecular biologic comparative analyses because similar cases were described previously in Asia (2).

We conducted an autopsy after the patient's death. For postmortem histopathological workup at the Institute of Pathology at Ulm University Hospital (Ulm, Germany), we fixed the patient's brain in 4% buffered formaldehyde and dissected according to our laboratory protocol. We paraffin-embedded tissue samples from 9 different regions according to our standard laboratory procedures. We conducted conventional (hematoxylin and eosin, Elastica van Gieson, Gram, periodic acid-Schiff, Berliner-Blau [Prussian blue]) and immunohistochemical (CD45, CD68, and GFAP proteins) staining on 3- μ m sections before microscopic evaluation.

We isolated *K. pneumoniae* from blood cultures by using the BACTEC system (BD, <https://www.bd.com>), postmortem swabs on casein-soy-peptone agar supplemented with 5% sheep blood (Oxoid, <http://www.oxoidshop.com>), and culture in brain-heart infusion broth (BD) for molecular workup. We used the Bruker (Bruker, <https://www.bruker.com>) matrix-assisted laser desorption/ionization time-of-flight mass spectrometry system for microbial identification and conducted antimicrobial testing by using VITEK2 (bioMérieux, <https://www.biomerieux.com>). We then conducted molecular analysis of 2 string test-positive isolates as previously described

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DOI: <https://doi.org/10.3201/eid3110.250877>

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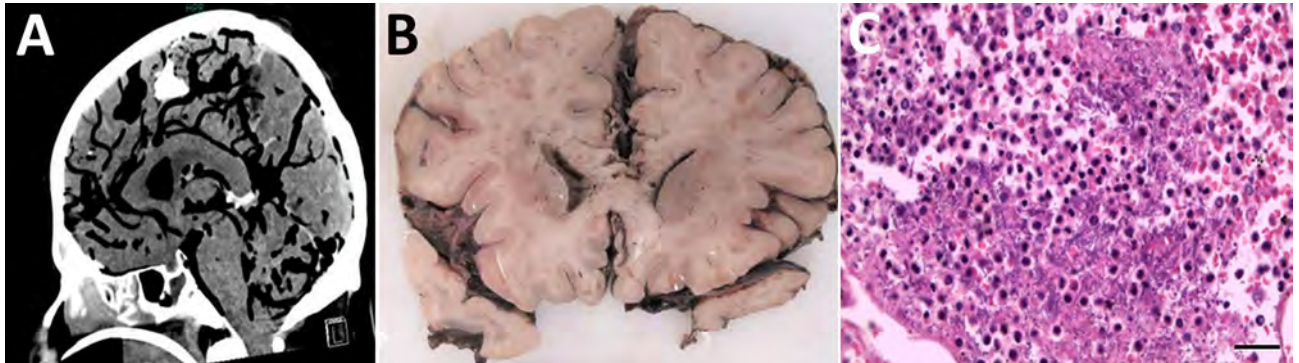


Figure 1. Radiologic and pathologic findings from a fatal pneumocephalus case caused by hypervirulent *Klebsiella pneumoniae* in Germany. A) Computed tomography imaging of the neurocranium revealing gas inclusions both in the arterial and venous system and the inner and outer cerebrospinal fluid spaces consistent with an intravital pneumocephalus. B) Postmortem coronal brain section revealing infiltrates of the leptomeninges and signs of global hypoxic-ischemic brain damage including blurred demarcation between the white and gray matter and discoloration. C) Microscopic appearance of purulent meningitis with bacilli accumulating in the leptomeninges. Hematoxylin and eosin stain. Scale bar indicates 50 μ m.

(3). For whole-genome sequencing, we extracted and quantified DNA by using the DNeasy Blood and Tissue Kit (QIAGEN, <https://www.qiagen.com>) and the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, <https://www.thermofisher.com>). We prepared sequencing libraries by using a Nextera XT DNA Library Prep kit (Illumina, <https://www.illumina.com>) and conducted sequencing on an Illumina NextSeq 550 (Illumina) by using a v2.5 chemistry kit (2 $\times$ 150 bp). We performed genome trimming and assembly as previously described (3). We compared the 2 iso-

lates from this patient (0354/24, 0355/25), an additional hypermucoviscous isolate from a patient with liver abscess in the same hospital (0074/24), and 26 recently published *K. pneumoniae* sequence type (ST) 23 isolates from hospitals in Germany (3,4) by using core genome multilocus sequence typing in SeqSphere 10.0.4 (Ridom, <https://www.ridom.de>) (5). We visualized the resulting tree with iTOL 6.8 (6). We identified contigs belonging to possible plasmids by MOB-suite 3.1.4 (5,7) and predicted resistance and virulence genes and capsular type by using Kleborate v3 (8,9).

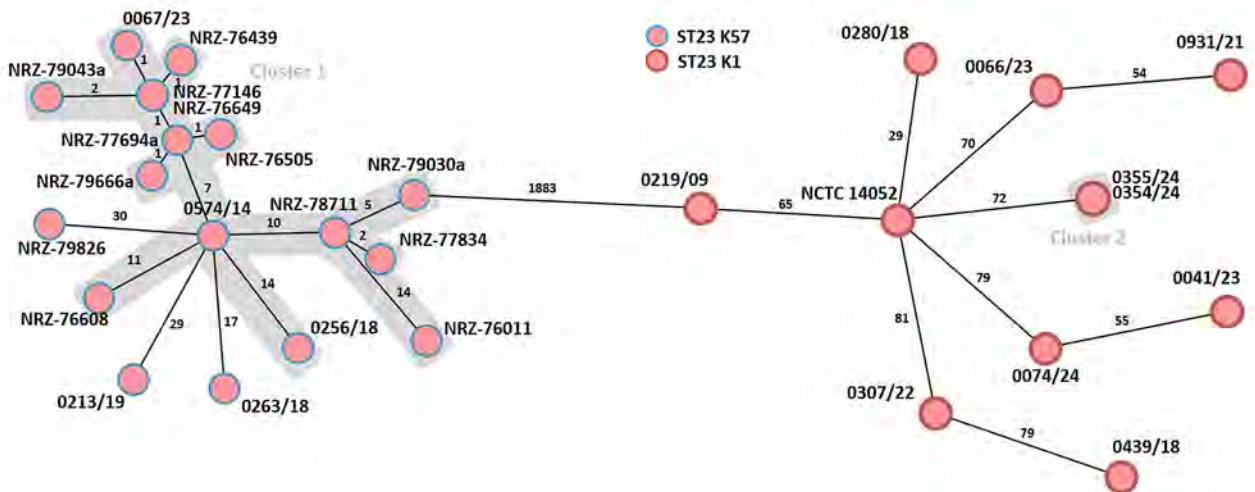


Figure 2. Minimum spanning tree generated to evaluate the genetic relationship of 29 *Klebsiella pneumoniae*-ST23 isolates (capsule types K1 and K57) during study of a fatal pneumocephalus case caused by hypervirulent *K. pneumoniae* in Germany (Appendix Table, <http://wwwnc.cdc.gov/EID/article/31/10/25-0877-App1.xlsx>). Ridom SeqSphere version 10.0.4 (ridom, <https://www.ridom.de>) was used to create tree for 29 samples isolated from hospitals in Germany on the basis of 2,358 columns, pairwise ignoring missing values, and logarithmic scale. Distance is on the basis of columns from *K. pneumoniae* sensu lato core genome multilocus sequence type (2,358 genes). Cluster distance threshold is 15 according to the Ridom SeqSphere. The patient isolates from this case (0354/24 and 0355/24) are labeled as cluster 2. Isolate 0074/24 is another *K. pneumoniae* ST23-K1 isolate from a patient with liver abscess admitted to the same hospital. Cluster 1 represents *K. pneumoniae* ST23-K57 isolates with virulence or convergence plasmids that were in part from patients previously hospitalized in Ukraine (3,4). ST, sequence type.

Clinical and radiologic findings supported the diagnosis of sepsis with central nervous system involvement. Although thoracic CT showed bronchial secretions and pulmonary consolidations, cranial CT revealed generalized hypoxic brain damage with intracranial gas inclusions (Figure 1, panel A). Abdominal CT revealed liver and prostate abscesses. On postmortem macroscopic examination, the brain showed signs of global hypoxic-ischemic brain damage, leptomeningitis, diffuse softening, oedematous swelling with signs of herniation (Figure 1, panel B), and numerous parenchymal gas inclusions. Histopathologic findings confirmed global hypoxic-ischemic damage and purulent leptomeningitis (Figure 1,

panel C). Gram-stained sections from different brain areas contained gram-negative bacilli consistent with the microbiologic detection of hypermucoviscous *K. pneumoniae* from postmortem brain swab specimens. Hypoxic brain damage because of pneumocephalus caused by hematogenous dissemination of gas-producing bacteria was confirmed as the cause of death. No anatomic or functional anomalies were identified to explain the pneumocephalus. In addition, the autopsy confirmed a chronic liver abscess with incipient connective tissue encapsulation and partly abscessing, phlegmonous prostatitis with proof of *K. pneumoniae*.

Genome analyses of the 2 hypermucoviscous *K. pneumoniae* isolates from blood cultures detected the

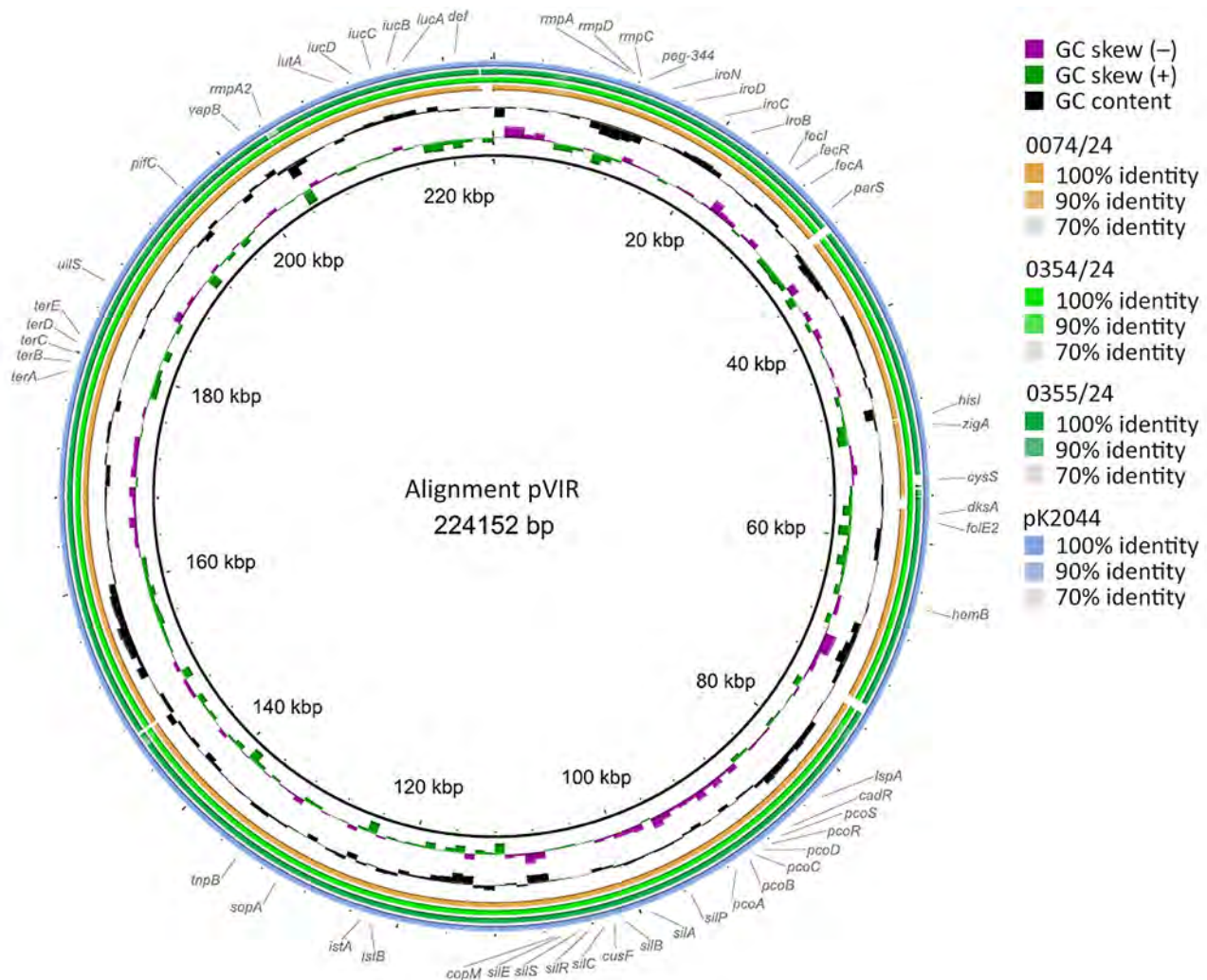


Figure 3. Comparative plasmid alignment of hypervirulent *Klebsiella pneumoniae* from the study of a fatal pneumocephalus case in Germany, completed by using BRIG (10). The innermost rings show guanine-cytosine content, and the outer ring shows the used reference virulence plasmid pK2044 (blue) from *K. pneumoniae* strain NTUH-K2044 from Taiwan (11). Concentric rings illustrate the virulence plasmids of the *K. pneumoniae* isolates from this case, 0354/24 (light green) and 0355/24 (dark green), and a patient isolate from the same hospital, 0074/24 (orange). Sequence similarity to the reference plasmid is indicated by the intensity and continuity of the colored rings. The gaps correspond to regions with no major sequence similarity. The locations of the typical virulence-associated genes (*iucA*, *iroB*, *rmpA*, *rmpA2*, *peg344*) (12) are visualized on the outermost ring.

magA gene, which is specific for capsule type K1, and a characteristic combination of virulence-associated genes (*iucA*, *iroB*, *peg-344*, *rmpA*, and *rmpA2*) (10). Multilocus sequence typing assigned both isolates to ST23. Subsequent comparison with 27 other *K. pneumoniae* ST23 isolates from Germany with capsule types K1 and K57 revealed that the isolates from this case were genetically identical (minimum spanning tree cluster 2) but not closely related to the other isolates (Figure 2). Plasmid analysis identified a type AA406 virulence plasmid in the 2 isolates from this study and 25 of the 27 other ST23 isolates (Appendix Table, <http://wwwnc.cdc.gov/EID/article/31/10/25-0877-App1.xlsx>). An alignment of the plasmids with the characteristic Pl virulence plasmid pK2044, which is almost exclusively found in ST23 isolates, confirmed a high sequence similarity (Figure 3) (10,11). In addition, 4 previous ST23-K57 isolates carried a convergence plasmid (AA405) containing both virulence-associated and antimicrobial resistance genes, highlighting the emerging clinical significance of ST23 (3,4).

Conclusions

We describe a lethal pneumocephalus case caused by hypervirulent *K. pneumoniae* ST23 in Germany. Similar cases were reported from Southeast Asia (13). To date, we have found only sporadic reports of hypervirulent and mostly imported *K. pneumoniae* strains in Europe (3). Of note, our patient had no history of travel, and his only well-described risk factor was type 2 diabetes mellitus. Poor glycemic control increases the risk for fulminant *K. pneumoniae* infections with hematogenous dissemination; mortality rates of meningitis in such cases exceed 50% (1,14).

Despite early intensive care and antiinfective treatment, this patient died within 72 hours of hospitalization. The pathogenesis of the fulminant pneumocephalus is likely the consequence of septic dissemination from his primary infection site to the central nervous system with subsequent gas production. The patient's *K. pneumoniae* ST23 isolates contained a virulence plasmid of type AA406 with typical virulence-associated genes and demonstrated no close genetic link to earlier reported *K. pneumoniae* ST23 strains in Germany (Figures 2, 3).

This case indicates a potentially underestimated prevalence of hypervirulent ST23 strains in Europe, which requires systematic surveillance to better assess the distribution and epidemiology of *K. pneumoniae* ST23 (15) (<https://op.europa.eu/en/publication-detail/-/publication/1e194058-d79e-11ee-b9d9-01aa75ed71a1>). Clinicians should consider hypervirulent strains in cases of severe or recurrent *K.*

pneumoniae infections, even without prior geographic exposure, and rapidly initiate microbiological diagnostics. Because not all hypervirulent *K. pneumoniae* strains are hypermucoviscous, and not all hypermucoviscous strains are hypervirulent (3), further molecular analyses should be implemented when hypervirulent *K. pneumoniae* infection is suspected. If molecular analysis is not possible despite clinical suspicion of infection with a hypervirulent strain, clinical management should be adjusted quickly (e.g., identification of occult abscesses).

Because of the dynamic global spread of pathogens, clinical and laboratory diagnostics are critical to efficient surveillance strategies for emerging pathogens such as hypervirulent *K. pneumoniae*, the epidemiologic relevance of which might still be underestimated. Further research is needed to clarify the reasons for different clinical courses in infections with hypervirulent *K. pneumoniae* isolates despite harboring identical virulence factors.

Against the background of globalization, complex trading networks, and increasing travel activities, enhanced genomic surveillance is crucial to detect and respond to novel pathogen threats such as hypervirulent *K. pneumoniae*. Close interdisciplinary collaboration between clinicians, pathologists, epidemiologists, and microbiologists is essential for a comprehensive understanding and sound surveillance practices.

Acknowledgments

We thank Sibylle Müller-Bertling and Kirstin Ganske for their excellent technical assistance. We thank the members of the Sequencing Core Facility of the Genome Competence Centre, Robert Koch Institute, for providing excellent sequencing service.

Author contributions: conceptualization, N.G., N.S.; methodology, A.E., A.O., J.B., J.B.H., Y.P.; validation, A.W., M.A.F., Y.P.; formal analysis, A.W., M.A.F.; investigation, A.E., A.O., A.W., M.A.F., Y.P.; resources, A.E., Y.P.; data curation, A.W., M.A.F.; writing: original draft preparation, A.W., J.B.H., N.G., N.S., Y.P.; writing: review and editing, A.E., A.I., A.O., J.B., M.A.F., T.F.E.B.; visualization, A.W., Y.P.; supervision, J.B.H.; project administration, A.I., J.B.H., Y.P. All authors have read and provided written consent to publish the current version of the manuscript.

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Zoonotic *Baylisascaris procyonis* Infection in Raccoons, Mississippi, USA, 2023–2024

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Baylisascaris procyonis, an emerging zoonotic parasite, causes clinically significant visceral, ophthalmologic, and neurologic disease in humans. We screened raccoons (n = 46) collected from central and southern Mississippi for *B. procyonis* by necropsy (13.0% prevalence) and droplet digital PCR of feces (26.7% prevalence). Further surveillance to determine raccoon infection rates throughout Mississippi is indicated.

Baylisascaris procyonis roundworms are emerging zoonotic parasites that cause clinically significant visceral, ophthalmologic, and neurologic disease in humans. The 3 major categories of human *Baylisascaris procyonis* infection are neural larva migrans, ocular larva migrans, and visceral larva migrans (1–3). Raccoons (*Procyon lotor*) are the definitive host for *B. procyonis* roundworms (2,3). Case reports in the literature describe life-threatening neurologic disease manifestations of eosinophilic meningitis, which causes substantial illness and death (3). North American raccoons are human-habituated and thrive in urban and suburban areas, posing a serious health risk to humans (4,5).

Human *B. procyonis* infection occurs after ingestion of eggs from raccoon feces in contaminated soil from the environment, commonly in latrine areas in which raccoons defecate (6). Female *B. procyonis* roundworms produce >100,000 eggs/day/worm, and infected raccoons shed millions of eggs through fecal matter at latrine locations (1,7). Younger

children and persons with developmental delays are more likely to become infected because of increased likelihood of ingesting eggs because of pica behaviors (3). In the United States, 33 cases of human *B. procyonis* infection have been reported (3). *B. procyonis* has previously been reported in states neighboring Mississippi, USA, in the southeast (5,8–10). We report the presence of *B. procyonis* roundworms in raccoons in Mississippi.

The Study

We collected raccoons (n = 46) during 2023–2024 from 8 different counties in central and southern Mississippi (Hancock, Harrison, Hinds, Humphreys, Lincoln, Pearl River, Yazoo, and Warren Counties). We used dog-proof traps or live traps with a scientific collection permit issued by the Mississippi Department of Wildlife Fisheries and Parks (permit no. 0207241); some collections were made from donated hunter-harvested raccoons. Raccoons were stored at –20°C until examined by necropsy. During necropsy, we examined the raccoon's small and large intestines for the presence of *B. procyonis* nematodes. We collected and stored *B. procyonis* adult and juvenile worms in 70% ethanol before DNA analysis. At the time of necropsy, 45 of the raccoons contained fecal matter; we collected fecal samples collected from the large intestines of those animals and stored at –20°C before DNA analysis (Appendix, <https://wwwnc.cdc.gov/EID/article/31/10/25-0658-App1.pdf>).

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DOI: <https://doi.org/10.3201/eid3110.250658>

Table. *Baylisascaris procyonis* positivity rates based on necropsy and ddPCR in study of zoonotic *Baylisascaris procyonis* in raccoons, Mississippi, USA, 2023–2024*

County	Necropsy positivity rate, no. (%)	No. necropsies, N = 46	ddPCR positivity rate, no. (%)	No. ddPCR analyses
Hancock	0	2	1 (50.0)	2
Harrison	0	3	0	3
Hinds	0	15	3 (17.6)	17
Humphreys	2 (50.0)	4	2 (50.0)	4
Lincoln	0	1	1 (100.0)	1
Pearl River	0	3	1 (33.3)	3
Yazoo	4 (57.1)	7	4 (57.1)	7
Warren	0	11	0	8
Total	6 (13.0)	46	12 (26.7)	45

* Bold indicates Mississippi counties with raccoons positive for *B. procyonis* nematodes or for *Baylisascaris* sp. DNA. ddPCR, digital droplet PCR.

All animal protocols used in this study were approved by the Mississippi College Institutional Animal Care and Use Committee (protocol no. 072723) and Institutional Review Board (protocol no. 111621).

We confirmed *B. procyonis* presence in necropsied raccoons by morphology (Appendix Figure, panel A), Sanger sequencing and droplet digital PCR (ddPCR) analysis of fecal samples (n = 45), and se-

quencing of helminth DNA (n = 46) (Table; Appendix). PCR and Sanger sequencing using *B. procyonis* *cox1*, *cox2*, *ITS1*, *18s*, and *28S* primers revealed 100% identity to *B. procyonis* sequences in GenBank (accession nos. MW385486, AF179908, NC_016200, U94368, MZ092854) (11,12). We submitted DNA sequences to Genbank (accession nos. PV593814 [18s], PV594051 [28s], PV594407 [ITS1], PV600642 [cox2],

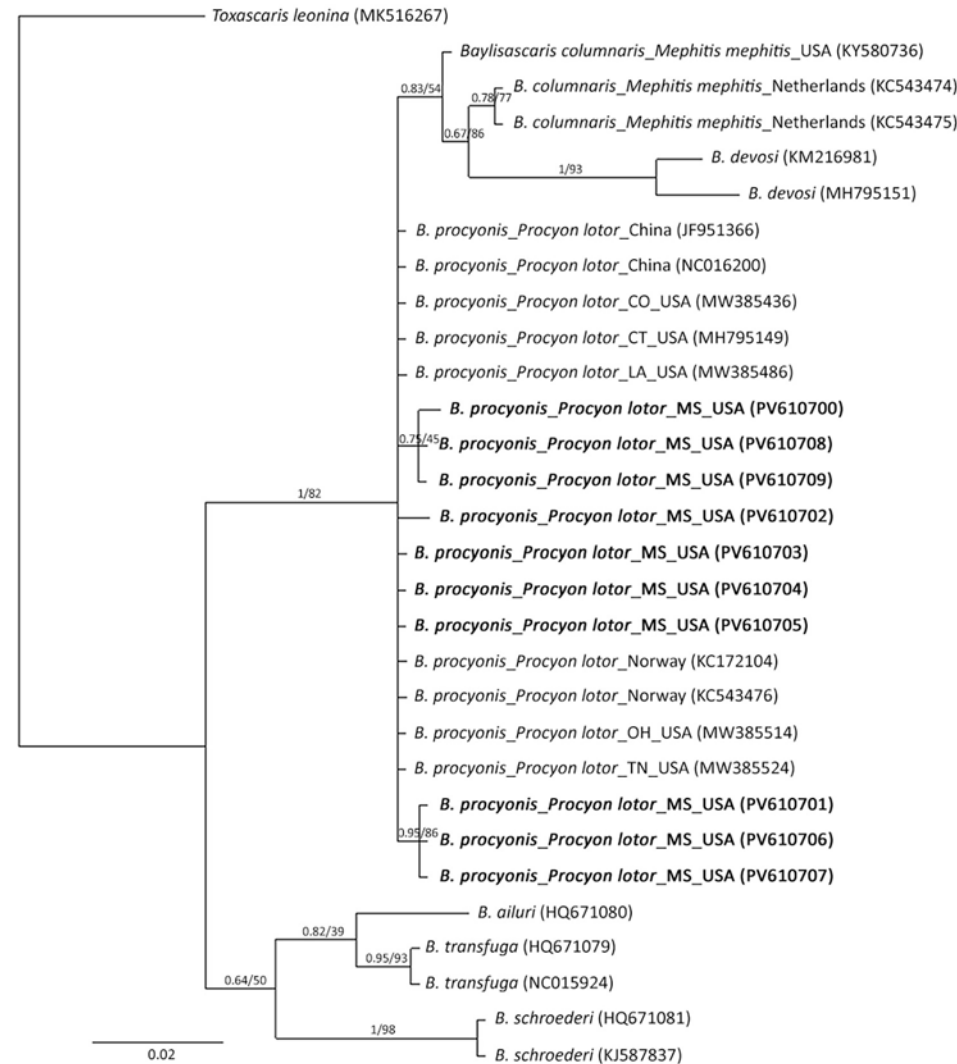


Figure 1. Phylogenetic trees of *Baylisascaris procyonis* *cox1* genes from nematodes (n = 10) collected by necropsy in raccoons, Mississippi, USA, 2023–2024. Analysis was conducted using the general time-reversible plus gamma model. Bayesian posterior probabilities and bootstrap support values (1,000 bootstrap replicates) are shown for each node. GenBank accession numbers are in parentheses. Scale bar indicates number of substitutions per site.

and PV610700–9 [*cox1*]). Phylogenetic analysis of the *cox1* sequences ($n = 10$) showed 100% identity with *B. procyonis* nematodes collected from raccoons in the United States, China, and Norway (Figure 1) (11,12). Necropsy of raccoons revealed *B. procyonis* positivity in 2/8 counties (Humphreys and Yazoo); positivity rates were $\geq 50\%$. The number and length of *B. procyonis* nematodes found in infected adult and juvenile raccoons varied (Appendix Table 1). Higher infection rates were seen in juvenile raccoons than in adult raccoons, as previously reported (1). Identification of *B. procyonis* eggs in fecal samples was hindered by freezing-induced morphological damage, making identification and positivity assessment difficult using traditional microscopic methods (Appendix Figure, panel B).

Our ddPCR analysis of fecal DNA revealed *B. procyonis* positivity in 6 of the 8 counties in Mississippi (Hancock, Hinds, Humphreys, Lincoln, Pearl River, and Yazoo) (Table). The ddPCR results estimated an infection rate of $\geq 50\%$ in Hancock, Humphreys, Lincoln, and Yazoo Counties; the mean infection rate was 26.7% across all 8 counties surveyed. The mean $\pm$ SE concentration of *Baylisascaris* sp. DNA in positive fecal samples was 2.28 ± 1.84 (range 0.16–22.17) (Appendix Table 2). Heatmapping of ddPCR results estimates *B. procyonis* positivity in raccoons throughout large portions of central and southern Mississippi and high positivity along the Mississippi-Louisiana border (Figure 2).

Conclusions

We report evidence of *B. procyonis* roundworms in Mississippi, previously reported in neighboring states in the southeastern United States, including Louisiana, Georgia, Tennessee, and Arkansas (5,8,9,13,14). Using necropsy, we found an overall *B. procyonis* roundworm statewide prevalence of 13.0% across all counties tested.

Fecal analysis using ddPCR detected more *B. procyonis* infections than did necropsy analyses; the average statewide positivity rate was 26.7% across all counties, similar to the 31.7% fecal prevalence reported in Louisiana (5) using quantitative PCR (qPCR) techniques. ddPCR analysis indicated positive results from raccoons in Hancock, Hinds, Humphreys, Lincoln, Pearl River, and Yazoo Counties. Fecal positivity using ddPCR ranged from 17.6% to 100.0%. The limitation of convenience sampling in some counties might account for higher detection rates; only 1–3 raccoons were sampled in 4 counties. Raccoon infection rates also varied by season, age, raccoon population crowding, and age of animals (6,15). Overall, the in-

fection rate estimate based on ddPCR data was higher and indicated the parasite was more widespread than was suggested by visual examination through necropsy alone. This discrepancy might be explained by the difficulty of conducting comprehensive necropsy across the entire intestine of raccoons, where some nematodes might have been overlooked; the difficulty of L3 and L4 larvae detection; or the migration of intestinal nematodes into the stomach after host death. We also found that freezing fecal samples altered *B. procyonis* egg morphology, making parasite eggs harder to identify using traditional microscopic methods. Overall, fecal analysis using ddPCR proved to be a simpler, more sensitive, and more comprehensive sampling strategy, supporting it as the preferred approach to estimate infection rates for this parasite of human-health concern.

The data represented in this study are limited by the small sample size from each county, and this study serves as proof of concept that this parasite is demonstrable in Mississippi raccoons, indicating the need for increased provider awareness and animal

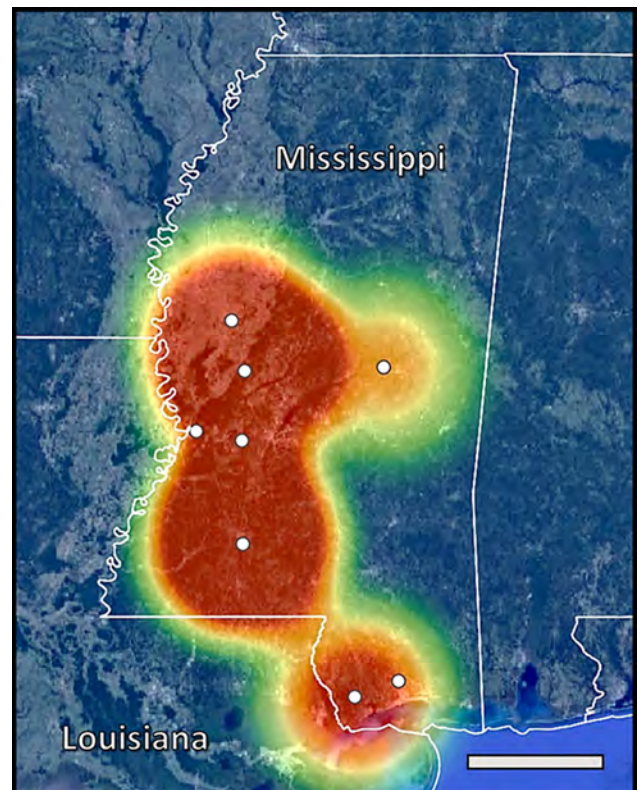


Figure 2. Locations of Mississippi counties sampled in study of zoonotic *Baylisascaris procyonis* in raccoons, Mississippi, USA, 2023–2024. Map indicates geographic center of counties sampled (white circles) and heatmapping estimates of *B. procyonis* positivity at the county level on the basis of fecal analysis using droplet digital PCR. Scale bar indicates 75.0 miles.

surveillance because of the human pathogenic potential of this parasite. Taken together, the data estimate low prevalence across much of the surveyed range and spots of hyperendemicity. Future large-scaled surveillance efforts to more accurately estimate infection rates and fully assess the geographic range of this parasite in Mississippi are needed. Overall, our data suggest that raccoons in Mississippi harbor *B. procyonis* infection, representing a danger to human health.

Acknowledgments

We thank the local Mississippi residents who donated raccoons to this study.

This work was supported by the Mississippi IDeA Network of Biomedical Research Excellence, funded by an Institutional Development Award from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476. This work was also funded by Mississippi College Office of Research.

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Genetic Cluster of Extended-Spectrum β -lactamase–Producing *Klebsiella pneumoniae* in Humans and Food, Switzerland, 2018–2019

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We describe a cluster of genetically related extended-spectrum β -lactamase–producing *Klebsiella pneumoniae* sequence type 14 recovered from human clinical samples and an alfalfa-cress sample collected by systematic food sampling in Basel, Switzerland. Our findings suggest food could represent a reservoir contributing to spread of extended-spectrum β -lactamase–producing Enterobacterales.

Since 2017, the World Health Organization has classified extended-spectrum β -lactamase (ESBL)–producing *Klebsiella pneumoniae* as a critical public health priority (1). Healthcare settings are one known reservoir (2), but food might represent an underestimated and insufficiently investigated source (3–5). We investigated ESBL-producing Enterobacterales (ESBL-PE) in humans and the environment in Basel, Switzerland.

The Study

We designed and conducted a prospective study during June 2017–June 2019 to investigate the epidemiology of ESBL-PE in Basel, Switzerland (6) (Appendix, <https://wwwnc.cdc.gov/EID/article/31/10/25-0253-App1.pdf>). We collected monthly food and

wastewater samples at predefined locations across Basel (7,8). The University Hospital Basel (USB) collected patient samples during routine clinical care. Microsynth AG (Balgach, Switzerland; <https://www.microsynth.com>) performed whole-genome sequencing of ESBL-PE isolates from clinical and food samples by using NextSeq 500 or 550 platforms (Illumina, <https://www.illumina.com>) on 150-bp paired-end reads, according to the manufacturer's protocols (Appendix). We used core-genome multilocus sequence typing (cgMLST) to determine genetic relatedness among sequences. We used the MinION Mk1B or GridION sequencer and R.10.4.1 flow cells (all Oxford Nanopore Technologies, <https://nanoporetech.com>) to conduct nanopore sequencing on selected isolates from genetically related clusters (Appendix).

We screened genomes from ESBL-PE isolates for antimicrobial resistance and plasmid genes. We conducted genomic comparisons against comprehensive international databases.

During the 13-month study period, 12 of 947 food isolates tested positive for *K. pneumoniae* and had ESBL subsequently confirmed through phenotyping. The first clinical ESBL-producing *K. pneumoniae* sequence type (ST) 14 isolate (P0745) was recovered in May 2018 from a urine sample collected from a female outpatient (patient A) in her 70s treated in the USB emergency department for abdominal pain and general weakness (Figure 1). She had a history of recurrent urinary tract infections. At that emergency department visit, catheter-associated urinary tract infection was diagnosed and treated with ertapenem for 14 days after ESBL-producing *K. pneumoniae* was confirmed (Appendix).

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DOI: <https://doi.org/10.3201/eid3110.250253>

¹These first authors contributed equally to this article.

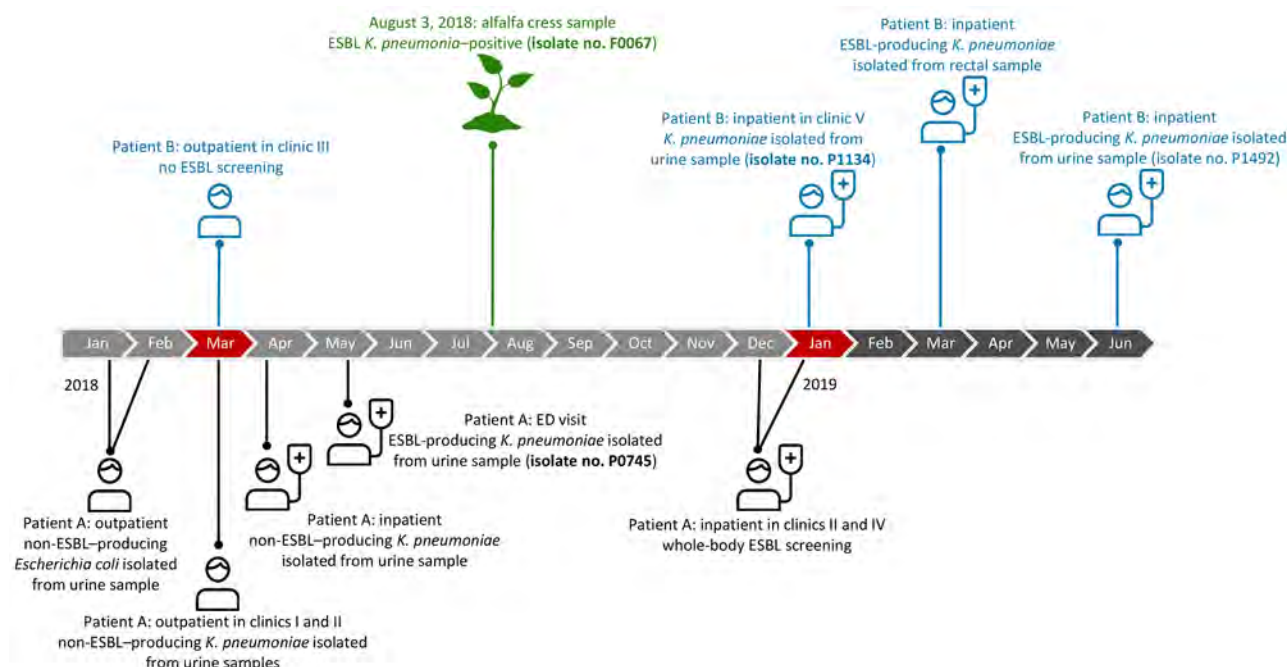


Figure 1. Timeline showing history of isolate collection in detection of ESBL-producing *Klebsiella pneumoniae* in human and food samples, Switzerland, 2018–2019. Bold font indicates isolates in the Basel cluster. Clinical ESBL-producing *K. pneumoniae* sequence type 14 isolates were collected from patient A (isolate no. P0745) in May 2018 and from patient B (isolate no. P1134) in January 2019. Both patients were treated at the University Hospital Basel (Basel, Switzerland) but at different times and in different clinics (Appendix, <https://wwwnc.cdc.gov/EID/article/31/10/25-0253-App1.pdf>). Isolates P1134 and P0745 formed a mixed-cluster of ESBL-producing *K. pneumoniae* isolates with food isolate (F0067), which was collected from alfalfa cress in August 2018. Months with overlapping visits or stays between both patients are depicted in red, and reference numbers of different clinics are provided to assess spatial proximity. ED, emergency department; ESBL, extended-spectrum β -lactamase.

Another clinical ESBL-producing *K. pneumoniae* ST14 isolate (P1134) was collected in January 2019 from a urine sample of another female patient (patient B) in her 70s admitted to USB for a cardiac disorder (Figure 1). Isolate P1134 was detected on day 6 of hospitalization and assessed as colonization. We investigated possible direct or indirect contact between the 2 patients but found no epidemiologic link (Appendix).

Phylogenetic analysis revealed an ESBL-producing *K. pneumoniae* ST14 isolate from 1 food sample that clustered with samples from the 2 patients. The food isolate (F0067) was recovered from 1 alfalfa cress sample organically produced in Switzerland and purchased from a Basel supermarket on August 3, 2018 (Figure 1). We identified 2 additional ESBL-producing *K. pneumoniae* isolates from samples collected from the same supermarket, 1 from mung beans purchased on November 9, 2018, and 1 from alfalfa cress purchased on February 8, 2019. All food samples were produced and packaged on the same farm in Switzerland. The 2 additional food isolates belonged to unrelated STs (the mung bean isolate was ST1310 and the second alfalfa cress isolate was ST2670), exhibited >1,990 allelic differences from F0067, and carried

different combinations of ESBL genes and plasmid replicons (Appendix).

Genome comparison of isolates P0745, P1134, and F0067 revealed 6 cgMLST allelic differences and 12 single-nucleotide polymorphisms (SNPs) between F0067 and the clinical isolate of patient A (P0745). We also detected 3 cgMLST allelic differences and 7 SNPs difference between F0067 and the isolate from patient B (P1134). SNPs were scattered along the chromosomes, but none were in plasmids (Appendix Table 1).

cgMLST comparison of 967 global publicly available *K. pneumoniae* ST14 genomes and 14 isolates from our collection (including the clustered isolates) revealed 2 additional clinical isolates grouped within our cluster. Those isolates, collected in 2019 and 2020 at Jena University Hospital (Jena, Germany), showed 4–9 allelic differences from the isolates from Switzerland (Appendix Figure 2, panel A). The cluster reported in this study differed from other *K. pneumoniae* ST14 genomes by ≥ 58 alleles, and other isolates from Switzerland were dispersed across the minimum spanning tree, clustering with isolates from different countries and continents (Figure 2, panel A). The

clustered isolates, including those from Germany, belonged to the same major lineage, indicating close phylogenetic relatedness (Appendix). Differences occurred only at the most specific level (10th node), suggesting minimal divergence within a clonal cluster.

The minimum mean average nucleotide identity among the 3 genomes from Basel was 99.99% (Appendix Figure 2, panel B). All isolates carried the KL2 capsule locus and the O1v2 locus, but we detected no additional known virulence genes. However, we detected a variant of the ESBL gene *bla*_{SHV-106}, along with *oqx*A, *oqx*B, and *fos*A6, in those chromosomes (Appendix Table 2). The 3 isolates from Basel also shared a 120,635-bp ESBL plasmid of the IncFIA replicon type (Figure 2, panel B; Appendix Table 3), containing the ESBL gene *bla*_{CTX-M-15}, and additional resistance genes for β -lactams (*bla*_{TEM-1B}), sulfonamides (*sul*2), trimethoprim (*dfr*A14), aminoglycosides (*aph*(6)-Ia, *aph*(3'')-Ib), quinolones (*qnr*B1), and tetracycline (*tet*A) (Appendix Table 4). In addition,

they shared a 193-kbp putative conjugative heavy metal resistance plasmid harboring genes for resistance to copper, arsenic, and silver (Appendix Table 3, Appendix Figure 3). We sequenced 1 additional *K. pneumoniae* ST14 isolate from patient B that shared most of those resistance genes and plasmid replicons, suggesting existence of a similar ESBL plasmid (Appendix Figure 1).

International comparison revealed plasmids from Basel had closest similarity (0.995 Mash identity [https://github.com/marbl/mash]) with a 130-kbp ESBL plasmid from a *K. pneumoniae* ST4 isolate collected in 2019 from wastewater in Japan (9) (Figure 2, panel B). That plasmid showed high synteny and included all antimicrobial resistance genes detected in the plasmids of the isolates from Switzerland and Germany (Appendix Table 4, Figure 4). However, the metal-resistant plasmid found in the isolates from Switzerland was absent from the isolates from Japan and Germany.

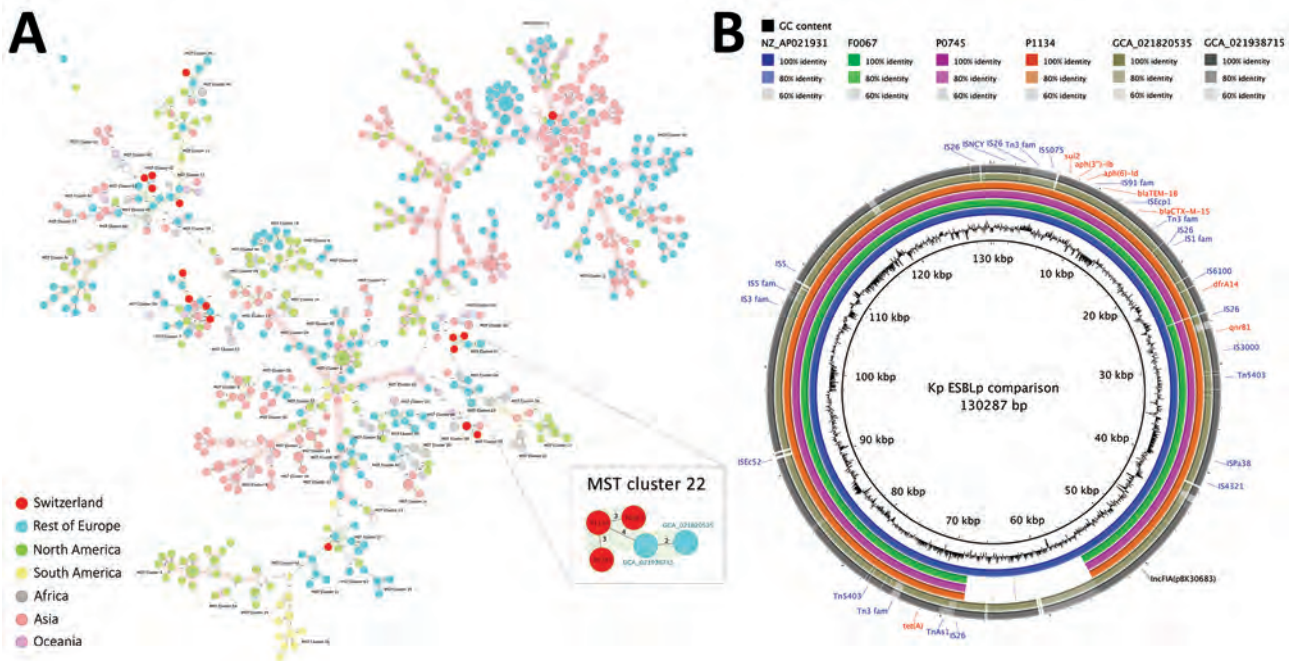


Figure 2. Phylogeny and related plasmids detected in ESBL-producing *Klebsiella pneumoniae* in human and food samples, Switzerland, 2018–2019. A) MST of 972 *Klebsiella pneumoniae* sequence type 14 international samples based on core-genome multilocus sequence typing (cgMLST) profiles (*K. pneumoniae* sensu lato cgMLST version 1.0 in SeqSphere+ version 9.0.0 [Ridom, https://www.ridom.de]). Inset image shows detail the mixed MST cluster 22. Color-filled circles denote the different geographic origin. Isolates of the same cluster (≤ 15 allelic differences) are surrounded by a colored shadow. Numbers in connecting lines represent allelic distance values. B) Circular BLAST Ring Image Generator (BRIG, https://github.com/happykhan/BRIG) comparison of the ESBL plasmids from isolates in this study. Plasmid pWP2-W18-ESBL-06_2 (accession no. NZ_AP021931; blue ring) was identified as the closest hit in the PLSOB database version 2023_11_03_v2 (Universität des Saarlands, https://ccb-microbe.cs.uni-saarland.de/plsodb2025). Plasmid of the food isolate (F0067) ESBL-producing *K. pneumoniae* shown in green. Patient A isolate (P0745) shown in pink ring; patient B isolate (P1134) shown in orange ring. Brown-gray rings show short-read assembly from isolates GCA_21938715 and GCA_021820535 from the University Hospital Jena, Jena, Germany. Color intensity of concentric rings represents percent identity to the reference plasmid, pWP2-W18-ESBL-06_2. GC content and plasmid length are displayed as inner rings. Outer rings show antimicrobial resistance genes (red font), replicons (black font), and IS and Tn (blue font) sequences. ESBL, extended-spectrum β -lactamase; IS, insertion sequence; MST, minimum spanning tree; Tn, transposon.

Conclusions

We describe a cluster of closely related ESBL-producing *K. pneumoniae* ST14 isolates from human clinical samples collected at 2 hospitals in Europe and an alfalfa cress sample collected during systematic food sampling. Although we could not confirm a direct link between the patients and the food source, the close genetic relatedness and the geographic proximity and sampling dates suggest a recent common ancestor. Those findings highlight that food could be a potential reservoir in the spread of ESBL-PE. The national antimicrobial resistance surveillance system in Switzerland indicated stable resistance trends in *E. coli* and *K. pneumoniae* over the past decade (<https://www.an-resis.ch/antibiotic-resistance/resistance-data-human-medicine>), supporting the relevance of our findings despite the time lapse after sample collection.

ESBL-producing *K. pneumoniae* clonal group 14, particularly CTX-M-15 producers, have caused human outbreaks globally (10,11) and have been detected in various fruits and vegetables (3), wastewater treatment plants, and rivers (12). The broad ecological range demonstrates that clonal group's adaptability and its potential to disseminate antimicrobial resistance genes among isolates from food, environmental, and human sources. The close relatedness of strains and plasmids from our study to others identified globally further supports that potential. The detection of a similar ESBL plasmid in a wastewater *K. pneumoniae* ST4 isolate from Japan suggests environmental dissemination among diverse genetic lineages. In addition, a plasmid with metal tolerance and resistance genes might enable those bacteria to persist in harsh environmental conditions. That case, combined with an ESBL-producing *K. pneumoniae* food-human cluster we reported previously (13), adds to the limited evidence that food might be an overlooked and underestimated source of clinically relevant ESBL-producing *K. pneumoniae* clones (4).

In conclusion, this study highlights the value of investigating foodborne transmission routes to control the spread of clinically relevant antimicrobial-resistant bacterial strains. Stricter hygiene practices might be required throughout the food production, handling, and consumption cycle to prevent further dissemination of ESBL-PE to the general population.

High-resolution images may be requested directly from the authors.

This work was financed by the University Hospital Basel, the University of Basel, and the Swiss National Science Foundation under National Research Program Antimicrobial Resistance (NRP 72, grant no. 407240_167060).

L.A.-B. and E.G.-S. collected and analyzed the data, created figures and tables and wrote the draft of the manuscript. I.V., M.A., R.S., L.M.P., R.F., L.E., I.S., A.B.G.-M., and T.S. contributed to data collection and revised the manuscript. C.B. conceptualized the study and critically revised the manuscript. S.T.S. conceptualized the study, obtained the funding and critically revised the manuscript. All authors approved the final version of the article.

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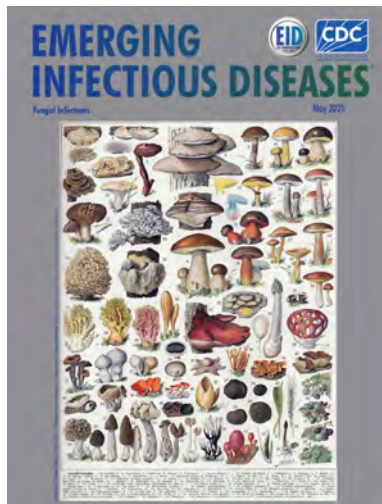
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May 2025

Fungal Infections

- Outbreak of Marburg Virus Disease, Equatorial Guinea, 2023
- Comprehensive Survival Analysis of Alveolar Echinococcosis Patients, University Hospital Zurich, Zurich, Switzerland, 1973–2022
- Features of Invasive Aspergillosis Caused by *Aspergillus flavus*, France, 2012–2018
- Nationwide Observational Case–Control Study of Risk Factors for *Aerococcus* Bloodstream Infections, Sweden
- Powassan and Eastern Equine Encephalitis Virus Seroprevalence in Endemic Areas, United States, 2019–2020
- Highly Pathogenic Avian Influenza A(H5N1) Outbreak in Endangered Cranes, Izumi Plain, Japan, 2022–23
- Metagenomic Identification of *Fusarium solani* Strain as Cause of US Fungal Meningitis Outbreak Associated with Surgical Procedures in Mexico, 2023
- Detection of SARS-CoV-2 Reinfections Using Nucleocapsid Antibody Boosting
- Postexposure Antimicrobial Drug Therapy in Goats Infected with *Burkholderia pseudomallei*
- Autochthonous *Leishmania (Viannia) lainsoni* in Dog, Rio de Janeiro State, Brazil, 2023



- Exponential Clonal Expansion of 5-Fluorocytosine–Resistant *Candida tropicalis* and New Insights into Underlying Molecular Mechanisms
- Administration of L-Type Bovine Spongiform Encephalopathy to Macaques to Evaluate Zoonotic Potential
- *Tropheryma whipplei* Infections, Mexico, 2019–2021
- Napoleon Bonaparte—A Possible Case of Trench Fever
- Rapid Transmission and Divergence of Vancomycin-Resistant *Enterococcus faecium* Sequence Type 80, China
- Self-Reported SARS-CoV-2 Infections among National Blood Donor Cohort, United States, 2020–2022
- Molecular Detection of Histoplasma in Bat-Inhabited Tunnels of Camino de Hierro Tourist Route, Spain
- Influenza A(H1N1)pdm09 Virus with Reduced Susceptibility to Baloxavir, Japan, 2024
- High Prevalence of Influenza D Virus Infection in Swine, Northern Ireland
- Recent and Forecasted Increases in Coccidioidomycosis Incidence Linked to Hydroclimatic Swings, California, USA
- Clade Ia Monkeypox Virus Linked to Sexual Transmission, Democratic Republic of the Congo, August 2024
- Unexpected Zoonotic and Hybrid Schistosome Egg Excretion Patterns, Malawi, 2024
- Emergence of Feline Sporotrichosis near Brazil Border, Argentina, 2023–2024
- *Trichophyton indotineae* Infection, São Paulo, Brazil, 2024

**EMERGING
INFECTIOUS DISEASES**

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Emergence of *Bordetella holmesii*–Associated Pertussis-Like Illness, Northern India, 2019–2023

Nishant Shekhar, Deepa Sharma, Surajit Chakraborty, Rajesh Kumar, Rajneesh Singh Rawat, Nirmal Kaundal, Pawan Kumar, Tigran Avagyan, Deeksha Chauhan, Neha Jain, Megha Sharma, Arun Kumar, Vikas Gautam

We investigated *Bordetella holmesii* and *Bordetella pertussis* in 935 suspected pertussis cases in northern India (2019–2023) using PCR and serology. *B. holmesii* showed increased prevalence in pertussis cases, particularly in older children, highlighting its emerging role and the need for ongoing surveillance and adjusted prevention strategies.

Pertussis, caused by *Bordetella pertussis*, is a serious, vaccine-preventable respiratory illness (1,2). India's immunization program recommends multiple tetanus-diphtheria-pertussis vaccine doses (3). The COVID-19 pandemic impacted vaccination rates and pertussis reporting across India, leading to a resurgence in pertussis cases (Appendix Figure 1, <https://wwwnc.cdc.gov/EID/article/31/10/24-1659-App1.pdf>). Concerns exist regarding other *Bordetella* species, such as *Bordetella holmesii*, a bacterium known to cause pertussis-like symptoms but not covered by current vaccines (4–7). Laboratory confirmation is crucial in differentiating *B. pertussis* from *B. holmesii* for accurate surveillance (8). Our study aimed to determine the incidence of *B. pertussis* and *B. holmesii* in pertussis-like cases in northern India.

The Study

We analyzed 935 respiratory specimens collected from case patients clinically suspected of having pertussis

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DOI: <http://doi.org/10.3201/eid3110.241659>

in northern India during January 2019–August 2023. We sourced specimens from 2 cohorts: group I (n = 213), comprising patients hospitalized with acute respiratory illness at Postgraduate Institute of Medical Education and Research's Advanced Pediatric Center; and group II (n = 722), obtained through the Vaccine-Preventable Disease Surveillance Network according to guidelines (9). We subjected 778 samples (178 from group I, 600 from group II) to real-time quantitative PCR (qPCR) (10) and examined 733 samples (101 from group I, 632 from group II) through serologic testing for pertussis toxin (PT) IgG using ELISA.

Of 778 swab samples tested by qPCR, we determined 383 (49.2%) to be positive for *Bordetella* IS481. Among those, we identified *B. pertussis* monoinfection in 52 (13.6%) samples, *B. holmesii* monoinfection in 143 (37.3%), and co-infection with both species in 15 (3.9%) samples (Table 1). Of note, *B. holmesii* positivity surpassed that of *B. pertussis* in 2022–2023 in the North India catchment. (Figure).

Both species predominantly affected infants <1 year of age. However, *B. holmesii* was significantly more prevalent in the 5–10-year age group ($\approx 30\%$ compared with $\approx 9\%$ *B. pertussis*; $\chi^2 = 16.22$; $p = 0.00102$). The mean patient age for *B. holmesii* infection was 3.4 years and for *B. pertussis* was 1.9 years (Appendix Figure 2). We detected PT IgG with overall seroprevalence (positive + intermediate) in 232 (31.6%) of the 733 samples tested. We collected serology samples from 174 of the 210 cases that tested positive by qPCR.

Stratifying the qPCR and serology results of the samples for which qPCR and serology results were obtained, we identified 121 *B. holmesii* qPCR-confirmed cases, 43 of which we determined to be positive for PT IgG by ELISA. By that same method, we identified 41 qPCR-confirmed *B. pertussis* cases, determining 9 to be positive for PT IgG by ELISA (Table 2). Among 91 cases with known diphtheria-pertussis-tetanus vaccination status, 28 ELISA-negative, unvaccinated

Table 1. Results of real-time quantitative PCR using the IS481, *ptxS1*, and *hIS1001* genes to differentiate *Bordetella* species in study of emergence of *Bordetella holmesii*–associated pertussis-like illness, northern India, 2019–2023

Category	No. samples
IS481	
Positive for <i>Bordetella</i> by multiplex PCR	197
Monoplex PCR, <i>ptxS1</i>	
Positive for <i>B. pertussis</i>	52
Negative	145
pIS1001	
Positive for <i>Bordetella</i> by multiplex PCR	17
Monoplex PCR, <i>ptxS1</i>	
Positive for <i>B. parapertussis</i>	2
Negative	15
IS481 and <i>hIS1001</i>	
Positive for <i>Bordetella</i> by multiplex PCR	143
Monoplex PCR, <i>ptxS1</i>	
Positive for <i>B. pertussis</i> and <i>B. holmesii</i>	15
Negative for <i>B. holmesii</i>	128
IS481 and pIS1001	
Multiplex PCR positive	26
Monoplex PCR, <i>ptxS1</i>	
Positive for <i>B. pertussis</i> and <i>B. parapertussis</i>	7
Negative	19
Total	778

case-patients (i.e., received no pertussis-containing vaccine [zero-dose]) tested positive for *B. holmesii* mono-infection and 8 ELISA-negative, unvaccinated case-patients tested positive for *B. pertussis* mono-infection.

Conclusions

Our study highlights the emergence of *B. holmesii* as a key contributor to pertussis-like illness in northern India during 2021–2023. Species-specific qPCR revealed that *B. holmesii* detection rates overtook those of *B. pertussis* in the 2022–2023 period, particularly among children 5–10 years of age, suggesting an evolving epi-

demiology distinct from classic pertussis and echoing observations noted by researchers investigating other *B. holmesii* outbreaks (11–13). Our study demonstrates evidence of notable *B. holmesii* circulation in the India subcontinent, underscoring this pathogen's potential role in co-infection and also as a primary etiologic agent, as demonstrated by the presence of this bacterium in unvaccinated PT IgG-negative case-patients (despite limitations in detailed clinical characterization of pertussis-like symptoms [e.g., cough duration, paroxysms] observed uniformly across cases).

In serology testing, PT IgG positivity (Table

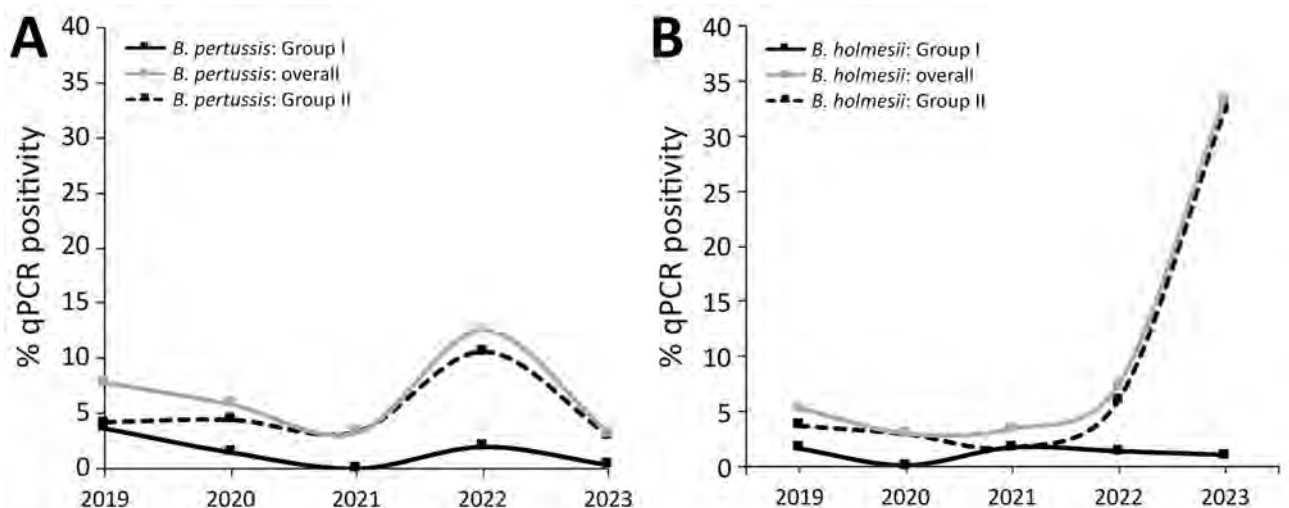


Figure. Yearwise trend of *Bordetella pertussis* and *B. holmesii* positivity from study of emergence of *B. holmesii*–associated pertussis-like illness, northern India, 2019–2023. Data based on real-time quantitative PCR using the IS481-*ptxS1*-*hIS1001* gene profile. Positivity is shown overall and for 2 cohorts: group I ($n = 213$), patients hospitalized with acute respiratory illness at Postgraduate Institute of Medical Education and Research's Advanced Pediatric Center; and group II ($n = 722$), obtained through the Vaccine-Preventable Disease Surveillance Network according to guidelines (9).

Table 2. Pertussis toxin IgG seroprevalence of real-time quantitative PCR-confirmed *Bordetella pertussis* and *B. holmesii* cases in study of emergence of *B. holmesii*-associated pertussis-like illness, northern India, 2019–2023

Serology	<i>B. pertussis</i>	<i>B. holmesii</i>	Co-infection with <i>B. pertussis</i> and <i>B. holmesii</i>
Positive	9	43	5
Negative	32	78	7
Total	41	121	12

1) among cases with *B. holmesii* monoinfection (qPCR-positive cases) might reflect recent *B. pertussis* exposure or vaccination. The absence of PT, pertactin, and fimbrial antigens from the *B. holmesii* genome and the lack of cross-protective immunity in whole-cell or acellular pertussis vaccines in animal models further reveals critical gaps in current immunization strategies (4,5). Although *B. holmesii* shares a filamentous hemagglutinin homologue and a conserved 66-kb pathogenicity island with *B. pertussis*, those shared elements have not yielded effective cross-protection or reliable serologic markers (14).

Our findings highlight the practicality of integrating molecular assays into routine surveillance to accurately distinguish *Bordetella* species and guide public health responses. Future research efforts should include more detailed clinical characterization of patients, population-based seroepidemiologic studies, and retrospective analysis of archived samples to clarify the historical prevalence of *B. holmesii*. Revisiting vaccine antigen composition to address nonpertussis *Bordetella* species might help close critical prevention gaps in pertussis-like disease control.

Acknowledgment

The authors thank the World Health Organization's Immunization Division's Vaccine Preventable Disease project team for their support.

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Escherichia coli Sequence Type 131-H22 in Parrots from Illegal Pet Trade, Brazil, 2024

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Escherichia coli sequence type 131:H:22 is a consequential lineage of extraintestinal pathogenic *E. coli*, associated with human pyelonephritis and sepsis. We report the transmission of avian pathogenic *E. coli* in a parrot rehabilitation center in Brazil and the presence of a high-risk zoonotic lineage of extraintestinal pathogenic *E. coli* sequence type 131-H22.

The regulation of the international wildlife trade is critical for the conservation of species and, according to the 2022 World Wildlife Trade Report (https://cites.org/sites/default/files/common/docs/Pilot_World_Wildlife_Trade_Report_for_CITES_CoP19.pdf), generates an annual revenue of USD ≈220 billion. However, in most countries, there is still an illegal wildlife trade that affects biodiversity, risks the extinction of some species, and leads to transmission of zoonotic diseases. According to the Convention on International Trade in Endangered Species of Wild Fauna and Flora, during 2011–2020, birds occupied the second place of most trafficked genera, behind only plants (1). Parrots are one of the most frequently sold birds in Brazil, and confiscated birds can act as reservoirs of bacteria such as avian pathogenic *Escherichia coli* (APEC) (2,3). In this article, we investigated the transmission of APEC in a group of 19 psittacine birds in Brazil.

The Study

Fifteen blue-fronted Amazon parrots (*Amazona aestiva*) and 4 macaws (*Ara ararauna*) were confiscated in the state of São Paulo, Brazil, by law enforcement authorities and kept for 4 weeks in a rescue center. At

the rescue center, fecal samples were collected at intervals of 5 days. After enriching the samples in brain-heart infusion broth (BD, <https://www.bd.com>), we cultured 20 µL of the broth on MacConkey agar (BD) at 37°C for 24 hours. We then conducted biochemical identification on the isolates. We screened *E. coli* strains by using PCR for minimal predictor virulence genes (*ompT*, *iss*, *hlyF*, *iutA*, and *iroN*), as described previously (4). This study was approved by the Ethics and Animal Use Committee of the School of Veterinary Medicine and Animal Science from the University of São Paulo (approval no. 1572080922).

We detected a total of 9 APEC strains. The transmission dynamic revealed that 1 parrot was colonized by APEC on day 1 (the day of seizure). One macaw became infected 5 days after entering the rescue center. After 30–35 days, 6 parrots and 1 macaw were positive for APEC. Next, we screened the strains for high-risk APEC, as proposed previously (5). We conducted whole-genome sequencing on 1 positive strain (RG299.1, isolated from an *A. aestiva* (parrot) by using MiSeq (Illumina, <https://www.illumina.com>). We used the Galaxy Europe platform (<https://usegalaxy.eu>) to analyze the genome and assess the quality of raw data and assemblies. To determine the sequence typing, plasmids, and virulence genes, we used the ResFinder (<https://genepi.food.dtu.dk/resfinder>), PlasmidFinder (<https://cge.food.dtu.dk/services/PlasmidFinder>), and Virulence Finder (<https://cge.food.dtu.dk/services/VirulenceFinder>) databases.

For the phylogenetic analysis, we selected *E. coli* sequence type (ST) 131 O25:H4 genomes available on Enterobase (<https://enterobase.warwick.ac.uk/species/ecoli>) and compared with the genome of the RG299.1 strain from our study. We included 63 genomes in total. We aligned single nucleotide polymorphisms by using snippy core v4.6.0. (<https://github.com/tseemann/>

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DOI: <https://doi.org/10.3201/eid3110.241279>

snippy). We inferred the tree by using IQ-Tree v2.1.2 (<https://iqtree.github.io>) to perform neighbor joining with 1,000 bootstraps and rooted them at the midpoint. We conducted visualization and metadata analysis by using iTOL (<https://itol.embl.de>). The RG299.1 strain showed a close relation with uropathogenic *E. coli* (UPEC) (Figure).

Sequencing confirmed that the study RG299.1 strain (GenBank accession no. SRR31896384) belongs to ST131 phylogroup B2 and serogroup O25:H4. Further characterization of the RG299.1 strain identified allele 22 of the *fimH* type 1 fimbrial adhesin gene (*H22*), confirming that strain belongs to ST131-H22

sublineage (6). Currently, ST131-H22 is described as a pandemic lineage of *E. coli*, drawing attention because of high levels of adaptation and resistance to multiple drugs, including fluoroquinolones, betalactams, and carbapenems (7,8). The RG299.1 strain did not have an antimicrobial resistance profile, possibly because of lower exposure to antimicrobial drugs in the environmental, compared with nosocomial isolates. PlasmidFinder revealed the presence of incompatibility groups IncFIB and IncFII_1 plasmid, but nontransferable mechanisms of resistance were also found. Only chromosomal genes correlated with antimicrobial resistance were present, such as the efflux pump

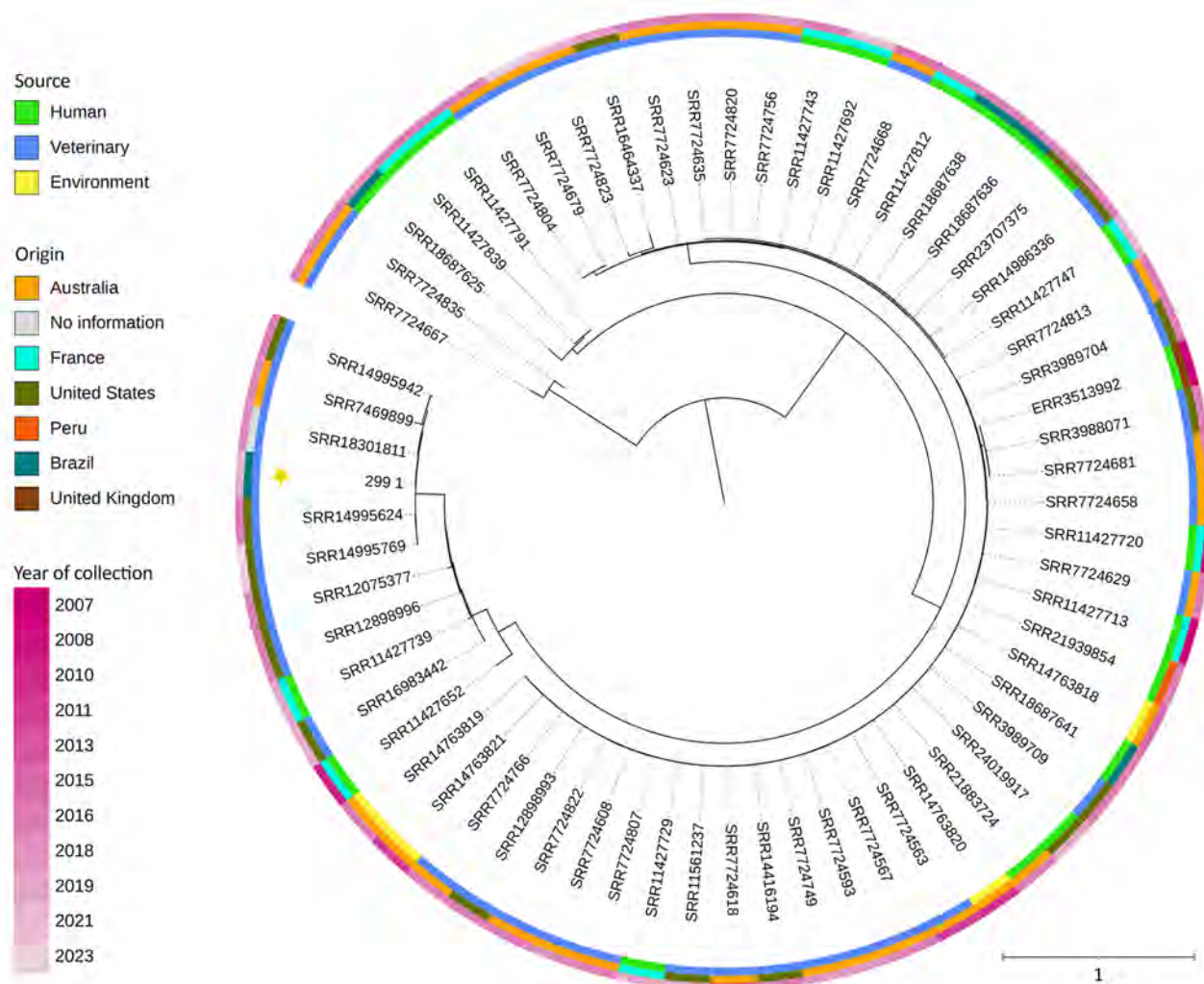


Figure. Phylogenetic analysis through a phylogenetic tree of *Escherichia coli* sequence type (ST) 131 isolated from different origins. The tree is rooted at the midpoint and generated to compare the studied strain with public genomes available in EnteroBase (<https://enterobase.warwick.ac.uk>), connecting 63 strains of ST131 *E. coli*. The subtypes from the database are subdivided into the avian and uropathogenic extraintestinal pathogenic *E. coli* pathotypes, along with their origin and the continent where they were isolated and described. The tree shows closely related strains from veterinary origins in South America and Oceania. Strain 299 from this study forms a monophyletic group with 5 sequences from GenBank (accession nos. SRR14995924, SRR7469899, SRR18301811, SRR14995624, and SRR1444995769). This group is related to 3 uropathogenic *E. coli* strains of human origin in Europe (GenBank accession nos. SRR11427839, SRR11427652 and SRR11427791).

encoder *mdfA*, which can result in quinolone resistance when associated with other acquired genes (9).

The RG299.1 ST131-H22 strain carried virulence genes associated with iron transport (*fpeA*, *entS*, *iutA*, *iroN*, and *fepG*), invasion (*ibeA* and *ompA*), and others related to adherence and pathogenicity, such as type 1 fimbriae genes (*fimA*, *fimB*, *fimF*, *fimG*, and *fimH*) (Table). The parrot colonized by strain RG299.1 on the day of seizure died after 15 days. Although the cause of the bird's death was not investigated, it is of note that the presence of virulent strains of *E. coli* in the intestines of Psittaciforme birds is considered undesirable because the microbiota of those birds typically consists of gram-positive bacteria (3).

APEC and extraintestinal pathogenic *E. coli* can share some virulence factors and plasmids associated with resistance (10–12). A previous study found that APEC and UPEC possess common virulence factors such as *papG II*, *iss*, and *ompT* genes (13), which encode an adhesin and 2 evasins related to colonization of the urinary tract and avoiding the host's immune system. Our results (Table) revealed that the RG299.1 strain carries genes associated with iron transport, invasion, adherence, and pathogenicity. The presence of those genes makes the strain potentially invasive and can contribute to other virulence traits such as biofilm formation (14). Genes involved in the biogenesis of capsules belonging to the capsular group II were also identified, such as *kpsD*, *kpsM*, and *kpsT*. Those genes are responsible for the transport of polysaccharide across the cytoplasmic membrane and their structure in the cell surface (14).

Conclusions

Six strains of *E. coli* ST131-H22 isolated from poultry with colibacillosis were identified in Brazil (2).

Whole-genome sequencing and phylogenetic analysis revealed a high similarity between those strains and strains found in other countries, including strains of human origin, revealing a threat to public health. In wild birds, colibacillosis is a high risk, because the infection by APEC can rapidly progress from airsacculitis to sepsis, leading to animal death.

Our phylogenetic data revealed a close relationship between the strain from the current study and strains of veterinary origin isolated from a chicken (GenBank accession no. SRR14995942) characterizing a food source type, from a turkey (GenBank accession no. SRR7469899), and from a domestic *Gallus gallus* (junglefowl) (GenBank accession no. SRR7469899). The geographic origin of the ST131 strains could be heterogeneous; however, our results demonstrate a major similarity in the variations of this ST among isolates from different continents.

Our findings suggest that illegal animal trade can play a major role in the dissemination of APEC, considering the poor hygiene conditions to which the animals are subjected. The overcrowded wildlife triage centers that receive seized animals might have difficulty controlling pathogen transmission through biosecurity failures. Even though the principal route of contamination for pathogenic *E. coli* strains is through foodborne transmission, domesticated wild birds can potentially serve as an additional contamination route (15).

ST131 is becoming the prevalent extraintestinal pathogenic *E. coli* multidrug-resistant lineage worldwide, highlighting the necessity for monitoring. More studies are needed to investigate the presence of high-risk lineages of APEC in wild birds. A PCR triage to detect ST with pandemic potential is necessary in the routine care of birds. This monitoring becomes

Table. Virulence and resistance data of avian pathogenic *Escherichia coli* RG299.1 ST131 strain from parrots in Brazil, encoding genes and their products found in PlasmidFinder, VirulenceFinder, and ResFinder databases*

Product	Gene or plasmid	Virulence association
Mobility elements	<i>IncFIB</i> , <i>IncFII_1</i>	Plasmid
Enterobactin components	<i>entD</i> , <i>fepA</i> , <i>fes</i> , <i>fepC</i> , <i>fepG</i> , <i>fepD</i> , <i>entS</i> , <i>fepB</i> , <i>entE</i> , <i>entF</i> , <i>iutA</i> , <i>iucD</i> , <i>iucC</i> , <i>iucB</i> , <i>iucA</i>	Iron acquisition
Iron transport	<i>chuS</i> , <i>chuA</i> , <i>chuT</i> , <i>chuX</i> , <i>chuU</i>	
Yersiniabactin siderophore biosynthetic protein	<i>ybtE</i> , <i>ybtT</i> , <i>ybtU</i> , <i>irp1</i> , <i>irp2</i> , <i>fyuA</i>	
Salmocheilin siderophore system	<i>iroB</i> , <i>iroC</i> , <i>iroD</i> , <i>iroE</i> , <i>iroN</i>	
K1 capsule	<i>kpsD</i> , <i>kpsT</i> , <i>kpsM</i>	Capsule
General secretion proteins	<i>gspM</i> , <i>gspL</i> , <i>gspK</i> , <i>gspJ</i> , <i>gspI</i> , <i>gspH</i> , <i>gspG</i> , <i>gspF</i> , <i>gspE</i> , <i>gspD</i> , <i>gspC</i>	General secretory pathway, type II secretion system
Outer membrane protein A	<i>ompA</i>	Invasion
Fimbrial proteins	<i>fimA</i> , <i>fimI</i> , <i>fimC</i> , <i>fimD</i> , <i>fimF</i> , <i>fimG</i> , <i>fimH</i> , <i>fimE</i> , <i>fimB</i>	Adhesins, biofilms
Fimbrial adhesins	<i>yagW/ecpD</i> , <i>fdeC</i>	
Invasion protein	<i>ibeA</i>	Invasion

*PlasmidFinder, <https://cge.food.dtu.dk/services/PlasmidFinder>; ResFinder, <https://genepi.food.dtu.dk/resfinder>; Virulence Finder, <https://cge.food.dtu.dk/services/VirulenceFinder>).

particularly critical when those birds are participating in a program to reintroduce and release. In addition, the next-generation sequencing of APEC varieties can clarify their origin and the relation between them, enabling better monitoring.

This work was funded by Fundação de Amparo a Pesquisa do Estado de São Paulo (grant no. 2022/11917-1), a Conselho Nacional de Desenvolvimento Científico e Tecnológico award to T.K. (grant no. 309921/2023-6), an award to V.G.P.R. (grant no. 141155/2022-1), and Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo (grant no. net 221RT0113).

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Zoonotic Soil-Transmitted Helminth Infections among Humans, Gabon

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We report human infections with *Necator gorillae* and *Strongyloides fuelleborni*, zoonotic helminths from nonhuman primates, in Gabon. We also detected a cryptic *Ancylostoma* species helminth. Infections occurred in settings of localized deforestation and environmental degradation, which increase human–animal contact. Surveillance to clarify the extent of human infections is needed.

Deforestation and environmental degradation across Central Africa, including Gabon, have increasingly brought nonhuman primates (NHPs) close to human settlements. In forest-edge communities, bushmeat hunting and forest resource exploitation further intensify human–NHP interactions (1). Those interactions increase the risk for zoonotic parasitic infections from NHP reservoirs to humans, including infections with soil-transmitted helminths (STHs) (1,2).

In reports from Europe, researchers were infected with *Necator gorillae* hookworm (3,4) and *Strongyloides fuelleborni fuelleborni* threadworm (5) during fieldwork with NHPs in Central Africa, suggesting that zoonotic STH infections might occur extensively in human communities near NHP habitats. A subsequent study among villagers living near NHP habitats in Gabon identified 2 additional human *N. gorillae* infections on the basis of internal transcribed spacer (ITS) 1 and 2 haplotyping analysis (6). To determine the extent of zoonotic STH infections in human populations, we conducted a survey of stool samples from villagers in Gabon.

The Study

We conducted a parasitologic survey of 226 human stool samples in July 2023, during the dry season in Ngounié Province, Gabon. We collected samples from persons in 6 forest-edge communities situated within a tropical savanna climate zone (Köppen classification Aw) (Appendix Figure 1, <https://wwwnc.cdc.gov/EID/article/31/10/25-0816-App1.pdf>).

Upon collection, we preserved stool specimens in 10% formalin and 70% ethanol and shipped them to Australia for analysis. Using formalin ethyl acetate sedimentation microscopy (7), we identified hookworm eggs in 15 samples, *S. f. fuelleborni* roundworm eggs in 6 samples, and *Strongyloides* spp. roundworm larvae in 1 sample (Figure 1). A total of 20 samples were helminth-positive, and some involved co-infections (Appendix Table 1).

We performed metabarcoding on DNA extracts from those 20 samples by targeting the mitochondrial cytochrome c oxidase subunit I (*cox1*) gene and the hypervariable region IV (HVR-IV) of 18S rDNA, 2 well-established genetic markers for helminth species identification (8). We targeted a 217-bp region of *cox1* to identify helminth species (8). Then, to further characterize *Strongyloides* species and genotypes, we conducted a second metabarcoding assay targeting 18S rDNA HVR-IV (≈255 bp) (8) on the 7 *Strongyloides* spp.-positive samples. For both assays, we performed sequencing on a MiSeq platform by using MiSeq Reagent Nano Kit v2 (Illumina, <https://www.illumina.com>) and 500 cycles for 250-bp paired-end reads. We used Geneious Prime version 2024.0.4

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National de Lutte contre les Maladies Parasitaires, Ministère de la Santé, Libreville (D. Nkoghe, J. Atsame); Westmead Hospital, Westmead, New South Wales, Australia (M. Watts); University of Sydney, Sydney, New South Wales, Australia (M. Watts); University of Queensland, Brisbane, Queensland, Australia (C. Gordon); QIMR Berghofer Medical Research Institute, Brisbane (C. Gordon)

DOI: <https://doi.org/10.3201/eid3110.250816>

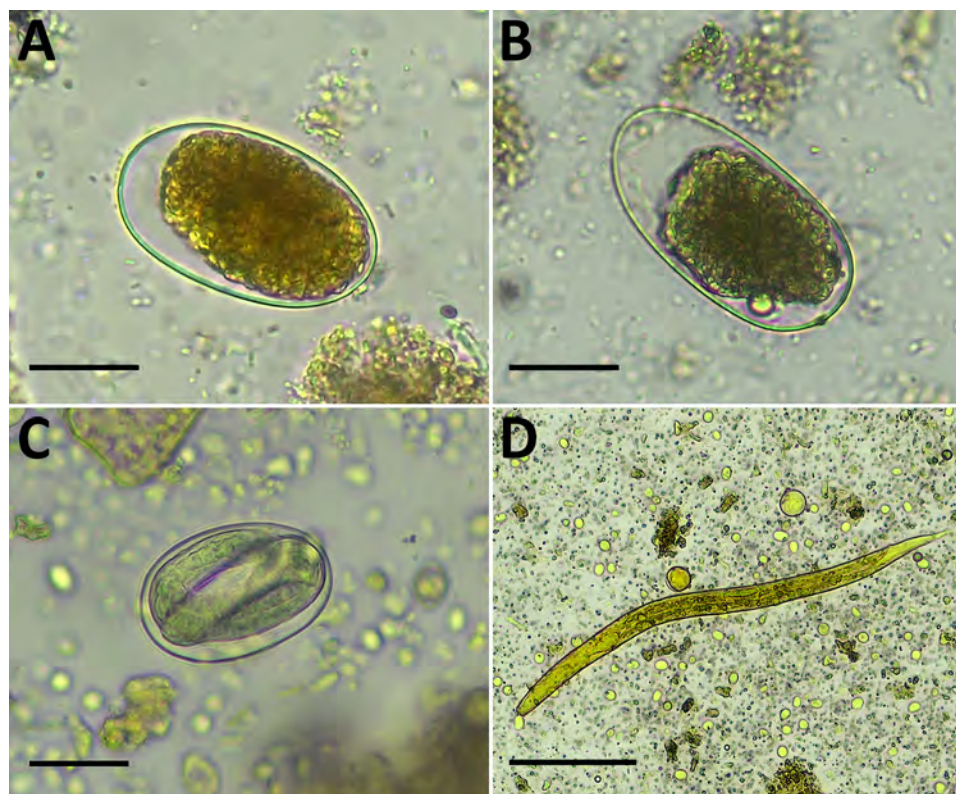


Figure 1. Light microscopy images of soil-transmitted helminths from human stool samples, Gabon. A, B) Hookworm eggs; scale bars indicate 25 μ m. C) *Strongyloides* spp. eggs; scale bar indicates 25 μ m. D) *Strongyloides* spp. larvae; scale bar indicates 50 μ m. Helminths were detected by using the formalin ethyl acetate sedimentation method. Molecular analysis of ethanol-preserved stool sample aliquots from the same patients indicated *Necator americanus* (A), *N. gorillae* (B), *Strongyloides fuelleborni fuelleborni* (C), and *S. stercoralis* (D).

(<https://www.geneious.com>) to analyze sequence data, using a custom workflow incorporating read quality control, contig assembly, and haplotype assignment. We conducted phylogenetic analyses of MUSCLE-aligned (<https://www.ebi.ac.uk/Tools/msa/muscle>) *cox1* sequences by using maximum-likelihood (MEGA 11, <https://www.megasoftware.net>) and Bayesian inference (MrBayes, <https://github.com/NBISweden/MrBayes>) methods and applying the general time-reversible nucleotide substitution model.

Eighteen of the 20 samples yielded *cox1* amplicons. Upon sequencing, 3 samples were dominated by reads from co-infecting *Ascaris lumbricoides* roundworms, but we did not detect sequences for hookworms or *Strongyloides* spp. roundworms. The other 15 samples yielded sequences assigned to *Necator* spp. ($n = 11$), *S. f. fuelleborni* ($n = 3$), or *Ancylostoma* spp. ($n = 1$) helminths (Appendix Table 1). Among the 11 *Necator* spp.-positive samples, 10 harbored *Necator americanus* hookworm, and 4 contained a *Necator* sp. hookworm with *cox1* sequences that had 100% identity to GenBank accession no. AB793562, a species previously detected in researchers from Europe who were infected in the Central African Republic (CAR); that species was later morphologically identified as *N. gorillae* (3). Three of the

4 *N. gorillae*-positive samples had *N. americanus* co-infection (Appendix Table 1).

Analysis of *cox1* sequence data revealed 15 haplotypes of *N. americanus* and 2 of *N. gorillae*. Maximum-likelihood and Bayesian inference phylogenetic analyses placed *N. americanus* sequences (217-bp) from this study within a clade containing previously published sequences for that species (Figure 2, panel A). The *N. gorillae* sequences clustered with isolates from NHPs from CAR and Gabon and with isolates recovered from the infected researchers from Europe (Figure 2, panel A). We also detected 1 cryptic *Ancylostoma* sp. hookworm that we could not confidently assign to any known species based on available data. At the *cox1* locus, that *Ancylostoma* sp. hookworm clustered basally to *Ancylostoma caninum* (GenBank accession no. AP017673) and another unidentified *Ancylostoma* sp. hookworm (GenBank accession no. MK434228) identified in dogs from Australia (Figure 2, panel A). We identified 4 *cox1* haplotypes of *S. f. fuelleborni* roundworms, all of which fell within the African clade of that species (Figure 2, panel B). An attempt to sequence the *cox1* of the *S. stercoralis*-positive sample was unsuccessful.

We obtained 18S rDNA HVR-IV sequences of 255–258-bp length from 6 *Strongyloides*-positive samples; we identified 5 as *S. f. fuelleborni* and 1 as *S. stercoralis* (Appendix Table 1). Haplotyping analysis

infection from NHPs or another animal, but detection of hookworm eggs in a fecal sample excludes transient passage of ingested DNA. Further investigations using longer read genotyping targets combined with morphologic analysis of harvested adult hookworms could provide more definitive speciation.

Our findings also suggest that *S. f. fuelleborni* roundworm infection is common among human populations in Central Africa. However, little *S. f. fuelleborni* roundworm infection epidemiologic surveillance has been performed in humans in Africa since 1980, when surveys of diagnostic specimens submitted to a hospital in Lusaka, Zambia, reported a 1.0% diagnostic prevalence of *S. f. fuelleborni* roundworm infections over a 7-month period (14). A 2024 molecular survey conducted in Asia identified a 3.0% (4/134) infection prevalence in some Bangladesh communities (15).

In our study, *S. f. fuelleborni* sequences clustered closely with isolates from Central Africa at both the *cox1* and 18S rDNA HVR-IV loci, supporting the hypothesis of geographic clustering for this species (10,11,15). The *S. stercoralis* 18S rDNA HVR-IV haplotype we identified is consistent with previous reports of that species in humans from Africa (8,10).

In summary, we report human infections with *N. gorillae* hookworms and *S. f. fuelleborni* roundworms in Gabon in Central Africa. Those infections occurred in a forest-edge region where localized environmental disturbance and anthropogenic activities, such as hunting and foraging in the adjacent forest, bring villagers into direct contact with NHP habitats, increasing exposure to NHP STHs (2). To determine the extent of human infections with zoonotic primate STH in areas where populations overlap and to define the clinical effects and most appropriate treatment strategies for infected persons, enhanced STH surveillance is needed.

Ethics approval for this study was provided by the Ministry of Health and Social Affairs Scientific and Ethics of the Centre hospitalier universitaire Mère-Enfant Fondation Jeanne Ebori, Gabon (approval no. 015 /MSAS/CHUMEFJE/DG/DAM/CS) and the Human Research Committee of James Cook University, Townsville, Australia (approval no. H9547).

H.Z.'s PhD studies are funded by the Australian Government Research Training Program Scholarship.

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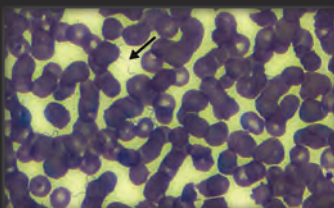
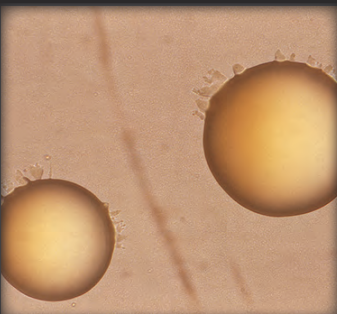
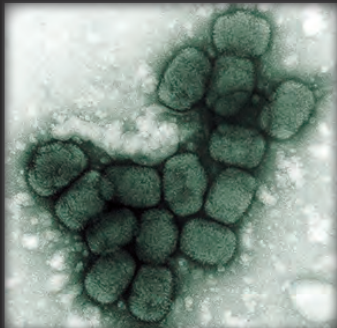
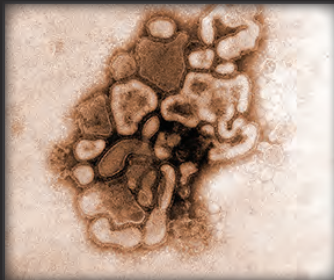
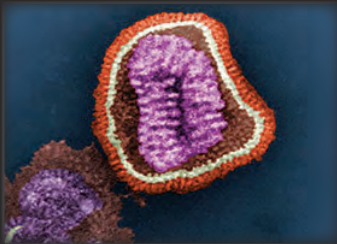
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Investigation of Possible Intraoperative Transmission of *Brucella melitensis*, Slovenia

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We report possible intraoperative transmission of *Brucella melitensis* in Slovenia, likely caused by aerosolized particles during wound irrigation. Whole-genome multilocus sequence typing revealed that isolates from the patient and the surgeon belonged to the same transmission cluster, differing by 1 allele. Our findings raise awareness of occupational risks faced by orthopedic surgeons.

Brucellosis causes >500,000 reported cases worldwide and is endemic in the Balkans, Central Asia, India, Central and South America, and the Middle East (1,2). Brucellosis is caused by gram-negative intracellular bacteria of the genus *Brucella*, most often *Brucella melitensis* (1). The primary routes of infection include having direct contact with infected animals, consuming unpasteurized dairy products or undercooked meat of infected animals, and, in rare cases, aerosol inhalation (3,4).

The prevailing symptoms of brucellosis are fever, night sweats, arthralgia, malaise, headache, and fatigue. Because of nonspecific symptoms and often prolonged incubation period, brucellosis presents a diagnostic challenge, particularly for healthcare providers in nonendemic areas where cases occur sporadically (1–3). Infection frequently affects osteoarticular structures; spinal involvement is estimated in 54% of brucellosis cases, mainly manifesting as spondylitis or spondylodiscitis (3,5). Animal-to-human transmission is most frequent in veterinary and agricultural workers; however, direct patient-to-patient transmission is rare. Laboratory workers face an increased occupational risk; most reported cases are

linked to handling *Brucella* isolates on an open laboratory bench (6).

In Slovenia, brucellosis cases are rare and mostly imported from the Balkans. Healthcare workers reported only 12 cases of brucellosis in Slovenia during 2017–2022, with 6 of those cases diagnosed in 2019 (7). We report possible intraoperative transmission of *B. melitensis* from patient to surgeon in Slovenia.

The Study

A 64-year-old woman with a history of back pain sought treatment at University Medical Center Ljubljana (Ljubljana, Slovenia), where physicians observed acute-onset left-sided femoralgia and motor deficit in the left foot dorsiflexion. Magnetic resonance imaging revealed a herniated disk at L4–5 and L5 neuroforaminal stenosis, prompting left-sided L4–5 discectomy and L5 foraminotomy. Postoperatively, the patient reported only a slight reduction in pain, despite uncomplicated rehabilitation. At 1-year follow-up, the patient reported persistent pain, following the preoperative pattern. Follow-up imaging, including radiography and magnetic resonance imaging, revealed a collapsed L4–5 disk and L4 anterolisthesis, accompanied by a minor seroma at the surgical site. Thus, surgeons performed an L4–5 posterior spinal fusion 20 months after the initial surgery, yielding a satisfactory recovery and hospital discharge of the patient 1 week after surgery.

At 1-month follow-up, physicians observed persistent serous wound drainage, and laboratory results revealed slightly elevated levels of C-reactive protein (20 mg/L; reference range <10 mg/L) and erythrocyte sedimentation rate (58 mm/h; reference range <30 mm/h). Leukocyte count remained unremarkable (7.7×10^9 cells/L; reference range $4.5\text{--}11.0 \times 10^9$ cells/L). Surgeons performed a revision surgery with microbiological sampling, administering amoxicillin with clavulanic acid and ciprofloxacin empirically. Laboratory analysis of a subcutaneous tissue sample

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DOI: <http://doi.org/10.3201/eid3110.250587>

identified *B. melitensis*, which was further confirmed by real-time PCR (Appendix, <https://wwwnc.cdc.gov/EID/article/31/10/25-0587-App1.pdf>). Results of blood analysis showed *Brucella* IgM and IgG. On the basis of those results, treating physicians changed the antibiotic regimen to intravenous gentamicin (240 mg/d) and oral doxycycline (200 mg/d) for 14 days, followed by doxycycline (200 mg/d) and rifampin (900 mg/d) for 6 months. C-reactive protein levels returned to reference range after 1 month.

Detailed retrospective analysis of the patient's medical history revealed that she frequently traveled to rural areas of Bosnia and Herzegovina and occasionally consumed unpasteurized cow milk and homemade goat cheese. Six months before the initial surgery, during one of those visits, she had a 5-day fever accompanied by dry cough and malaise (Figure 1).

Three months after the patient's initial surgery, the lead surgeon experienced fever, malaise, and joint pain lasting 7 days, initially attributed to a viral infection. He underwent no microbiologic investigation. Nine months later, the surgeon experienced a recurrence of fever, malaise, and joint pain; symptoms persisted and gradually worsened over 9 days. Given his recent travel to Moldova, the surgeon underwent a comprehensive microbiologic investigation, revealing *B. melitensis* in blood cultures and *Brucella* spp. antibodies in the blood sample. After receiving the established antibiotic regimen, he recovered without complications.

Of note, the surgeon had no travel history to endemic regions in the previous 2 years, except for Moldova, and recalled no food source of infection. Retrospective serologic investigation revealed the absence of *Brucella* spp. antibodies in a blood sample taken 1 month before the initial surgery. Thus, when the surgeon's laboratory results showed *B. melitensis* infection following the third revision surgery, retrospective analysis indicated that transmission might have occurred during the first operation. Surgical staff involved in any of the operations underwent serologic testing, revealing IgG in 1 additional asymptomatic person, who subsequently received antibiotic treatment.

To confirm this suspected patient-to-surgeon transmission of *B. melitensis*, we performed whole-genome sequencing (WGS) on the isolates from both the patient and the surgeon, along with 8 additional human *B. melitensis* isolates identified in Slovenia during 2017–2020. To determine the putative geographic origin of the Slovenia isolates, we included in the analysis 15 closely related (differing by <80 alleles from the Slovenia isolates), publicly available *B. melitensis* sequence type 8 genomes belonging to the Eastern Mediterranean clade (genotype II) (Appendix Table) (8). All Slovenia isolates were sequence type 8. We observed 3 whole-genome multilocus sequence typing (wgMLST) clusters (Figure 2), of which 2 also encompassed publicly available but epidemiologically unrelated isolates from neighboring countries.

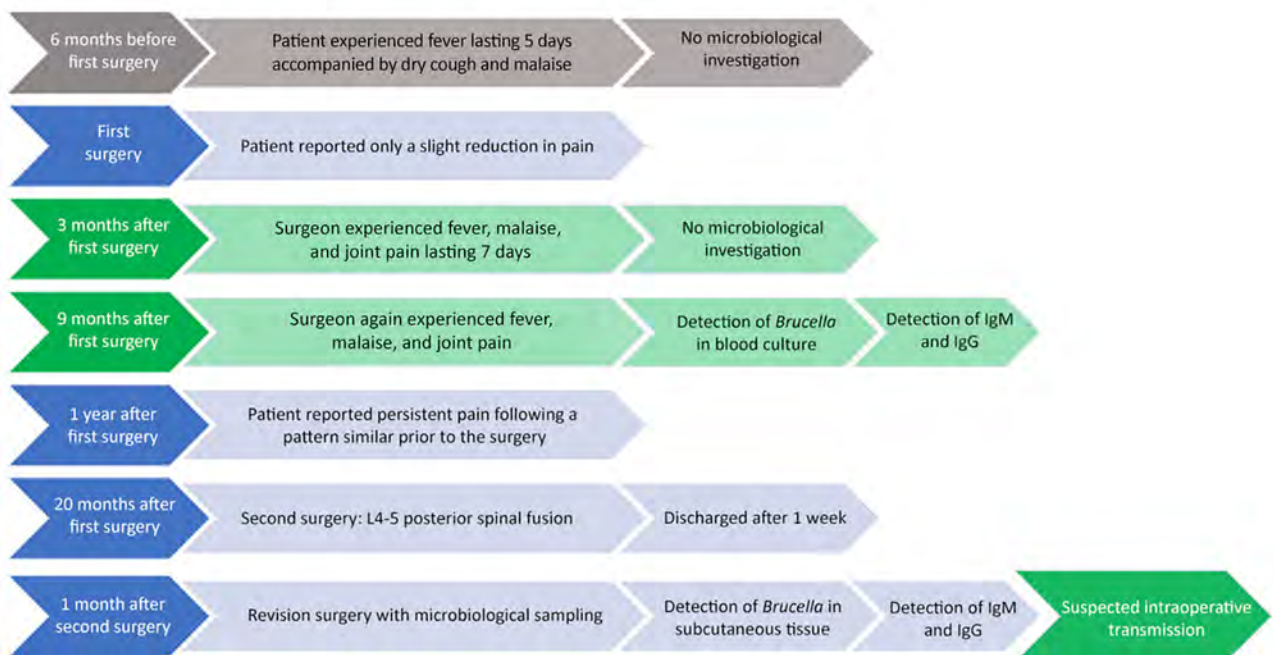


Figure 1. Patient-to-surgeon transmission timeline in study investigating possible intraoperative transmission of *Brucella melitensis*, Slovenia.

Two isolates formed no clusters. The isolates from the patient (BM7) and the surgeon (BM3) formed a common cluster, differing by only 1 wgMLST allele (Figure 2). That cluster also encompassed 2 other isolates (BM9 and BM10) from Bosnia and Herzegovina and was most closely related (≥ 4 wgMLST allele differences) to the isolates from Croatia (CVI_6 and CVI_7). The patients from isolates BM7, BM9, and BM10 reported travel history to Bosnia and Herzegovina, so we cannot exclude the possibility that their infections are related. However, the surgeon (isolate BM3) neither traveled to the Western Balkans (including Bosnia and Herzegovina) nor consumed milk or cheese made from unpasteurized milk from that region.

Conclusions

Most documented cases of brucellosis result from high-risk exposures or laboratory-acquired infections (1,6,7); knowledge is limited regarding exposure to *Brucella* spp. in other hospital settings (8,9). Although the surgeon in this report strictly followed standard intraoperative infection control measures,

we suspect patient-to-surgeon transmission occurred during a discectomy, when wound irrigation caused small droplets to aerosolize, producing an environment in which a standard surgical mask provided insufficient protection.

The overall incidence of human brucellosis in Europe has declined substantially over the past decade, but the Mediterranean region remains highly affected (7) (Appendix). Furthermore, brucellosis is difficult to diagnose early because of nonspecific symptoms (10), and the infected tissue is nonpyogenic, meaning the infection can easily be overlooked in the absence of epidemiologic suspicion. In this case, it was only after the third operation, after brucellosis in the patient had been confirmed, that a retrospective review of magnetic resonance imaging suggested subtle signs of a possible low-grade infection. In similar cases, it would be prudent to assess the patient's serostatus and initiate appropriate antibiotic treatment before surgery and to ensure surgical staff uses appropriate personal protective equipment to reduce the risk of aerosol transmission.

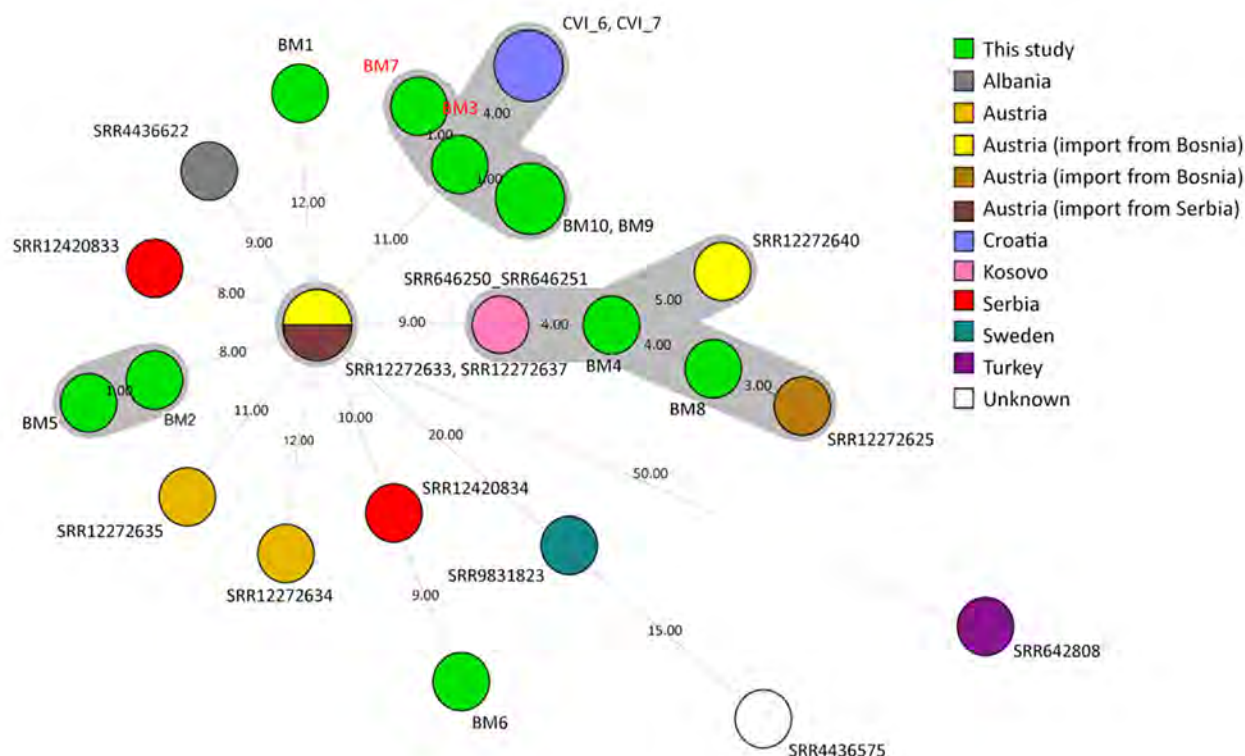


Figure 2. Minimum spanning tree based on whole-genome multilocus sequence typing allele profiles showing the relatedness of 25 *Brucella melitensis* isolates in study investigating possible intraoperative transmission of *Brucella melitensis*, Slovenia. Numbers above the branches correspond to the number of allele differences. Gray shading indicates clusters of isolates differing by ≤ 6 alleles. Isolates sequenced in this study shown in green: BM1 (National Center for Biotechnology Information Sequence Read Archive accession no. SRR32696437), BM2 (accession no. SRR32696436), BM4 (accession no. SRR32696434), BM5 (accession no. SRR32696433), BM7 (red text, the patient; accession no. SRR32696431), BM9 (accession no. SRR32696429), BM10 (imported from Bosnia and Herzegovina; accession no. SRR32696428), BM8 (imported from Croatia; accession no. SRR32696430), BM6 (imported from North Macedonia; accession no. SRR32696432), and BM3 (red text, the surgeon; accession no. SRR32696435).

Although WGS has discriminatory power to trace outbreaks and investigate human-to-human transmission (11,12), this type of analysis can be limited by small numbers of *Brucella* isolates available for WGS typing. The scarcity of isolates in this study primarily resulted from low incidence of confirmed cases of human brucellosis in Slovenia, as well as the absence of isolates from other sources (e.g., food) collected within the same geographic region and time period. Nevertheless, our study highlights the effectiveness of WGS in confirming that, although all Slovenia isolates were closely related *B. melitensis* genotype II isolates, only the isolates from the patient and surgeon were almost identical. This specific finding underscores the potential risk of *B. melitensis* transmission in the context of orthopedic surgery.

Acknowledgments

We acknowledge the EU (European Union)/OIE (World Organization for Animal Health)/FAO (Food and Agriculture Organization of the United Nations) Brucellosis Reference Laboratory (ANSES [French national agency for food, environmental and occupational health and safety], France) for providing the computational resources needed to perform WSG analyses in BioNumerics software program (bioMérieux, <https://www.biomerieux.com>). We thank the patient for sharing her medical history and allowing us to publish the data.

The sequencing data from this investigation are available in the National Center for Biotechnology information BioProject database (accession no. PRJNA1236232). The individual sequence accession numbers used in our study are listed in the Appendix Table.

The highest ethical standard was maintained during the study. A written informed consent was obtained by the patient for the publication of this case report. All procedures involving human participants followed the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

The authors acknowledge the financial support from the Slovenian Research and Innovation Agency (research core funding No. P4-0092; P3-0083) and Network of Infrastructure Centres of the University of Ljubljana (MRIC-UL-IC-BSL3+). The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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Cutaneous Coccidioidomycosis Mimicking Rosacea in Immunosuppressed Patient, Arizona, USA, 2024

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DOI: <https://doi.org/10.3201/eid3110.250010>

An immunocompromised patient in Arizona, USA, experienced cutaneous coccidioidomycosis mimicking rosacea-like dermatitis; she was receiving vandetanib therapy. Histopathology confirmed the diagnosis despite inconclusive serologic results. Lesions resolved with fluconazole treatment, but blue-gray hyperpigmentation persisted, likely linked to vandetanib. This case highlights diagnostic challenges in endemic fungal infections and rare drug-associated hyperpigmentation.

Coccidioidomycosis is caused by dustborne and soilborne *Coccidioides* spp. fungi that are endemic to the southwestern United States (1). *Coccidioides* spores become aerosolized and, when inhaled, develop into spherules within the lungs. The spherules can rupture and release endospores, enabling pulmonary spread and dissemination to other organs (1).

Coccidioidomycosis manifests nonspecific symptoms: fever, cough, dyspnea, fatigue, arthralgias, myalgias, and cutaneous lesions. The differential diagnosis for cutaneous coccidioidomycosis includes

rosacea, discoid lupus, psoriasis, sarcoidosis, granuloma faciale, and other disseminated fungal infections such as tuberculosis, paracoccidioidomycosis, and blastomycosis. We report cutaneous coccidioidomycosis mimicking rosacea-like dermatitis in an immunocompromised patient in Arizona, USA.

A 70-year-old woman with stage IV medullary thyroid cancer being treated with the multikinase inhibitor vandetanib who had undergone thyroidectomy and radiation therapy was referred for an 8-month history of progressive pruritic, inflammatory papules and pustules on the central forehead and bilateral cheeks (Figure 1, panel A). The patient denied facial trauma. Before referral, she received a 10-day course of prednisolone, followed by empiric oral antibiotics (cephalexin, levofloxacin), antiviral drugs (acyclovir), and topical agents (clindamycin, clotrimazole), prescribed over 4 months by various providers, with transient or no improvement. Oral prednisolone treatment led to temporary improvement in the inflammatory lesions but subsequent worsening upon discontinuation. A review of systems revealed occasional diarrhea and night sweats.

The patient, originally from Iran, had lived in Arizona for 5 years after previous residency in California and Virginia. At the time of her first dermatology visit (8 months after lesion onset), she was started on a 1-month course of doxycycline (100 mg 2×/d) and topical metronidazole (0.75% 2×/d) for a working diagnosis of rosacea, with no clinical improvement.

Given the lack of response to standard rosacea therapies, at her second dermatology visit (≈9 months after initial symptoms), we performed a punch biopsy of a representative pustule on the left glabella. Histopathology demonstrated granulomatous inflammation with lymphocytes, neutrophils, and eosinophils, plus periodic acid-Schiff-positive spherules containing endospores (Figure 2),



Figure 1. Clinical manifestations in case of cutaneous coccidioidomycosis mimicking rosacea in immunosuppressed patient, Arizona, USA, 2024. A) Inflammatory papules and pustules on the forehead and cheeks before treatment. B) Persistent blue-gray hyperpigmentation after 3 months of fluconazole therapy.

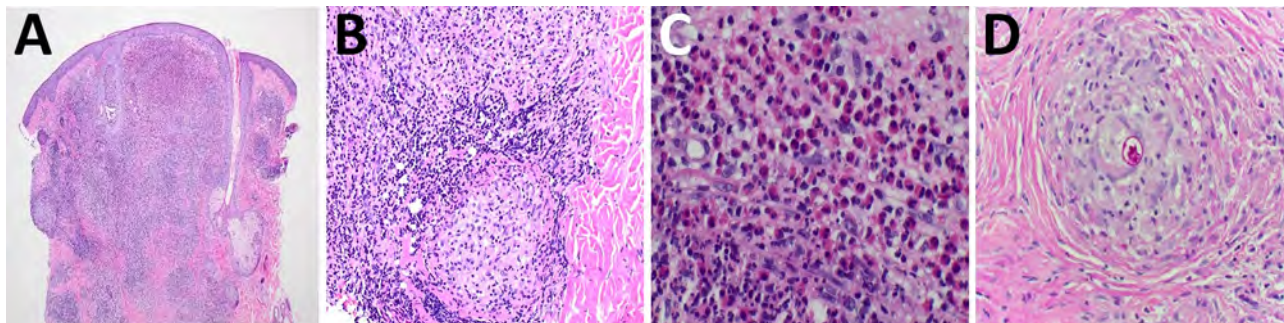


Figure 2. Histopathologic findings in case of cutaneous coccidioidomycosis mimicking rosacea in immunosuppressed patient, Arizona, USA, 2024. A–C) Granulomatous inflammation with mixed infiltrate of lymphocytes, histiocytes, neutrophils, and eosinophils. Hematoxylin and eosin stain; original magnifications $\times 2$ (A), $\times 20$ (B), and $\times 40$ (C). D) Periodic acid–Schiff staining demonstrating a coccidioidal spherule containing endospores within a granuloma. Original magnification $\times 40$.

confirming a diagnosis of cutaneous coccidioidomycosis. Serologic testing yielded negative IgM and indeterminate IgG results. However, additional confirmatory tests (tissue culture and PCR) were deemed unnecessary given the patient's residence in an endemic region and characteristic histopathologic findings.

Chest imaging revealed a new 1.3×1.3 -cm pulmonary nodule within the left lower lobe among otherwise stable nodules. The nodules were subcentimeter and not biopsied. Given the patient's oncologic history, it was unclear whether they represented metastatic disease or pulmonary coccidioidomycosis. Without definitive pathology, empiric antifungal therapy was initiated. After 2 weeks of fluconazole (400 mg/d), the papules and pustules showed substantial regression. However, after 3 months of oral fluconazole, blue-gray hyperpigmentation persisted at sites of prior papulopustules (Figure 1, panel B). After multidisciplinary discussion among infectious disease and oncology specialists, continuation of vandetanib and concurrent indefinite fluconazole treatment was recommended.

Given the absence of preceding trauma in this patient, direct inoculation with *Coccidioides* spp. is unlikely. Hematogenous dissemination is favored, particularly considering the patient's immunosuppression from both malignancy and tyrosine kinase inhibitor therapy. Her recent relocation to Arizona without early-life endemic exposure might have limited immune priming and increased susceptibility.

We histopathologically identified coccidioidomycosis in this case. Coccidioidomycosis shows refractile spherules with endospores, whereas paracoccidioidomycosis shows pilot wheel budding and blastomycosis shows broad-based budding. Histopathology may show granulomatous dermatitis, eosinophilic inflammation, micro-abscesses,

perivascular inflammation, and gummatous necrosis. Spherules are typically identifiable by using methenamine silver, periodic acid–Schiff stains, or Papanicolaou stains (2). Immunosuppression increases risk for disseminated coccidioidomycosis; only 1% of immunocompetent persons have disseminated disease develop, compared with 30%–50% of immunosuppressed persons (2). Patients who are immunocompromised (e.g., by HIV, malignancy, organ transplantation, antiinflammatory biologics, or chemotherapy) face 50% mortality with disseminated disease (3). Coccidioidomycosis can relapse during immunosuppressive treatment after antifungal discontinuation (4). Treatment varies by host immunity and organ involvement, ranging from observation to amphotericin B (5). Secondary cutaneous involvement occurs in up to 15% of extrapulmonary cases, especially in immunocompromised patients (6). Lesions mimic other dermatologic conditions, requiring histologic confirmation.

Azoles represent first-line therapy for cutaneous coccidioidomycosis; response rates are 25%–91% (5). This patient improved substantially with fluconazole at a dose of 400 mg/day. Immunocompetent patients typically need 6–12 months of therapy, whereas immunocompromised patients with disseminated disease often require lifelong treatment, depending on severity and immunosuppression (5). Given variable responses and relapse risk, careful monitoring is essential after treatment discontinuation.

This patient had persistent blue-gray hyperpigmentation develop at sites of prior papulopustules, intensifying after 3 months of fluconazole (Figure 1, panel B). Tetracyclines, especially minocycline, can cause hyperpigmentation, and blue-gray hyperpigmentation has been reported with vandetanib. Evidence suggests potential synergistic hyperpigmentation with concurrent vandetanib and doxycycline use (7).

In summary, this case highlights diagnostic challenges in endemic fungal infections and rare drug-associated hyperpigmentation. Diagnostic stewardship, particularly in endemic regions, is critical to reduce delays in appropriate antifungal therapy and minimize unnecessary antimicrobial exposure.

The patient provided written informed consent for the publication of her photograph.

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Jorge Lobo's Disease in Child with Tick Exposure, Brazil

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DOI: <https://doi.org/10.3201/eid3110.250209>

Jorge Lobo's disease (JLD), caused by *Paracoccidioides lobogeorgii*, primarily affects inhabitants of the Amazon Forest. We report a 9-year-old boy in Brazil who had JLD diagnosed after a tick bite. The rarity of pediatric cases likely reflects surveillance gaps. Increased clinical awareness is crucial for early JLD detection and intervention, especially in endemic regions.

Jorge Lobo's disease (JLD) is a chronic infection caused by the noncultivable fungus *Paracoccidioides lobogeorgii* (previously *Lacazia loboi*) (1). JLD primarily affects persons in the Amazon Basin and parts of Central America, and most reported cases originate from Acre state in Brazil (2). Infection is thought to occur when skin trauma involving contaminated vegetation or arthropods permits direct fungal inoculation or enables pathogen implantation from secondary environmental sources (3). Initial lesions can appear as subtle papules or nodules, often on the ears, arms, or legs, and might evolve into extensive verrucous plaques (3,4). The hypothesis of environmental transmission is supported by the cessation of new cases after relocation of the Caiabi Indigenous population from endemic to nonendemic areas.

JLD disproportionately affects men involved in rural occupations and forest extractivism. Left undiagnosed, JLD can cause visible disfigurement, functional impairment, and psychosocial stigma, which can undermine the ability to work and can contribute to social isolation. Although few pediatric cases have been documented, the long disease course, which can persist for decades, supports the hypothesis of early-life acquisition and delayed clinical recognition (4–6). We describe a pediatric male patient with JLD, providing insight into early acquisition and clinical appearance.

In February 2018, a child from a forested area in Brasília, Acre state, Brazil, was seen at the Serviço Estadual de Dermatologia do Acre (Rio Branco, Brazil) for a single lesion on his right ear. His mother, who frequently brought him to forest work areas, said that a tick had been attached at the site for 2 days in 2017. Initial treatment with topical dexamethasone (1 mg/g) was ineffective. Over 6 months, the lesion evolved from mild inflammation to a nodular lesion. One year later, a dermatologist suspected JLD on the basis of a solitary indurated lesion on the ear (Figure 1, panel A). Histopathology confirmed the JLD diagnosis, revealing granulomatous inflammation with spherical, double-walled yeast cells in branching chains (Figure 1, panel B) and prominent histiocytes and

multinucleated giant cells (Figure 1, panel C).

Treatment began with pediatric multidrug therapy for multibacillary leprosy, as previously described (7): monthly doses of rifampin (300 mg), clofazimine (150 mg), and dapsone (50 mg), combined with daily dapsone (50 mg) and clofazimine (50 mg on alternate days) for 2 years, and quarterly follow-up visits (7). After 4 months, lesion atrophy made surgical excision possible. By November 2020, the patient was lesion-free but was lost to follow-up. In August 2022, during a health outreach mission for leprosy surveillance in the Amazon, the patient was relocated, and a small recurrent lesion on the pinna of the same ear was observed and excised. Itraconazole (200 mg/d) was prescribed for 3 months, but adherence to follow-up consultations was irregular. By June 2024, a 5-cm lesion recurred at the same site. A novel biopsy confirmed JLD recurrence showing chronic granulomatous dermatitis with multinucleated giant cells, fibrotic stroma, and yeast-like cells (Figure 1, panel D). Treatment with itraconazole continued, along with regular medical monitoring.

JLD in children is underreported, although evidence suggests many cases originate in childhood (8,9). JLD progression, often spanning decades, underscores the need for early detection in endemic

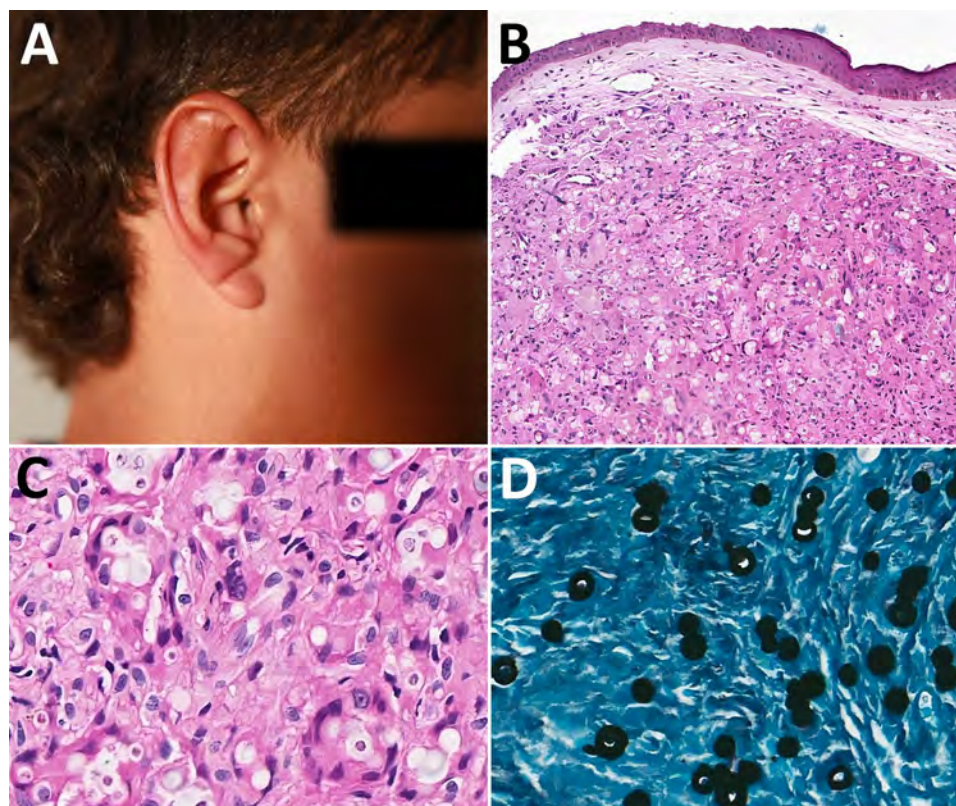


Figure 1. Nodule and histopathology in a case of Jorge Lobo's disease in child with tick exposure, Brazil. A) Single granulomatous lesion on the right ear, which was surgically excised and histologically examined in 2018. B, C) Hematoxylin and eosin stain of nodule showing cells of the mononuclear phagocyte system, including monocytes, macrophages, epithelioid cells, and multinucleated giant cells, revealing granulomatous inflammation with spherical, double-walled yeast cells in branching chains (B) and prominent histiocytes and multinucleated giant cells (C), which are predominantly dense with areas of mild neovascularization, stromal hyalinization, and a slight infiltrate of lymphocytes and neutrophils. D) Grocott–Gomori methenamine silver stain of recurrent nodule from 2024 in which yeast-like fungal structures are diffusely distributed throughout the stroma and in the cytoplasm of phagocytic cells.



Figure 2. Location of a case of Jorge Lobo's disease in child with tick exposure, Brazil. The patient resided near the Brazil–Bolivia border and was diagnosed in Brasiléia, Acre state, Brazil. Brasiléia is situated between the municipalities of Xapuri and Epitaciolândia in Acre, also known as Brasiléia–Epitaciolândia–Xapuri immediate region. This zone borders Bolivia within a dense rainforest region of the western Amazon and is marked by rubber plantations and extractive activities.

regions. Long latency periods support the hypothesis of early-life acquisition (3,4). The patient's slow lesion development, despite a year without treatment, further suggests a gradual pediatric course. Timely diagnosis combined with surgical and antifungal therapy can prevent complications (5,10). JLD elicits a robust inflammatory response with histiocytic infiltrates, multinucleated giant cells, and fungal elements, progressing to fibrosis, chronicity, and immune containment. In this case, a tick bite might have led to fungal entry, as observed in other reports linking JLD onset to arthropod trauma (3). Despite several attempts, molecular confirmation (rDNA sequencing) failed because of poorly preserved paraffin-embedded material. Histopathological examination confirmed the diagnosis.

Brasiléia is a municipality in western Acre state, Brazil, bordering Bolivia. It falls under the Brasiléia–Epitaciolândia–Xapuri immediate region (Figure 2), an area marked by rubber plantations and extractive activities, which might increase environmental exposure. Because river transport is seasonal, remote communities face year-round delays in healthcare; floods during the wet season wash out roads and make navigation hazardous, whereas low water in the dry season strands boats and slows travel.

In summary, treatment for JLD remains empirical, and no standardized protocols are available (4,5). This case illustrates a 9-year disease course marked by relapses and partial responses and echoes adult reports of forest exposure during childhood, suggesting underdiagnosed childhood infections. Polychemotherapy regimens used for multibacillary leprosy have shown efficacy in localized JLD cases, although prolonged treatment is often necessary (7). In this case, therapy lasted 2 years and was complicated by irregular follow-up. Itraconazole regimens in adults typically span 12–24 months (5), but data on pediatric treatments are scarce. The patient's rediscovery during a targeted field mission highlights the need to strengthen active surveillance and outreach in endemic forested areas to enable early detection, timely treatment, and improved management and to mitigate the long-term effect of JLD on at-risk populations.

Funding was provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico (grant no. 445376/2023-6).

Ethics committee approval was obtained by Fundação Hospital Estadual do Acre–FUNDHACRE (IRB no. 7.104.488). Patient data were handled in accordance with ethical standards.

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Dr. Gonçalves is a professor at the Federal University of Acre, Brazil, specializing in neglected diseases in the Amazon, particularly Jorge Lobo's disease and leprosy. Her research interests focus on epidemiology and public health strategies to improve healthcare outcomes in the Amazon region.

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Neonatal Gonococcal Conjunctivitis Caused by Fluoroquinolone-Resistant *Neisseria gonorrhoeae*

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DOI: <https://doi.org/10.3201/eid3110.250895>

Prophylaxis for ophthalmia neonatorum remains in use despite decreased incidence of the condition. We report a breakthrough case of neonatal conjunctivitis in Japan caused by a levofloxacin-resistant *Neisseria gonorrhoeae* bacteria strain, co-infected with *Chlamydia trachomatis* bacteria. This case highlights failures in screening, prophylaxis, and treatment, underscoring the need to reassess prevention strategies.

Since the introduction of prophylactic ophthalmic solutions by Carl Credé in 1881, the incidence of neonatal gonococcal conjunctivitis has declined markedly (1,2). Over time, the agents used for ocular prophylaxis have shifted from silver nitrate to erythromycin or tetracycline ophthalmic ointments and povidone/iodine (3).

In Japan, erythromycin/colistin ophthalmic formulations had been widely used since 1970. However, production of erythromycin/colistin preparations was discontinued in 2015, prompting some institutions to adopt fluoroquinolone-based ophthalmic agents for neonatal prophylaxis. Although fluoroquinolone-resistant *Neisseria gonorrhoeae* bacteria strains have been reported in adults, neonatal infections with such strains remain rare (2). We describe a breakthrough case of neonatal gonococcal conjunctivitis caused by a fluoroquinolone-resistant *N. gonorrhoeae* strain and further complicated by concurrent *Chlamydia trachomatis* bacteria infection. This case highlights the need to reevaluate current strategies for preventing and managing neonatal conjunctivitis.

In 2023, a 12-day-old female infant was brought to a hospital in Shizuoka, Japan, where she was observed to have purulent ocular discharge and peri-orbital swelling. She was born at full-term through spontaneous vaginal delivery. The mother had a negative *C. trachomatis* nucleic acid amplification test at 12 weeks' gestation; however, no screening for *N. gonorrhoeae* was performed. The infant received prophylactic levofloxacin ophthalmic solution immediately after birth. From day 4 of life, purulent ocular

discharge started occurring and progressively worsened, despite use of levofloxacin ophthalmic solution, applied 3 times daily, that was prescribed by a local pediatrician on day 8. By day 10, bilateral eyelid edema and marked periorbital inflammation were evident, prompting referral to the hospital’s ophthalmology department.

We obtained conjunctival cultures and added a cephalosporin-based ophthalmic preparation to the patient’s treatment plan. Culture of the ocular discharge confirmed *N. gonorrhoeae* infection, leading to a diagnosis of neonatal gonococcal conjunctivitis. We then admitted the infant for treatment.

At admission, we recorded vital signs, laboratory test results, and urine test results (Appendix, <https://wwwnc.cdc.gov/EID/article/31/10/25-0895-App1.pdf>). Physical examination revealed bilateral conjunctival injection, pronounced left eyelid edema, and periorbital erythema (Figure). The corneas were clear, having no evidence of epithelial defect, ulceration, or perforation. We noted no anterior chamber abnormalities, and ocular motility was preserved. We observed a conjunctival hemorrhage in the left eye.

We initiated intravenous cefotaxime after hospitalization. We discontinued antimicrobial therapy after 48 hours of negative blood culture results. Ocular symptoms began improving by hospital day 3.

A PCR test performed on a pharyngeal swab sample on hospital day 3 was positive for *C. trachomatis*, and we administered azithromycin. The infant recovered fully without recurrence.

The *N. gonorrhoeae* isolate, which we designated as B196-JP22, was resistant to levofloxacin (MIC 12 µg/mL), had elevated MIC for azithromycin (MIC 0.75 µg/mL), and had reduced susceptibility to penicillin G



Figure. Bilateral conjunctival infection, pronounced left eyelid edema, and periorbital erythema observed in a 12-day-old infant in case of neonatal gonococcal conjunctivitis caused by fluoroquinolone-resistant *Neisseria gonorrhoeae*, Japan, 2023.

(1 µg/mL) (Table). We performed full-genome analysis on the isolate (Appendix) and deposited it to the National Center for Biotechnology Information BioProject database (project no. PRJNA1277472) and BioSample database (accession no. SAMN49109531). We found missense mutations in the *gyrA* and *parC* genes consistent with the levofloxacin-resistance phenotype (5) and antimicrobial resistance genes (Table) (Appendix).

According to the multilocus sequence typing analysis at the Center of Genomic Epidemiology (<https://www.genomicepidemiology.org>) the sequence type was 7371 (Appendix Tables 1, 2). The phylogenetic tree clustered our B196-JP22 isolate in a monophyletic terminal clade together with a

Table. MICs of antimicrobial drugs for *Neisseria gonorrhoeae* isolate B196-JP22 from a case of neonatal gonococcal conjunctivitis caused by fluoroquinolone-resistant *N. gonorrhoeae*, Japan

Antimicrobial drug	MIC, mg/L	Interpretation*	Antimicrobial resistance genes (mutation details)
Penicillin G	1	Reduced susceptibility	<i>mtrR</i> (mtrR_A39T), † <i>mtrR</i> (mtrR_promoter_a-57del), ‡ <i>ponA</i> (ponA_L421P), † <i>penA</i> gene§
Piperacillin/tazobactam	<0.01	Susceptible	
Ceftriaxone	0.05	Susceptible	
Meropenem	0.01	Susceptible	
Levofloxacin	12	Resistant	<i>gyrA</i> (gyrA_S91F/D95N), † <i>parC</i> (parC_S87I)†
Azithromycin	0.75	Elevated MIC¶	<i>mtrA</i> , <i>macA</i> , <i>macB</i> , <i>farA</i> #

*Antimicrobial MIC tested by using Etest (bioMérieux, <https://www.biomerieux.com>). Interpretation according to Clinical and Laboratory Standards Institute (CLSI) standards (4). CLSI cutoff for susceptible is MIC <0.06 µg/mL, for intermediate is MIC 0.12–1 µg/mL, for resistant is MIC ≥2 µg/mL. A MIC of 1 was described as reduced susceptibility.

†Missense mutation.

‡Promoter deletion.

§The *penA* gene that encodes the PBP2 shared 99.77% identity with the *penA* encoded by the World Health Organization Alfa strain. However, it could not be classified by using the NG-STAR platform (<https://ngstar.canada.ca>) because of 2 gaps in the sequence, and the closest NG STAR type was the nonmosaic allele 18.001.

¶MIC 0.75 µg/mL was within susceptible range, although elevated compared with local reference strains. CLSI MIC cutoff for *N. gonorrhoeae* azithromycin susceptibility is MIC ≤1 µg/mL. However, CLSI has set a susceptible only interpretive breakpoint at this value, with the recommendation that this applies when azithromycin (1 g single dose) is used in combination with another antimicrobial, such as ceftriaxone.

#Efflux pumps encoded in this strain include *mtrA*, *macA*, *macB*, and *farA*.

urogenital strain (BioProject no. PRJNA560592; BioSample accession no. SAMN12591021) isolated in the city of Shenzhen, Guangdong Province, China, in January 2017 (6). Isolates in neighboring terminal clades were reported from Australia, Hong Kong, mainland China, and Vietnam (Appendix Figure).

This case demonstrates a triple failure: inadequate maternal screening for sexually transmitted infections and ineffective prophylaxis and treatment using fluoroquinolone-based ophthalmic agents against a multidrug-resistant *N. gonorrhoeae* strain. Robust maternal screening is critical to prevent perinatal transmission of sexually transmitted infections. US Centers for Disease Control and Prevention guidelines recommend repeat testing for *N. gonorrhoeae* and *C. trachomatis* during the second trimester for all pregnant women <25 years of age at increased risk (7). In contrast, prenatal care in Japan typically involves a single screening early in pregnancy, and routine screening for *N. gonorrhoeae* is uncommon (8).

In developed countries, the necessity of routine neonatal ocular prophylaxis is increasingly debated because it does not prevent *C. trachomatis* infection and the number of cases of gonococcal conjunctivitis is small (9). Several countries in Europe have discontinued prophylaxis without observing increased incidence of neonatal ophthalmia (10).

In Japan, fluoroquinolone-resistant *N. gonorrhoeae* strains are highly prevalent among adults; resistance rates are >80% for fluoroquinolones and ≈20% for macrolides (2). Genomic analysis of our isolate suggests further spread of resistant organisms in Asia, highlighting the importance of identifying this isolate in the region.

Given the low incidence of neonatal gonococcal conjunctivitis and the increasing prevalence of antimicrobial resistance in Japan, routine use of prophylactic ophthalmic solutions appears insufficient. Instead, systematic screening for maternal gonococcal infection, especially in late pregnancy, might be considered a more effective strategy to prevent vertical transmission.

The study was funded by Japan's Ministry of Health, Labour and Welfare (grant nos. MHLW 23HA1002 and 30 E-1, awarded to I.M.). Written permission for publication was obtained from the parents of the patient. An artificial intelligence tool (Chat GPT-4o, <https://openai.com/index/hello-gpt-4o>) was used only for grammatical correction of the manuscript.

All authors declare that they do not have any potential, perceived, or real conflicts of interest relevant to this study. H.M. and I.M. wrote the first draft of the manuscript, and C.A.Y. performed the analysis of the isolate. M.K. provided data. All authors have critically reviewed

the paper and are accountable for all aspects of the work, meeting International Committee of Medical Journal Editors criteria for authorship.

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Disseminated Blastomycosis Mimicking Tuberculosis, China

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DOI: <https://doi.org/10.3201/eid3110.250671>

Blastomycosis is endemic in central and southern North America but rare in China. It can mimic community-acquired pneumonia, tuberculosis, or cancer. We describe a patient who initially had tuberculosis diagnosed and later had blastomycosis diagnosed through metagenomic detection, which aided diagnosis and treatment. Clinicians should consider blastomycosis in differential diagnoses for respiratory diseases.

Blastomycosis is a dimorphic fungal infection endemic to North America but rarely reported in China. (1). The pathogen is not directly transmissible between persons (1). Disseminated blastomycosis should be considered in cases with multisystemic involvement, even when typical symptoms such as cutaneous or central nervous system lesions are absent (2). Clinical manifestations of blastomycosis often mimic those of tuberculosis (TB), malignancy, or bacterial pneumonia, complicating diagnosis. *Blastomyces percursorus*, a novel species phenotypically and epidemiologically distinct from *B. dermatitidis*, has been linked to infections in patients from Israel, South Africa, and other regions in Africa (3,4). We report an unusual case of *B. percursorus* infection in China that was initially diagnosed as pulmonary tuberculosis.

A 38-year-old man from Xinxiang, Henan Province, China, sought care after 4 months of cough, hemoptysis, and weight loss. He had been treated empirically with antimicrobial drugs at a local hospital without substantial improvement. He had no history

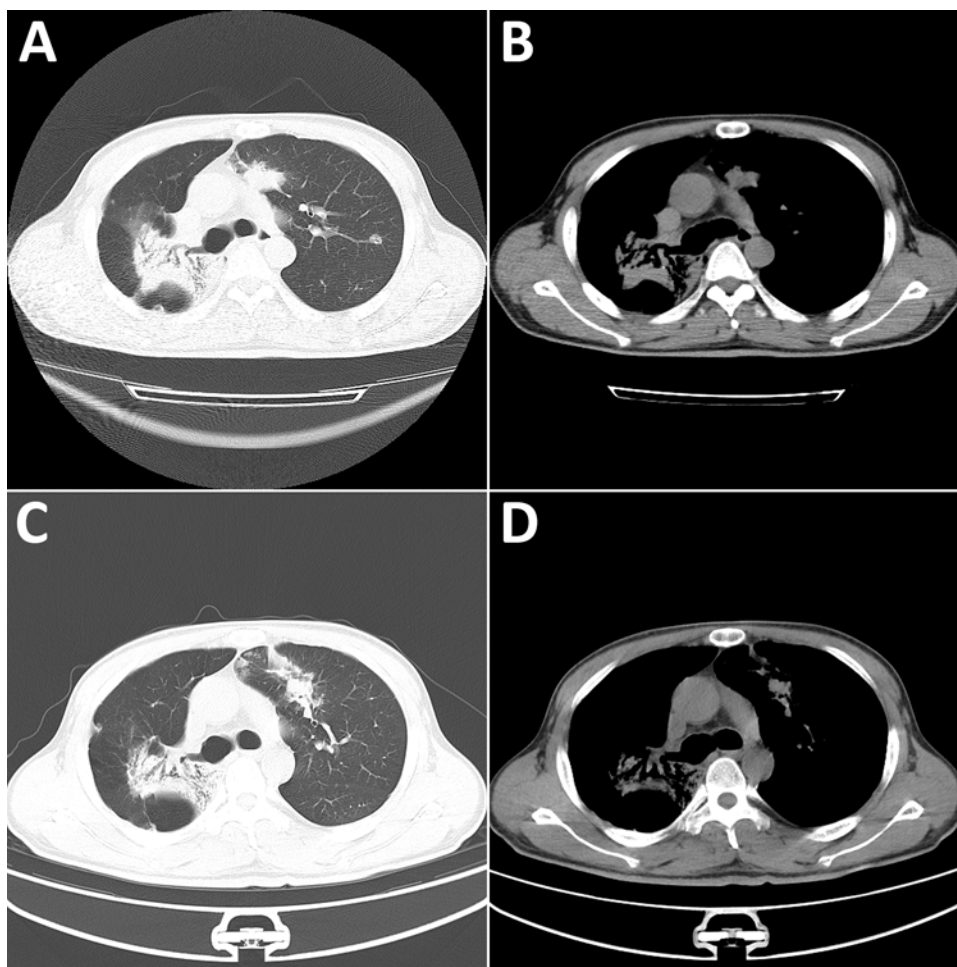


Figure 1. Chest computed tomography (CT) images from a patient with disseminated blastomycosis mimicking tuberculosis, China. A, B) Chest CT at first admission revealed bilateral lobar infiltrates (A) and patchy opacities (B). C, D) Chest CT taken 2 months later showed resolution of left lung lesions but progression of right lung lesions.

of incarceration, foreign travel, or known immunosuppression. He worked as a truck driver and had lived locally for years.

Chest computed tomography scans revealed bilateral lobar infiltrates and patchy opacities (Figure 1, panels A, B). Tests for *Aspergillus galactomannan*, β -D-glucan, and respiratory viruses were negative. Bronchoscopy revealed noncaseating granulomatous inflammation, and all stains and cultures for mycobacteria and fungi were negative. Because of clinical suspicion, we started the patient on a 4-drug TB treatment regimen (rifampin 600 mg, isoniazid 300 mg, ethambutol 1,000 mg, and pyrazinamide 1,500 mg daily).

Two months later, the patient was readmitted with new cutaneous lesions on his abdomen and lower limbs, which were nodular and verrucous (Figure 2). Laboratory studies showed leukocytosis (leukocyte count 14,160/ μ L) and 85.9% neutrophils. Results of ELISA testing for HIV were negative, and results for tumor markers and autoimmune antibody panels were unremarkable. Repeat chest computed tomography scans demonstrated resolution of left lung lesions but progression on the right (Figure 1, panels C, D).

Repeat bronchoscopy was performed. GeneXpert MTB/RIF (Cepheid, <https://www.cephheid.com>) testing of bronchoalveolar lavage fluid was negative. However, 1 week later, metagenomic next-generation sequencing of bronchoalveolar lavage fluid identified *B. percursorus*.

The patient received intravenous liposomal amphotericin B (3 mg/kg/d) for 2 weeks, then oral itraconazole (200 mg 2 $\times$ /d) for 1 month. After treatment, both



Figure 2. Cutaneous lesion on the abdomen from a case of disseminated blastomycosis mimicking tuberculosis, China. Two months after treatment for tuberculosis, new cutaneous lesions developed on the patient's abdomen and lower limbs; molecular methods on bronchoalveolar lavage fluid detected *Blastomyces percursorus*.

pulmonary and cutaneous lesions resolved. Itraconazole remains a preferred agent for treating mild-to-moderate pulmonary and disseminated blastomycosis (8).

Blastomycosis can involve virtually any organ and often masquerades as TB, leading to frequent misdiagnoses. In this case, the patient was treated with multiple drugs for TB, delaying accurate diagnosis by ≥ 1 month. Ultimately, metagenomic sequencing identified *B. percursorus*. Metagenomic approaches increasingly have been applied to clinical microbiology for detecting pathogens in respiratory infections (5), bloodstream infections (6), and central nervous system infections (7). Those methods make unbiased, broad-spectrum detection possible even when conventional tests fail.

The delay in diagnosis led to unnecessary drug exposure and progressive dissemination, including cutaneous lesions. Medications for TB carry substantial risks, including hepatotoxicity and bone marrow suppression. The lack of reliable rapid diagnostic tests for *B. percursorus* remains a barrier to timely treatment. Comprehensive physical examination, especially of the skin, is crucial, because cutaneous findings can aid in early recognition. In this case, clinicians adhered to a TB diagnosis despite a lack of microbiological evidence, and skin lesions developed in the patient while he was receiving empirical TB therapy only. Earlier consideration of alternative etiologies, such as blastomycosis, nocardiosis, or actinomycosis, might have expedited appropriate care.

This case illustrates how blastomycosis, although rare in China, can mimic pulmonary TB and lead to delayed diagnosis and treatment. Clinicians should include blastomycosis in the differential diagnosis of unexplained pulmonary infections. Increasing awareness of endemic mycoses and integrating metagenomic tools into routine diagnostics could improve the precision and timeliness of care.

Author contributions: C.G., M.Q.G., and Y.P. contributed to the writing of this manuscript. C.G., Y.J.P., J.J.Y., L.Y.Y., Y.H.H., and J.W.C. contributed to patient care. All authors approved the final version of this manuscript.

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Crimean-Congo Hemorrhagic Fever Virus Circulation in Wild European Rabbits, Portugal, 2018–2023

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DOI: <http://doi.org/10.3201/eid3110.250184>

Crimean-Congo hemorrhagic fever virus is considered a public health risk in southwestern Europe. We surveyed serum samples from 667 European rabbits across Portugal, a rabbit species known to host immature *Hyalomma lusitanicum* ticks. We found low levels of virus antibodies (>1%), with a localized cluster reaching 5.77% in southern populations.

Crimean-Congo hemorrhagic fever virus (CCHFV) is a highly pathogenic tickborne pathogen able to cause severe hemorrhagic fever that has a high case-fatality rate in humans (1). Since the virus' first detection in southwestern Europe in 2010 (2), CCHFV has emerged as a formidable public health risk. Reports from Spain have identified *Hyalomma lusitanicum* ticks as reservoirs and vectors of CCHFV (2–4) and have suggested circulation and maintenance of the virus at local levels to be related to animal abundance (5). Mammals infected by tick bites become viremic for 2–10 days and develop a persistent immune response (4), making serologic surveys an effective tool for monitoring CCHFV dynamics (2,4–6).

Wild lagomorphs, and particularly European rabbits (*Oryctolagus cuniculus*), are key hosts of immature stages of *H. lusitanicum* ticks (7) and are expected to play a critical role in CCHFV epidemiology (8). However, prior reports have not established clear evidence of natural exposure of lagomorphs to CCHFV in Europe (8). Our study aimed to fill this gap through a serologic survey of rabbit populations from Portugal.

During May 2018–December 2023, we sampled 667 wild rabbits across 20 sites throughout mainland Portugal (average 33.4 ± 46.1 [standard deviation] rabbits per site) (Figure). We selected 8 longitudinal sites on the basis of their high rabbit abundance.

Twelve cross-sectional sites included hunted rabbits from ongoing studies. Study sites consisted of mixed agro-forestry landscapes with variable rabbit and wild ungulate abundance. Samples (1 per animal) encompassed blood from live-captured rabbits in a longitudinal capture-recapture study ($n = 472$) and hunted rabbits ($n = 195$), including 71 dried blood spots (DBS). We collected blood samples by way of the saphenous vein (live rabbits) or the thoracic cavity (hunted rabbits) and centrifuged samples at $2,000 \times g$ for 10 minutes to obtain serum. We collected blood from the thoracic or abdominal cavities as DBS in Whatman Protein Saver 903 cards (Cytiva, <https://www.cytivalifesciences.com>), dried at room temperature (2–6 weeks) before being shipped to the laboratory. We stored serum samples and DBS at -20°C prior to processing them for CCHFV detection using a commercial ELISA with recombinant purified CCHFV nucleoprotein (IDScreen, Double Antigen Multi

species; <https://www.innovative-diagnostics.com>), following the manufacturer's instructions.

We performed a univariate analysis because of a low number of positive ELISA results. We evaluated associations between seropositivity and individual categorical variables (year, month, sex, and age class) using Fisher exact test. We considered a p value <0.05 to be significant. We conducted statistical analyses in R v4.3.1 (The R Project for Statistical Computing, <https://www.r-project.org>) and QGIS v3.38.2 (<https://qgis.org>) for mapping the results.

We detected 4 animals with CCHFV antibodies ($n = 667$), resulting in an overall prevalence of 0.60% (95% CI 0.23%–1.53%). We collected the 4 positive samples from 2 sites located 74 km apart in southern Portugal (Figure), within an area previously identified as highly active for the circulation of CCHFV in wildlife (5). Those samples encompassed a DBS from a rabbit in 2023 (data not available) and 3 serum samples from

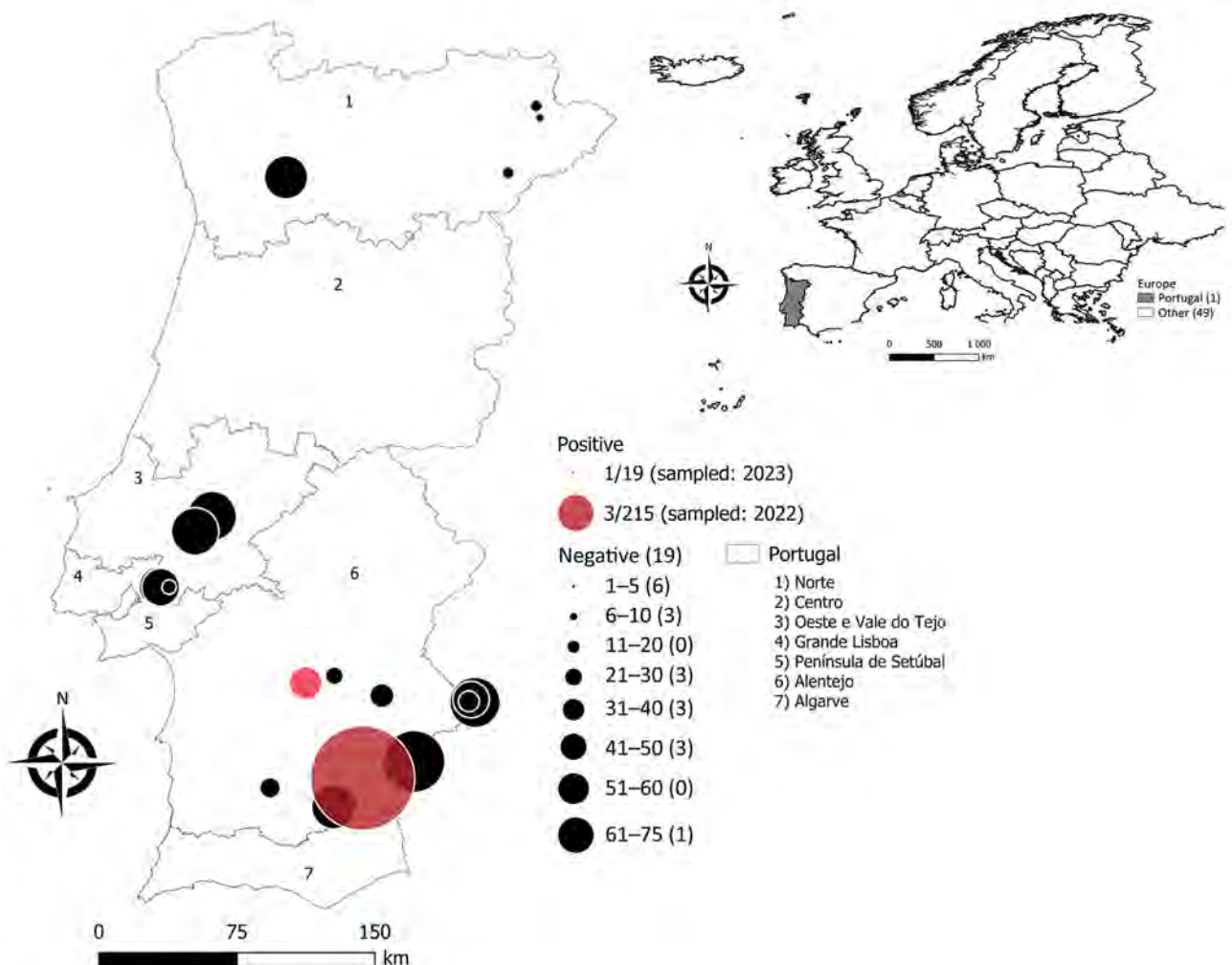


Figure. Study sites in Portugal, sample sizes, and locations of seropositive rabbits in study of Crimean-Congo hemorrhagic fever virus circulation in wild European rabbits, Portugal, 2018–2023. Inset map shows location of Portugal in Europe (gray shading).

Table. Univariate analysis of binomial serologic results in study of Crimean-Congo hemorrhagic fever virus circulation in wild European rabbits, Portugal

Category	Seropositive, no.	Sample size, no.	Prevalence, % (95% CI)	p value*
Sex				Referent
M	1	260	0.37 (0.07–2.15)	
F	2	289	0.71 (0.21–2.76)	
Unknown	1	122	0.82 (0.14–4.50)	
Age				0.041
Adult	0	326	0 (0–1.16)	
Subadult	0	104	0 (0–3.56)	
Juvenile	3	127	2.36 (0.81–6.72)	
Unknown	1	110	0.90 (0.16–4.97)	
Year				0.743
2018	0	60	0 (0–6.02)	
2019	0	51	0 (0–7.00)	
2020	0	59	0 (0–6.11)	
2021	0	127	0 (0–2.94)	
2022	3	195	1.54 (0.52–4.42)	
2023	1	175	0.57 (0.10–3.17)	
Month of sample collection				0.519
Jan–Apr	0	45	0 (0–7.87)	
May–Aug	3	299	1.00 (0.34–2.91)	
Sep–Dec	1	314	0.31 (0.06–1.78)	
Unknown	0	9	0 (0–29.91)	

By Fisher exact test. Bold indicates significance (p<0.05).

rabbits captured alive in 2022 (1 female and 1 male in June, 1 female in July, all juveniles). None of the seropositive rabbits were later recaptured. In this location, longitudinal monitoring of the same population identified a sudden increase of seroprevalence between 2 consecutive years, ranging from 0 (0/86 rabbits) in 2021 to 5.77% (95% CI 1.98–15.64%; 3/52 rabbits) in 2022 (Table), before dropping again to undetectable levels in 2023 (0/78 rabbits). We analyzed 143 rabbits in 9 other sites from Portugal collected in 2022 without detecting any seropositivity, suggesting that CCHFV circulation was very localized. Given the rabbit density data obtained by capture-recapture methods in that same population (peak densities of 3.0–3.8 rabbits/hectare in 2021–2023) (9), we estimated the captured rabbits to represent 40%–75% of the total population yearly. Therefore, we inferred that the probability of CCHFV being undetected in 2021 and 2023 ranged from 1% to 10%. (<https://epitools.ausvet.com.au/freedomss>). Overall, our data suggest that CCHFV circulation in rabbit populations in Portugal was highly localized in space and time, with a sudden increase in a specific southern location (Mértola) in 2022.

Our results highlight the need for further studies to understand the ecologic and epidemiologic role of wild rabbits in the dynamics of *Hyalomma* tick populations and CCHFV circulation. Considering the preference of immature *H. lusitanicum* ticks for lagomorphs in the Iberian Peninsula, we anticipated the detected exposure of rabbits to CCHFV (7). Nevertheless, previous surveys in wild rabbit populations from areas of suspected active circulation in Spain (5)

did not detect any evidence of CCHFV exposure. Given our results of 10% between-cluster prevalence and 5% within-cluster seroprevalence, we estimated that aiming for 95% cluster sensitivity, a minimum sample of 31 rabbits per cluster would be required to detect exposure to CCHFV in areas of active viral circulation (<https://epitools.ausvet.com.au/twostage-freedomsstwo>). Therefore, when planning future surveys in wild rabbits, we recommend implementing a 2-stage cluster sampling approach (10) to better detect spatiotemporally clustered CCHFV antibodies.

The live capture protocol was approved by CIBIO Animal Welfare committee (ORBEA/2023_01) and performed under the nature conservation authority licenses (08/2019, 1/2020, 197/2020, 23/2021, and 574/2022), according to Portuguese and European legislation. No animals were killed for the purpose of this study.

Acknowledgments

The authors acknowledge the support of Parque de Natureza de Noudar S. A., Empresa de Desenvolvimento e Infra-estruturas do Alqueva S. A., and Companhia das Lezírias S.A., as well as the hunters and students who assisted with the collection of samples.

This work was funded by Fundação para a Ciência e Tecnologia (Grant SFRH/BPD/116596/2016 to N.S.). Work supported by the European Union’s Horizon 2020 Research and Innovation Programme (grant agreement no. 857251) and funds from provided by Direction Generale de l’Alimentation.

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Increased Rates of *Purpureocillium lilacinum* Mold among Laboratory Culture Results, United States

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DOI: <https://doi.org/10.3201/eid3110.250715>

Purpureocillium lilacinum, a common environmental mold and bionematicide, can cause human infections. At a major US commercial laboratory during March 2019–February 2025, *P. lilacinum* culture rates increased; rates were highest in the South Atlantic and Pacific states. Nonculture-based diagnostic tools such as microscopy may help identify and confirm clinical infection earlier.

Purpureocillium lilacinum (formerly *Paecilomyces lilacinus*) is a naturally occurring filamentous fungus that is common in the environment, particularly in soil and decaying vegetation (1). Two strains of *P. lilacinum* registered in 2005 and 2021 are used as agricultural bionematicides in the United States (2,3). The fungus rarely causes human infection but can cause hyalohyphomycosis, an infection with varied clinical soft-tissue, ocular, or pulmonary manifestations. Infection most frequently affects immunocompromised persons but can also occur in immunocompetent persons. Mortality rates can reach 20% (1).

P. lilacinum infection is clinically indistinguishable from other mold infections, and the organism resembles other molds on cytology, histopathology, and culture, potentially leading to misidentification and to delayed or inappropriate treatment (1). *P. lilacinum* is intrinsically resistant to amphotericin B and can be correlated with poorer treatment outcomes (1,4).

Worldwide, clinical characteristics and outcomes of 101 *P. lilacinum* infections have been described, 31 of which were from the United States, but epidemiology of the infection in the United States is poorly understood (1). We explored *P. lilacinum* culture data from a large national commercial laboratory to describe this organism in the United States.

We used data from the Centers for Disease Control and Prevention's National Syndromic Surveillance Program (NSSP) (<https://www.cdc.gov/nssp/index.html>), which collects data from Labcorp (<https://www.labcorp.com>), a major national commercial laboratory network. Labcorp transmits test orders and results for all reportable diseases in the United States to NSSP. Although *P. lilacinum* infection is not reportable to public

health authorities, NSSP receives data on all fungal cultures performed at Labcorp because other fungal diseases are reportable (5). We identified culture results for *P. lilacinum* ordered during March 1, 2019 (earliest available data) through February 28, 2025.

We examined demographic characteristics, geographic location of submitting provider's state (US Census division), specialty or setting of the ordering healthcare provider, specimen type, and time from test order to culture result. Unique patient identifiers were unavailable; therefore, we conducted analyses at the test-result level.

We identified 1,180 *P. lilacinum* cultures (Table; Appendix Figure 1, <https://wwwnc.cdc.gov/EID/article/31/10/25-0715-App1.pdf>). Rates of *P. lilacinum* per 100,000 fungal cultures per year increased from 56.6 in 2019 to 74.3 in 2024 and peaked at >90 in the third quarter of 2024 (Figure). Overall, male persons (68.8/100,000 population) and persons ≥65 years of age (89.7/100,000 population) had the highest culture rates. By US Census division, the South Atlantic (110.6/100,000 population) and Pacific (100.3/100,000 population) divisions had the highest rates. Most (52%) *P. lilacinum* cultures were ordered from hospital settings. Specimen type was available for 57% of cultures, among which respiratory specimens were most common (38%). The median time from collection to result was 23 (interquartile range 15.0–33.0) days.

Those commercial laboratory data suggest a recent increase in *P. lilacinum* cultures in the United States and a wide geographic distribution. Higher rates among male persons and older persons align with a previous study of *P. lilacinum* infections (1). The long time (>3 weeks) for culture growth and identification for this mold might lead to diagnostic delays and potentially inappropriate treatment (1,4).

The South Atlantic and Pacific Census divisions had substantially higher *P. lilacinum* culture rates than other regions of the United States. The high rates in the Pacific region could reflect a substantial uptick in cultures from that area during 2024–2025, including 60 cultures from a single facility that was the subject of a public health investigation (6). That event represented a pseudo-outbreak from environmental contamination rather than a true clinical outbreak, which is a rare occurrence. A previous *P. lilacinum* outbreak was linked to contaminated skin lotion (7). Two strains of *P. lilacinum* are used in the United States as agricultural bionematicides, PL251 registered in 2005 and PL11 registered in 2021 (2,3). Use of those bionematicides could possibly contribute to increased environmental presence and culture contamination during collection or in laboratories.

Table. Patient characteristics in study of increased rates of *Purpureocillium lilacinum* mold among laboratory culture results, United States*

Characteristic	Value	Rate†
Year of sample collection, n = 1,180		
2019	136 (12)	56.6
2020	134 (11)	50.5
2021	182 (15)	59.3
2022	224 (19)	71.7
2023	210 (18)	62.8
2024	258 (22)	74.3
2025	36 (3)	61.9
Median patient age, y (IQR)	65.0 (53.0–74.0)	
Age group, y, n = 1,175		
0–17	40 (3)	21.6
18–44	154 (13)	41.0
45–64	357 (30)	59.7
≥65	624 (53)	89.7
Sex, n = 1,159		
M	607 (52)	68.8
F	552 (48)	58.9
US Census division, n = 1,172‡		
East North Central	91 (8)	69.1
East South Central	139 (12)	78.9
Middle Atlantic	11 (1)	3.6
Mountain	13 (1)	11.6
New England	<10 (<1)	28.7
Pacific	263 (22)	100.3
South Atlantic	603 (51)	110.6
West North Central	<10 (<1)	6.7
West South Central	44 (4)	17.3
Provider type, n = 1,100		
Hospital	567 (52)	
Family practice or internal medicine	132 (12)	
Dermatology	111 (10)	
Podiatry	71 (6)	
Pulmonary disease	55 (5)	
Otolaryngology	45 (4)	
Ophthalmology	14 (1)	
Other	105 (10)	
Median days from collection to result (IQR)	23.0 (15.0–33.0)	
Specimen type, n = 670		
Respiratory	254 (38)	
Nail	106 (16)	
Skin	82 (12)	
Sinonasal	13 (2)	
Eye	12 (2)	
Isolate	183 (27)	
Other	20 (3)	

*Values are no. (%) except as indicated. IQR, interquartile range.

†Positive results/100,000 cultures. Fungal cultures identified by Logical Observation Identifier Names and Codes nos. 188243, 188573, 188805, 42804–5, and 580–1.

‡US Census, https://www2.census.gov/geo/pdfs/maps-data/maps/reference/us_regdiv.pdf.

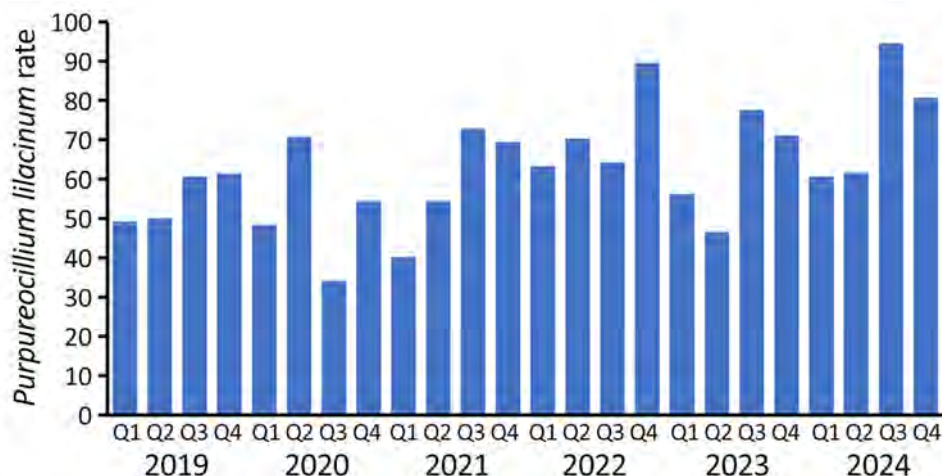


Figure. Rates of *Purpureocillium lilacinum* mold among laboratory culture results, United States. Graph shows *P. lilacinum* cultures per 100,000 fungal cultures by year and quarter during March 2019–February 2024.

Internationally, at least 1 case of subcutaneous *P. lilacinum* infection has been reported in association with bionematicide use (8). We could not assess environmental exposures in patients from whom the cultures in our study were derived; more data are needed on potential *P. lilacinum* environmental sources and their correlation with clinical culture positivity and infection risk.

Without clinical data, we were unable to determine patients' underlying medical conditions and whether cultures represented true infection versus colonization. The most common specimen source was the respiratory tract, which can be colonized with various molds. Furthermore, nearly half of cultures were missing specimen type information, likely skewing our results; however, another study of confirmed infections found that skin was the most common infection site (1). Last, our data are a convenience sample and might not necessarily represent the entire US population.

In summary, mold infections generally are associated with substantially delayed and missed diagnoses (9). Further investigations are needed to understand the increased *P. lilacinum* culture rates, including examining bionematicide use, environmental changes, and clinical effects. Because *P. lilacinum* culture rates appear to be increasing, clinicians could consider nonculture-based diagnostic tools, such as microscopy (Appendix Figure 2), to help identify and confirm clinical infection earlier.

Acknowledgments

The authors thank Stephanie Dietz for assistance with NSSP data.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the US Department of Agriculture.

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Angiostrongylus cantonensis Lungworms in Definitive and Intermediate Hosts, Madagascar, 2024

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DOI: <https://doi.org/10.3201/eid3110.241741>

We assessed the prevalence of the rat lungworm, *Angiostrongylus cantonensis*, in rats and snails in Toamasina, Madagascar, using molecular techniques. Although no human cases of neuroangiostrongyliasis have been reported in Madagascar, the pathogen's presence in definitive hosts (2.5%, 2/78) and intermediate hosts (26.9%, 35/130) reveals active circulation and potential zoonotic risk.

The rat lungworm, *Angiostrongylus cantonensis* (Strongylida: Angiostrongylidae), first identified and described in China in 1935 (1), is responsible for neuroangiostrongyliasis, which can cause neurologic damage, such as eosinophilic meningitis or encephalitis, in humans. The life cycle of this parasitic nematode involves rats as definitive host and snails and slugs as intermediate hosts; humans act as incidental hosts only in this system (2).

Neuroangiostrongyliasis is a foodborne disease, contracted by humans through accidental ingestion of the *A. cantonensis* infective larvae; several thousand human cases have been documented worldwide (3). The infective larva can be found not only in intermediate hosts but also in paratenic hosts (e.g., crustaceans, frogs), on contaminated vegetables, and in water (2,4).

In Madagascar, *A. cantonensis* worms were first sampled in 1964 (5) in rats from Ambavaniasy, Alaotra Mangoro Region, and later confirmed in *Rattus* spp. rats (6). The parasite was reported in rats captured in Antananarivo in 1982 (7); prevalence was 12% during the rainy season and 5% during the dry season. Human cases of neuroangiostrongyliasis on the neighboring islands of Mayotte and La Reunion have been reported (2). In addition, human breeding and consumption of intermediate hosts of this parasite, specifically snails of the genus *Achatina*, are still taking place on the island; however, data on the potential role of this snail as a carrier of *A. cantonensis* worms are currently unavailable. In this context, the aim of this study was to update the occurrence of *A. cantonensis* worms in rats and investigate their presence in snails.

We trapped rats and collected snails in March 2024 in 3 communes in Toamasina district: Fanandrana, Antetazambaro, and Ankirihiry (Appendix, <https://wwwnc.cdc.gov/EID/article/31/10/24-1741-App1.pdf>). Further, we collected snails of the genus *Achatina* (Figure 1, panel A) and sampled and preserved a portion of the foot of each snail for molecular screening for the presence of *A. cantonensis* worms. After DNA extraction, we performed conventional PCR targeting the cytochrome c gene to confirm the identity of adult lungworms (Appendix). Further, we performed molecular screening of *A. cantonensis* worms using TaqMan quantitative PCR (Thermo Fisher Scientific, <https://www.thermofisher.com>) targeting the internal transcribed spacer gene (Appendix). We used the χ^2 test to compare the statistical differences in intermediate host infection by localities.

We captured a total of 78 individual rats of 2 species, 76 *Rattus rattus* rats and 2 *R. norvegicus* rats; 61.5% (48/78) were male and 38.4% (30/78) were

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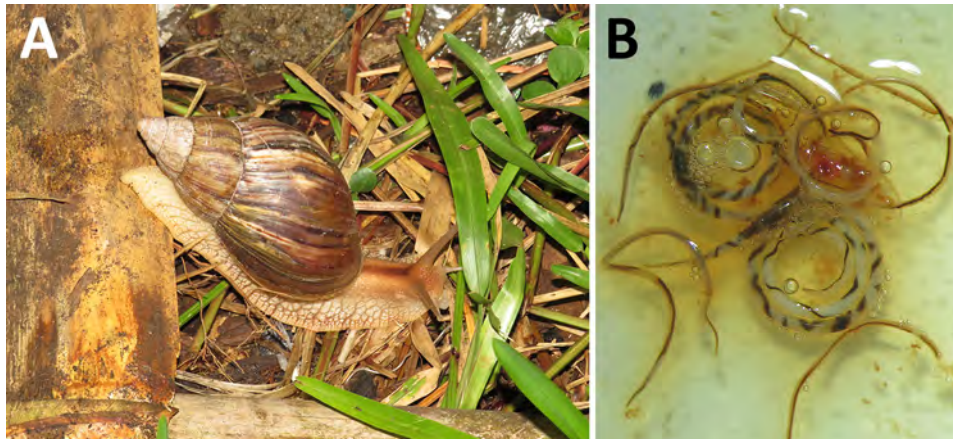


Figure 1. Specimens obtained in study of *Angiostrongylus cantonensis* lungworms in definitive and intermediate hosts, Madagascar, 2024. A) An *A. cantonensis* worm intermediate host giant African land snail (*Achatina* sp.) in Madagascar; B) adult male and female *A. cantonensis* worms extracted from the lung of an infected *Rattus norvegicus* rat.

female, and the age distribution was 55.1% (43/78) juvenile and 44.8% (35/78) adult (Table 2; Appendix). Two *R. norvegicus* rats captured in Ankirihiry harbored 12 adult lungworms; 8 were isolated from a pregnant female rat and 4 from a juvenile male rat (Figure 1, panel B). We amplified and sequenced 2 adult lungworms from each of the 2 rats (GenBank

accession nos. PV185895, PV185896). Those sequences showed 100% similarity with a partial genome sequence of an isolate from Spain (accession no. PP748576) and clustered with nucleotide sequences from Spain, Australia, the United States, Japan, and Brazil (Figure 2). The overall prevalence of infection in rats was 2.5% (2/78).

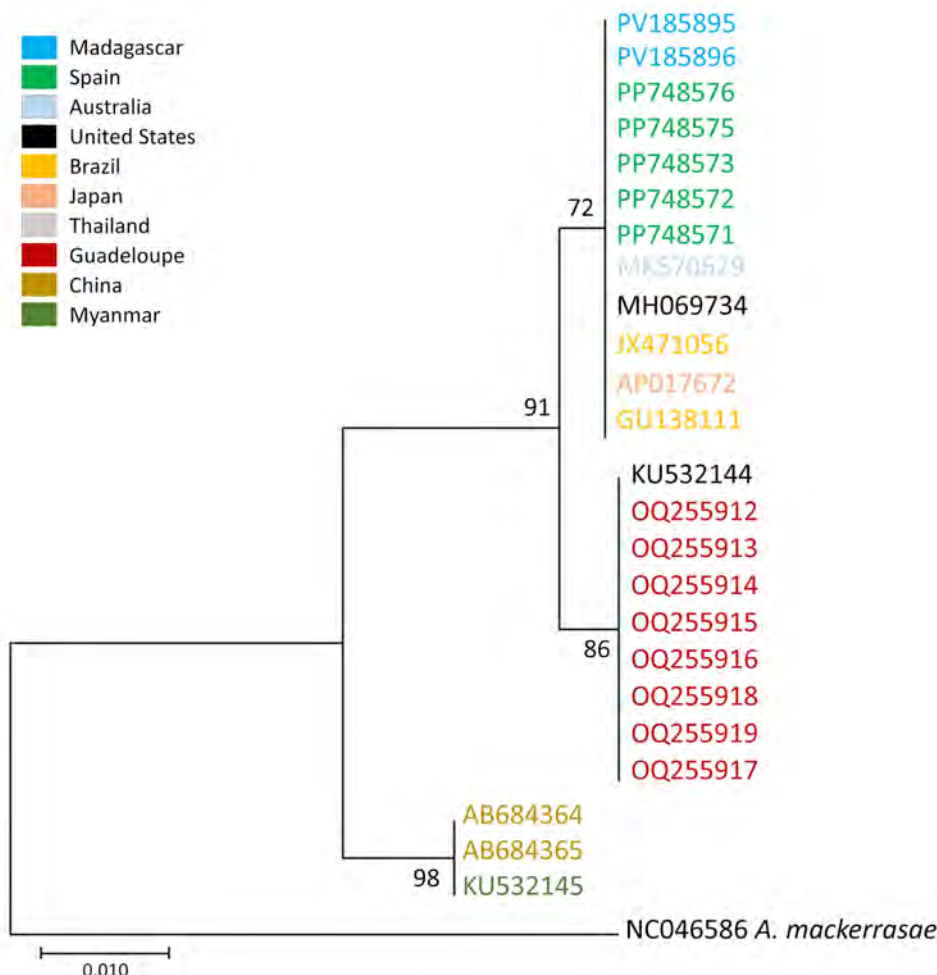


Figure 2. Phylogeny of adult *Angiostrongylus cantonensis* lungworm based on cytochrome c sequences in definitive and intermediate hosts, Madagascar, 2024. Labels are GenBank accession numbers, colored by location. The phylogenetic tree was constructed using the Tamura-Nei 1993 plus gamma distribution model with 10,000 iterations in MEGA X (<https://www.megasoftware.net>). All bootstrap values are shown on corresponding nodes. *Angiostrongylus mackerrasae* was used as the outgroup. Scale bar indicates number of substitutions per site.

For the intermediate host, we collected 130 snails of the *Achatina* genus. A total of 35 individual snails tested positive by quantitative PCR, reaching an overall prevalence of 26.9% (35/130); rates of prevalence were 51.5% (17/33) in Ankirihiry, 29.6% (16/54) in Antetetzambaro, and 4.6% (2/43) in Fanandrana. Statistical analysis revealed a significant difference in prevalence of *A. cantonensis* worms in *Achatina* snails collected in those localities ($\chi^2 = 38.343$, degrees of freedom = 2; $p < 0.0001$).

Our data report an overall prevalence of 26.9% of *A. cantonensis* worms in *Achatina* snails from the east coast of Madagascar, which is higher than in other *A. cantonensis*-endemic regions such as China (21.5%) (8), Brazil (21.7%) (9), and Ecuador (15.2%) (10). The high prevalence of *A. cantonensis* worms in the *Achatina* genus demonstrates their involvement in the life cycle of this zoonotic parasite in Madagascar. Furthermore, 2 *R. norvegicus* rats (a juvenile male and pregnant female) caught in Ankirihiry were parasitized by *A. cantonensis* worms. The presence of this lungworm in *R. norvegicus* rats and snails in Ankirihiry suggest an active circulation of this parasite and a potential risk for human neuroangiostrongyliasis on the east coast of Madagascar because of the abundance of hosts (intermediate or definitive) and the consumption of either infected snails or food contaminated by infective larva. Properly boiling water to cook snail meat thoroughly before consumption is critical to avoid infection. Because *Rattus* spp. rats are found everywhere in Madagascar, future research should investigate other locations to survey the prevalence of *A. cantonensis* worms in rats, as well as the distribution of *Achatina* spp. snails and the prevalence of the parasite in those and other snail species to implement surveillance. Although no human infections have been reported in Madagascar, our findings indicate active circulation of the *A. cantonensis* rat lungworm, posing a potential zoonotic risk.

Financial support to conduct this research was given by Institut Pasteur de Paris through ACIP ORACAN (ACIP no. 569-2022).

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Detection of *Burkholderia thailandensis* in Soil Samples, Suriname

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DOI: <https://doi.org/10.3201/eid3110.251114>

Melioidosis, caused by the highly lethal pathogen *Burkholderia pseudomallei*, is emerging in North and South America. We studied soil samples in Suriname to determine endemicity of *Burkholderia* species. *B. thailandensis* was isolated, but *B. pseudomallei* was not. A multidisciplinary approach could establish clinical and ecologic distribution of both *Burkholderia* species in Suriname.

The epidemiology of the environmental gram-negative bacteria *Burkholderia pseudomallei*, the highly lethal pathogen causing melioidosis, and less lethal *B. thailandensis* is changing because of climatic conditions (1,2). During 2019–2022, *B. pseudomallei* and *B. thailandensis*, both part of the *B. pseudomallei* complex, were isolated from the environment in the continental United States (2,3). A large set of whole-genome sequence data indicates that *B. pseudomallei* could have been introduced from Africa to

North and South America between 1650 and 1850 through the slave trade (4), and that might also be true for *B. thailandensis*.

Suriname, a tropical country in South America with an ethnically diverse population of <1 million inhabitants, could be a historic port of entry for *Burkholderia* species, especially considering *B. pseudomallei* is established in neighboring countries Brazil and (presumably) French Guiana (5,6). However, neither *Burkholderia* species has yet been identified in Suriname. We performed an environmental sampling study to determine whether *B. pseudomallei* or *B. thailandensis* could be isolated from soil samples in Suriname.

We collected soil samples in Suriname during the major wet season in May–July 2023 (<https://climateknowledgeportal.worldbank.org/country/suriname/climate-data-historical>) by using methods from the consensus guidelines for isolation of *B. pseudomallei* and our previous work in Nigeria (7,8). We selected 4 sites in the northern districts Paramaribo, Commewijne, and Nickerie and collected 100 samples at each site at a depth of 60–80 cm (Table). We determined the soil acidity and alkalinity and the salinity of air-dried mixed soil samples by measuring the pH and electrical conductivity. Next, we cultured 10 g of soil by using selective broth and agar and inspected agar plates daily for suspect isolates. We subcultured morphologically suspect isolates onto blood agar plates and screened for oxidase positivity by using oxidase strips (Sigma-Aldrich, <https://www.sigmaaldrich.com>) and antimicrobial resistance testing by using disks (Thermo Fisher Scientific, <https://www.thermofisher.com>) to assess susceptibility to amoxicillin/clavulanic acid at 30 µg (defined by the presence of an inhibition zone) and resistance to colistin at 10 µg and gentamicin at 10 µg (defined by the absence of an inhibition zone), considered an adjustment of the standard operating procedure of the consensus methods (7,9). Next, we subjected isolates matching those criteria to a real-time multiplex

Table. Site and sample characteristics for detection of *Burkholderia thailandensis* in soil samples, Suriname*

Site	Region	District	Location	Site characteristics	pH	Electrical conductivity, µS/cm	No. positive samples
A	Northern	Paramaribo	Centre for Agricultural Research in Suriname	Inceptisol soil type; brackish field from which former crops included sem bean, banana, cassava, peanut, corn, and soybeans	7.30	769	0
B	Northern	Commewijne	Plantation Rust en Werk	Inceptisol soil type; field with cattle	4.42	1,313	0
C	Northwest	Nickerie	Europolder	Inceptisol soil type; brackish rice field	7.66	908	0
D	Northwest	Nickerie	Middenstandspolder	Inceptisol soil type; brackish rice field	6.83	1,097	4

*At each site, 100 soil samples were collected at a depth of 60–80 cm.

PCR on the LightCycler 480 II (Roche, <https://www.lifescience.roche.com>) and subsequent presumed *B. thailandensis* isolates were confirmed by using whole-genome sequencing on the NextSeq 500 platform (Illumina, <https://www.illumina.com>) (Appendix, <https://wwwnc.cdc.gov/EID/article/31/10/25-1114-App1.pdf>). We uploaded whole-genome sequences of the *B. thailandensis* isolates to the European Nucleotide Archive database (<https://www.ebi.ac.uk/ena>; project no. PRJEB79520, sample accession nos. ERS20924713–6).

We collected 400 soil samples across 4 different sites and identified 4 *B. thailandensis* isolates at 1 site in the Nickerie district (Table). However, we did not detect *B. pseudomallei*. Sequence data revealed that the *B. thailandensis* isolates were phylogenetically distinct because they differed by 2,965–3,226 single-nucleotide polymorphisms from each other, suggesting multiple introductory events. We downloaded paired-end sequences of other *B. thailandensis* isolates with data on country and source from the European Nucleotide Archive database, including isolates identified in the continental United States and Nigeria (2,8), and constructed a phylogenetic tree (Figure). The isolates from Suriname clustered in the same clade with the isolates from the United States and Nigeria, suggesting the Suriname isolates descended from Africa.

We might have underestimated the true distribution of *B. thailandensis* and cannot rule out the

presence of *B. pseudomallei* because of the small sample size and reliance on outdated soil-focused consensus guidelines based on soil sampling in highly endemic areas (7). Furthermore, additional water and air sampling might have yielded a higher success rate in determining endemicity for *B. pseudomallei* yet not an integral part of the adopted guidelines (7).

A strength of our study is performing an environmental sampling study in different districts in the northern parts of Suriname, where the population and agricultural practices are dense and interaction between humans and soil is highly likely. However, we did not find *B. pseudomallei*, which might reflect a low soil abundance. In line with this finding, a previous study of traveler-associated melioidosis cases in the Netherlands did not identify any cases that originated in Suriname (10), despite frequent family visits between Suriname and the Netherlands. Our phylogenetic results support the theory that *B. thailandensis* was introduced to the Americas through slave trade, similar to the continental introduction of *B. pseudomallei*, suggested by others on the basis of sequence data of 469 isolates (4).

In conclusion, we detected *B. thailandensis* in soil samples from Suriname. Although we did not detect *B. pseudomallei*, a multidisciplinary approach could clarify the clinical and ecologic distribution of both *Burkholderia* species in Suriname.

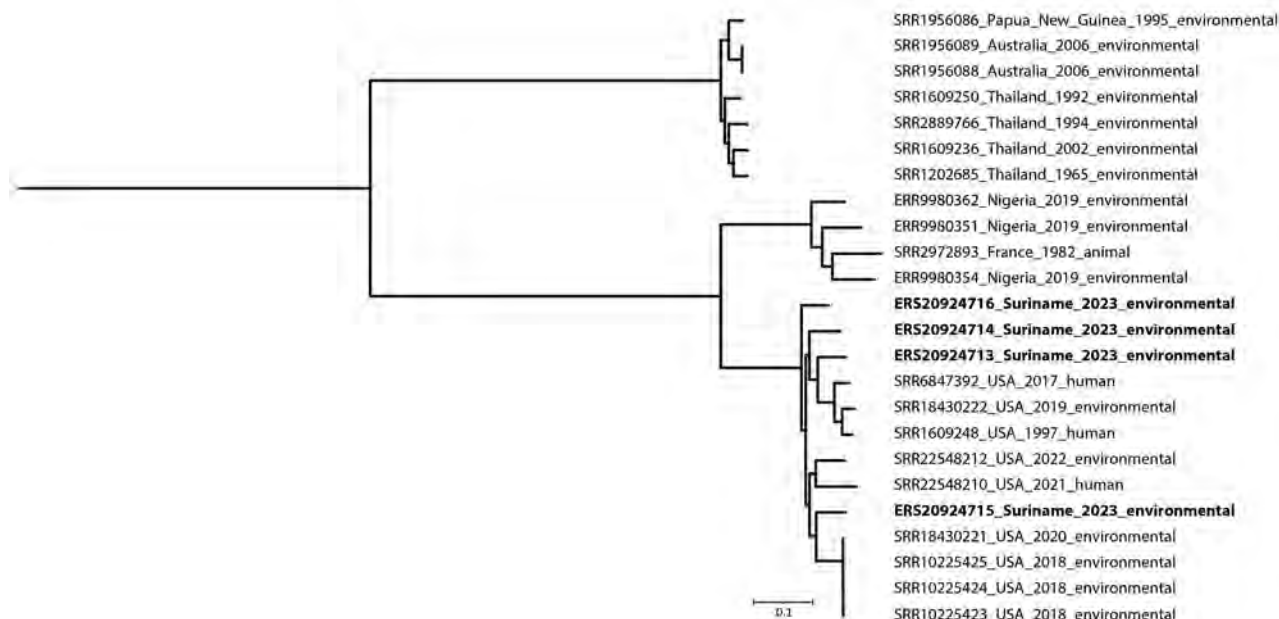


Figure. Phylogeny of *Burkholderia thailandensis* detected in soil samples, Suriname. Maximum-likelihood tree generated by FastTree (<http://www.microbesonline.org/fasttree>) on the basis of core single-nucleotide polymorphism distance and visualized with iTOL (<https://itol.embl.de>). Bold indicates isolates from this study. Additional global genomes, all retrieved from the European Nucleotide Archive database (<https://www.ebi.ac.uk/ena>), are indicated by accession number, country name, year of isolation, and isolate source. Scale bar indicates nucleotide substitutions per site.

Acknowledgments

We are grateful to everyone who helped with the collection, processing, and analysis of the samples. We especially thank the team of Verenigde Cultuur Maatschappijen at Rust en Werk for their kind gestures.

This work was financially supported by the Stichting Studiefonds KETEL 1 and the Cultuurfonds. J.S. was supported by an Amsterdam UMC PhD scholarship and E.B. by a Veni grant of the Dutch Research Council (reference no. 09150162210184).

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Detection of Immunity Gap before Measles Outbreak, Ho Chi Minh City, Vietnam, 2024

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DOI: <http://doi.org/10.3201/eid3110.250234>

In 2022, we established a residual sample serosurveillance program in Ho Chi Minh City, Vietnam. During September 2022–April 2024, we found low measles antibody seroprevalence in children in the city's western region, where a measles outbreak began in May 2024. Serosurveillance could be a useful tool for outbreak prediction and prevention.

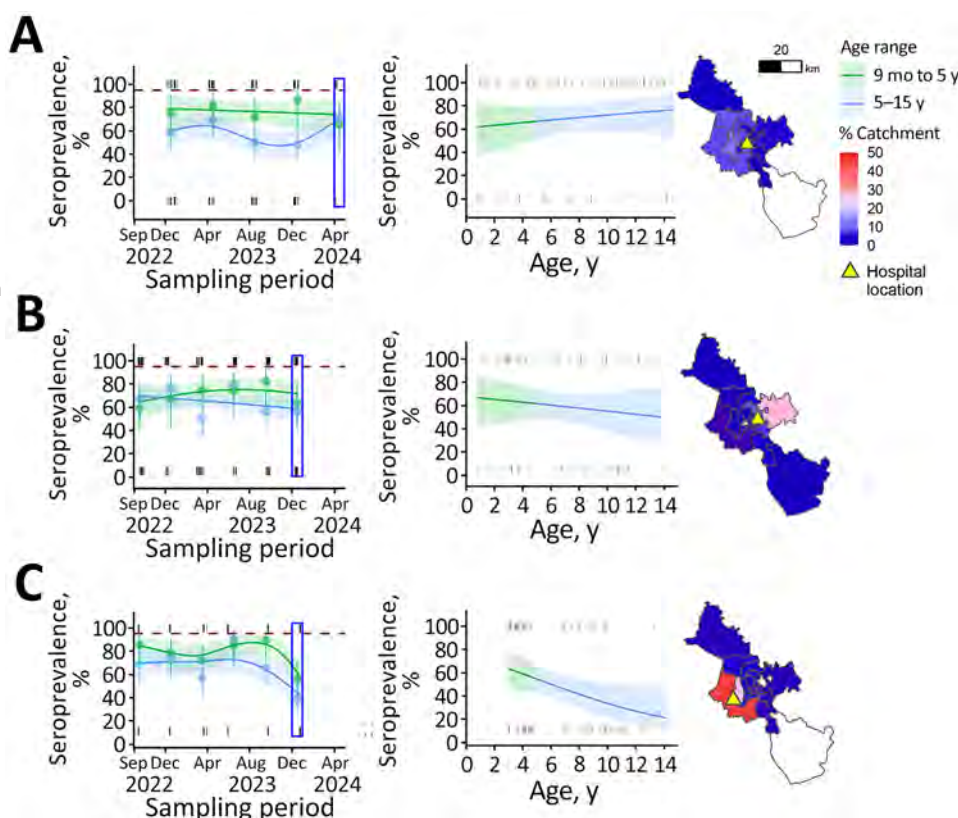
Measles is a highly contagious vaccine-preventable disease. Ho Chi Minh City (HCMC), Vietnam, a city of 11 million persons, experienced a 20-month vaccination disruption during COVID-19 lockdowns and mass COVID-19 vaccination campaigns (June–November 2021) and postpandemic vaccine shortages (October 2022–December 2023). The last major measles outbreak in HCMC occurred in 2019. In 2024, measles cases increased in May, leading to an official outbreak declaration on August 27, 2024, in which 4,133 cases were reported by December 2024. Case-patients had a median age of 5.1 years (interquartile range 9 months to 12.6 years; range 1 month to 76 years). HCMC began a measles vaccination campaign on August 31, 2024, targeting children

1–5 years of age, and expanding to children 1–10 years of age on October 1, 2024.

Serosurveillance of antibody titers can be used to assess population immunity and relies on repeated serosurveys to monitor immunity changes over time (1). In HCMC, we established a serosurveillance system using anonymized residual serum samples from hospitals, cataloged and stored for future use. Sampling followed an age-stratified serial cross-sectional scheme every 3 to 4 months at Children's Hospital 1, Children's Hospital 2, and City Children's Hospital. The only public pediatric hospitals in HCMC, those hospitals handle most hospitalized children 0–15 years of age across HCMC.

We analyzed 1,097 serum samples from children 0–15 years of age collected during September 2022–April 2024 by using a measles IgG ELISA assay (SERION Immunologics, <https://www.serion-immunologics.com>). We defined seropositivity as antibody titer >200 mIU/mL (sensitivity 99.0%; specificity 95.0%). We used generalized additive models with a binomial error distribution, logit link, and thin plate spline smooth terms to model seroprevalences

Figure 1. Hospital locations, catchment areas, and number of cases per age in detection of immunity gap before a measles outbreak, Ho Chi Minh City, Vietnam, 2024. A) Children's Hospital 1; B) Children's Hospital 2; C) City Children's Hospital. The first column shows seroprevalence as a function of time of collection for 2 age bands from each hospital; blue rectangles indicate the latest collections; dashed red lines represent the 95% critical vaccination threshold for measles; tick marks at top and bottom of graphs denote seropositive and negative samples; points represent monthly aggregated seroprevalence; error bars indicate 95% CI of the aggregated seroprevalence; shaded areas indicate 95% CI of the seroprevalence estimates by time of collection. Before the measles outbreak, the levels of seroprevalences were 70.7% (95% CI 57.6–81.1) for Children's Hospital 1 in April 2024 (A), 58.1% (95% CI 44.1–70.9) for Children's Hospital 2 in December 2023 (B), and 41.5% (95% CI 29.4–54.1) for City Children's Hospital in December 2023 (C). The middle column shows age-stratified seroprevalence at the most recent timepoint (represented in blue rectangles from the first column). The third column maps the catchment areas based on sample addresses.



as functions of age or time. For time analysis, the models applied population weights to each age. We corrected all models for sensitivity and specificity using the Rogan-Gladen estimate (2). We used line list data of patients residing in HCMC to investigate the outbreak. We estimated time-varying reproduction numbers, using previously described methods (3), with gamma-distributed serial intervals (mean 14.5 days; SD 3.25 days) (4) (Appendix, <https://wwwnc.cdc.gov/EID/article/31/10/25-0234-App1.pdf>).

During September 2022–April 2024, seroprevalence of measles antibodies among children <15 years of age remained consistently <90% across all 3 hospitals (Figure 1). Such low seroprevalences in the 0–5-year age group, likely resulting from vaccination disruptions, translates to high risk for a measles outbreak. Seroprevalence was lower in the 5–15-year age group (Figure 1), possibly because of incomplete vaccination in the past. The western part of HCMC, corresponding to City Children’s Hospital catchment area (Figure 1), had the lowest seroprevalence and reported the first outbreak cases, highest cumulative incidence rates, and consistently high attack rates compared with other districts (Appendix Figure 1, panels A, C). Seroprevalence remained below the administrative coverage estimates for children who received both first and second measles vaccine doses (Figure 2; Appendix Figure 2, Tables 1, 2).

Reproduction numbers peaked at 1.99 (95% CI 1.65–2.36) on August 13, 2024, and declined to 0.97–1.50 after the vaccination campaign (Appendix Figure 1). The percentage of infected patients 1–10 years of age decreased after the vaccination campaign targeting that group, and the percentage of infected 10–15-year-olds increased, and attack rates rapidly rose. Children 10–15 years of age and adults were infected later than other groups, but their attack rates increased quicker than for other age groups.

Measles antibody seroprevalence gaps might have served as an early warning for this measles outbreak. The lowest seroprevalence, observed in the city’s western region, corresponded to the earliest cases and highest attack rates. Low seroprevalence in persons 5–15 years of age might explain the number of measles cases in that group. Because that age group is the most socially connected in the country’s population (5), low immunity can accelerate community spread. Of note, researchers studying a 2014 measles outbreak in northern Vietnam recommended serosurveillance to assess outbreak risks and improve responses (6). Strengthening serosurveillance to detect immunity gaps early could enhance preparedness for future outbreaks.

The first limitation of our study is that hospital-based samples might underestimate community immunity. However, small pockets of susceptibility pose high outbreak risks, especially in communities with high average vaccination coverages (7). Estimating coverage from registry data is challeng-

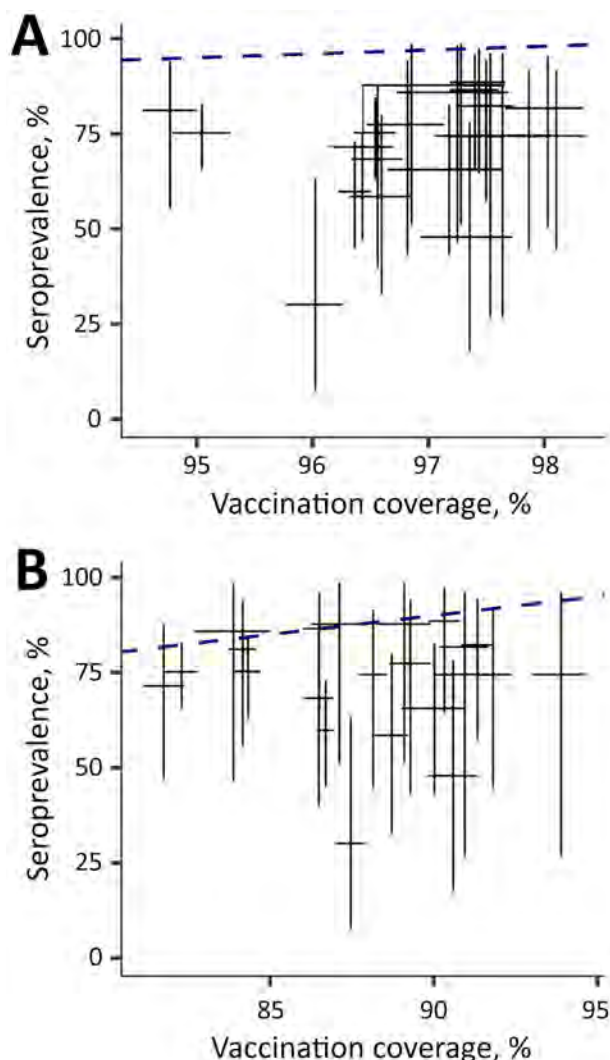


Figure 2. Comparison of seroprevalence and vaccination coverage in a study of immunity gap before a measles outbreak, Ho Chi Minh City, Vietnam, 2024. A) Seroprevalence among children receiving first measles vaccine dose only; B) seroprevalence among children receiving first and second measles vaccine doses. Bars show the 95% CI of seroprevalence (vertical) and vaccine coverage (horizontal). Seroprevalence remained below the administrative vaccination coverage estimates for children who received both first and second measles doses but corresponded more closely with second-dose coverage. The blue dashed line indicates 1:1 relationship of vaccination coverage to seroprevalence. A wider view, administrative coverage data for each district and neighboring provinces, and antibody concentration of 17 children <9 months of age is available in the Appendix (<https://wwwnc.cdc.gov/EID/article/31/10/25-0234-App1.pdf>).

ing because of variables such as uncertain residency and unrecorded migration from areas with lower vaccination coverage. Our hospital-based serosurveillance system is a valuable example of sentinel surveillance, especially where hospital-acquired infections have triggered previous outbreaks (8). A key advantage is efficiency and sustainability in sample collection. Second, although measles antibody titers correlate with protection, limited data are available to establish a protective threshold (9). We used the manufacturer-recommended cutoff of >200 mIU/mL to classify seropositivity.

In summary, our results suggest that low-cost, hospital-based serosurveillance data could detect risk for measles outbreaks ≥ 6 months in advance. Acknowledging that this approach to surveillance should be further validated to assess the specificity of such a system (false-positive alarms), we are confident that such an approach could effectively identify populations at high outbreak risk, potentially informing optimal vaccination strategies.

Acknowledgments

We thank Children's Hospital 1, Children's Hospital 2, and City Children's Hospital for providing the residual serum samples.

This study was approved by the institutional review board of the Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam (approval no. 1735/QD-BVBND), and the Oxford Tropical Research Ethics Committee (OxTREC reference no. 577-24), University of Oxford, United Kingdom.

L.V.T. is supported by Wellcome (grant no. 226120/Z/22/Z). The OUCRU serum bank, measles assays, T.O., P.N.T.N., H.N.N., and M.C. are supported by Wellcome through the core grant of OUCRU (no. 225167/A/22/Z).

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etymologia

Malassezia [mal"ə-sē'zhə]

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Malassezia is a genus of basidiomycetous yeasts (Figure 1) that includes 18 validly published species, most of which are strictly lipophilic. These organisms are considered part of the normal cutaneous microbiota in humans and many animals. *Malassezia* spp. have also been associated with many skin diseases, including pityriasis versicolor, folliculitis, seborrheic dermatitis, dandruff, atopic dermatitis, and psoriasis.

In 1874, French histologist and anatomist Louis-Charles Malassez (Figure 2) isolated yeast

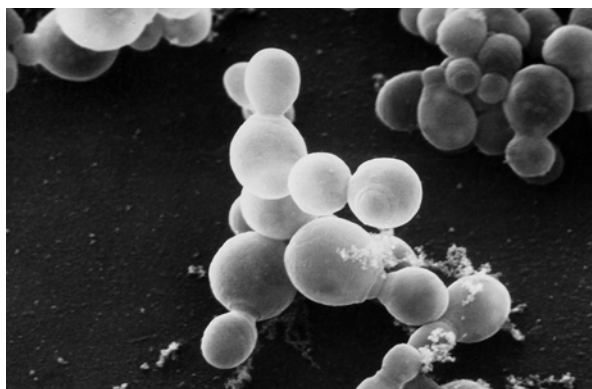


Figure 1. Scanning electron microscopic image of *Malassezia furfur* reproductive fungal spores. Source: Public Health Image Library, Centers for Disease Control and Prevention (<https://phil.cdc.gov>). Photograph credit: Janice Haney Carr.

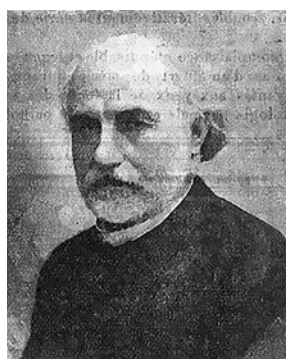


Figure 2. Louis-Charles Malassez (1842–1909), after whom *Malassezia* spp. fungi are named. Image reproduced under open license. Source: Paris City University Image and Portrait Bank (<https://numerabilis.u-paris.fr/medica/banque-images/index.php>).

cells from human dandruff scales and observed yeast with spherical and oval forms. Decades earlier, in 1846, Carl Ferdinand Eichstedt was the first to recognize the infectious nature of this fungus. He observed a journeyman with extensive pityriasis, which became subsequently known as Eichstedt's disease.

The naming of this fungus was controversial because of disagreements over its classification, and it was initially named under the genus *Pityrosporum*. After years of debate, the genus was renamed *Malassezia* in 1984, honoring Malassez, who made major contributions to understanding this group of fungi, particularly in their taxonomic classification and lipophilic nature.

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DOI: <https://doi.org/10.3201/eid3106.221743>

Emerging Sexual Transmission of *Trichophyton mentagrophytes* Genotype VII Infections, United States

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DOI: <https://doi.org/10.3201/eid3110.251056>

Trichophyton mentagrophytes genotype VII (TMVII) is an emerging dermatophyte strain associated with sexual transmission among men who have sex with men. A hypothesis-generating query of US infectious diseases specialists found that 56% had heard of TMVII and 23% knew how to treat TMVII infections, underscoring a need for increased clinician education.

Trichophyton mentagrophytes genotype VII (TMVII) is an emerging fungus that causes dermatophy-

tosis (particularly tinea pubogenitalis, glutealis, or faciei) and is associated with sexual transmission. Initially described in travelers returning from Thailand after sexual contact with sex workers, TMVII infections more recently were reported among men who have sex with men (MSM) in Europe (1–3). TMVII infections also have been reported in China, where cases were diagnosed in a family operating a rabbit farm, including 1 child who had Majocchi's granuloma, a condition characterized by the formation of nodules in the skin that results from a deep fungal infection (4,5). The first US case of TMVII was reported in June 2024, after which public health authorities were alerted to several additional cases, all among MSM (6,7).

Recognizing and appropriately treating TMVII infections (typically with oral terbinafine) is essential to improve outcomes. TMVII infections might be confused with noninfectious conditions (e.g., psoriasis) and other sexually transmitted infections (7), and delayed treatment might result in scarring or secondary bacterial infection and continued spread (2). Patients with TMVII infection might seek care in sexual health clinics or HIV preexposure prophylaxis or HIV care settings, often under the supervision of infectious disease (ID) clinicians.

Table 1. General awareness of TMVII infection among 117 polled member clinicians of the Emerging Infections Network, by selected characteristics, United States, 2025*

Characteristic	No. (%)
Primary setting of clinical practice	
University hospital	42 (36)
Community hospital	17 (15)
Outpatient only	15 (13)
Nonuniversity teaching hospital	10 (9)
Veterans Affairs hospital or Department of Defense	11 (9)
City, county, or public hospital	10 (9)
Children's hospital	1 (1)
Other	11 (9)
Type of provider	
Infectious disease physician	105 (90)
Physician assistant, physician associate, or nurse practitioner	2 (2)
PharmD/Doctor of Pharmacy	5 (4)
Other	5 (4)
How did you first hear about TMVII infections?	
I had not previously heard of this issue	52 (44)
I had heard of this issue	65 (56)
Source	
CDC's Morbidity and Mortality Weekly Report	30 (46)
Emerging Infections Network emails	27 (42)
Public health department communication	9 (14)
CDC website	8 (12)
Clinician education website	8 (12)
Clinical colleagues	8 (12)
Scientific publication	8 (12)
Other professional listserv	7 (11)
News media	6 (9)
Social media	2 (3)
Patient	1 (2)

*CDC, Centers for Disease Control and Prevention; TMVII, *Trichophyton mentagrophytes* genotype VII.

Table 2. Awareness of TMVII infection, diagnosis, laboratory testing, and treatment among 117 polled member clinicians of the Emerging Infections Network, United States, 2025*

Survey question	Yes	No	Not sure or not answered
In the past year, I have seen or consulted on a patient with a dermatophyte infection that was suspicious for TMVII infection	5 (4)	105 (90)	7 (6)
In the past year, I have seen or managed a patient with a laboratory-confirmed TMVII infection	1 (1)	110 (94)	6 (5)
I know when to suspect TMVII infection in clinical practice	42 (36)	42 (36)	33 (28)
If I saw a patient with potential TMVII infection, I would be able to obtain laboratory testing to determine the species	33 (28)	41 (35)	43 (37)
I know how to treat TMVII infection	27 (23)	53 (45)	37 (32)
I know how to counsel patients with TMVII infection about preventing spread	34 (29)	45 (38)	38 (33)

*Values are no. (%) respondents. TMVII, *Trichophyton mentagrophytes* genotype VII.

To help explore strategies to increase disease recognition and prompt early treatment, we polled member clinicians of the Emerging Infections Network (EIN) (<https://ein.idsociety.org>), a sentinel network of ID physicians and other ID specialists. This exploratory poll was not designed to represent the knowledge and practices of the membership of EIN but only to generate hypotheses for future study.

During May 2025, EIN distributed a poll that assessed clinician knowledge of TMVII to ≈3,000 member subscribers on 3 separate occasions ≈1 week apart (Tables 1, 2). EIN queries are designated as non-human subjects research by the institutional review board of the University of Iowa.

Of the 117 respondents, 65 (56%) reported having heard of TMVII infection. Of those, 30 (46%) reported hearing about TMVII from the Centers for Disease Control and Prevention's Morbidity and Mortality Weekly Report (7) and 27 (42%) from EIN emails. Only 5 (4%) of the 117 respondents reported seeing a patient with a dermatophyte infection that was suspicious for TMVII involvement in the previous year, and only 1 respondent reported seeing a patient with confirmed TMVII infection. A total of 42 (36%) respondents stated they knew when to suspect TMVII infection, how to obtain laboratory testing specific for TMVII (33 respondents [28%]), how to treat TMVII infection (27 [23%]), or how to counsel patients about preventing spread of TMVII (34 [29%]).

The findings of this poll cannot be used to draw conclusions about the knowledge and practices of all EIN members. However, the results suggest that, although most members have heard of TMVII infection, only a minority have experience with it or know how to diagnose or treat it. TMVII is an emerging dermatophyte infection that can cause severe pain and scarring and requires prolonged treatment, particularly if diagnosis is delayed (2).

Although direct microscopic examination at the point of care can help confirm a dermatophyte infection, it cannot distinguish dermatophyte species (8). Fungal

culture can be insensitive and can require weeks before results are obtained; in addition, genotyping to identify dermatophyte species such as TMVII is not routinely available (7,9). Therefore, clinicians should be aware of TMVII and consider initiating empiric treatment with oral terbinafine (250 mg/d) in patients with consistent epidemiologic context and clinical manifestations without waiting for species confirmation (7).

Recognition of potential TMVII infection by clinicians can enable appropriate management and counseling, including initiation of empiric oral treatment, testing for sexually transmitted infections, and partner notification (7). Representative clinical images of TMVII infection are available from the Centers for Disease Control and Prevention's Morbidity and Mortality Weekly Report (7). Although TMVII has not been associated with antimicrobial resistance, ID clinicians can serve an important role in recognizing and monitoring emergence of this infection in their communities.

If the knowledge of this sample of ID physicians reflects that of the larger clinician population, then a need exists for greater education about TMVII infections and their management. Healthcare professionals who suspect a TMVII infection can contact their state or local health departments for assistance (<https://www.cdc.gov/publichealthgateway/healthdirectories/index.html>) and can email fungaloutbreaks@cdc.gov for assistance with recommendations and testing.

Acknowledgments

We thank Kaitlin Benedict for her contribution to survey design and review of this manuscript.

This work was supported by the Cooperative Agreement No. 5 (grant no. NU50CK000574), funded by the Centers for Disease Control and Prevention.

This activity was reviewed by CDC and was conducted consistently with applicable federal laws and CDC policy (see e.g., 45 C.F.R. part 46.102(l) (2), 21 C.F.R. part 56; 42 U.S.C. §241(d); 5 U.S.C. §552a; 44 U.S.C. §3501 et seq).

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Invasive Urogenital *Neisseria meningitidis* Serogroup Y Multilocus Sequence Type 1466

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DOI: <https://doi.org/10.3201/eid3110.250902>

To the Editor: Coincident outbreaks of *Neisseria meningitidis* serogroup Y (MenY) multilocus sequence type (ST) 1466 were detected in 2024, manifesting as invasive meningococcal disease (IMD) in the United States and urogenital infections in Australia (1). We read with interest the genomic analysis of globally available MenY ST1466 sequences that found that isolates from outbreaks in the United States and Australia were closely related (2). Although the study provided evidence that the urogenital site could act as a reservoir for IMD cases, risk estimates of urogenital MenY ST1466 ability to cause IMD or the propensity for the invasive isolates to inhabit the urogenital niche were lacking (2). Here, we report the case of a previously healthy 29-year-old woman who sought care at an emergency department for postprocedural pelvic inflammatory syndrome 3 days after the insertion of an intrauterine device.

¹These senior authors contributed equally to this article.

We collected microbiological samples as the initial case workup. Vaginal swab specimens were negative for *Chlamydia trachomatis* and *N. gonorrhoeae*. Oropharyngeal specimens were not collected. MenY was isolated from a positive blood culture, and subsequent investigation included additional vaginal samples for culture. Although culture results were negative, MenY was detected by PCR from 5 vaginal and intrauterine device fluid samples. We conducted whole-genome sequencing on the blood culture after DNA extraction by using Illumina iSeq (Illumina, <https://www.illumina.com>), according to manufacturer instructions. In silico typing confirmed MenY ST1466. The patient was treated with ceftriaxone and made a full recovery.

This case represents presumptive progression of urogenital carriage of MenY ST1466 to invasive disease, supporting the premise that urogenital carriage is a risk factor for IMD (2). On the basis of the link between upper airway inflammation associated with influenza and IMD, it is biologically plausible that meningococcal urethritis similarly poses an increased risk for IMD (3). Concerns remain in Australia regarding the risk posed by urogenital MenY cases being detected through enhanced laboratory practices.

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Mija-tesse Ververs (1962–), *The Pathogen Quilt*, 2021. Fabric, beads, and iron wire. 43 in x 45 in/110 cm x 114 cm. Personal collection. Digital image courtesy of Mija-tesse Ververs.

A Personal Journey Through the Microbial World in Quilting

Lesli Mitchell

This month's cover features *The Pathogen Quilt*, created by Mija-tesse Ververs, MMed, MPH, a health scientist at the Centers for Disease Control and Prevention. The quilt created by the artist serves as a poignant reflection on global health challenges, particularly in the wake of the COVID-19 pandemic. It highlights a variety of pathogens from the microbial world, including *Vibrio cholerae*, Ebola virus, HIV, SARS-CoV-2, influenza virus, Nipah virus, and monkeypox virus.

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DOI: <https://doi.org/10.3201/eid3110.AC3110>

Each pathogen is represented not only as a symbol of disease but also as a reminder of the resilience and creativity that can emerge in times of crisis.

The word quilt is linked to the Latin word *culcita*, meaning sack or stuffed. Quilting is a method of stitching layers of material together, typically involving 2 layers of fabric with a layer of padding (wadding) in between, held together by lines of stitching based on a pattern or design. Some sources trace the first evidence of quilting back to ancient Egypt, but the history of quilting can be traced at least to medieval times. Quilt-making reflects both the domestic practicality of creating a bedcover through repurposed

fabric and a visual demonstration of the design, stitching skills, and cultural traditions of the artist.

In crafting this quilt, Ververs incorporated general features of pathogens, such as viral nucleic acids, as well as the intricate processes of DNA and RNA replication. The building blocks of life—nucleotides—are represented with beads, which also convey a sense of dynamic movement, illustrating particles entering or leaving a cell's cytoplasm or nucleus or transitioning between pathogen and human tissue. This representation highlights the complex interactions within the microbial world and their impact on human health.

The materials used in this quilt were sourced from the artist's personal collection during the early months of the COVID-19 pandemic in 2020. The constraint on shopping because of quarantine led to creative choices, including the use of a small pouch from an airline amenity kit. The color palette selected is deliberately muted; Ververs felt that bright, cheerful colors were out of place amidst the somber realities of the time. However, she allowed for the freedom to invent fantasy pathogens, purely for their visual appeal, which became one of the most enjoyable aspects for her of the creative process.

Quilt-making throughout history has combined the practical craft of repurposing fabric with designs and needlework skills that reflect the quilter's cultural traditions. Ververs' quilt reflects this rich tradition of quilting as a means of storytelling and is deeply personal to her. Ververs (pers. comm., email, 2025 Aug 12) said:

My mother passed away in late 2019, just before COVID-19. She was an avid quilter, and I had never quilted before. In 2020 and 2021, while working intensively on Ebola Virus Disease and COVID-19 outbreaks, I decided to use some of her fabrics. Quilting became both a way to grieve and a way to feel close to her.

Ververs learned new appliqué techniques while immersed in her work, which focused heavily on nutrition, including breastfeeding and breastmilk, in outbreak contexts. During her 9 years at the Centers for Disease Control and Prevention, her work also focused on diseases such as Ebola virus disease, Marburg virus disease, COVID-19, cholera, dengue fever, Oropouche virus disease, and, more recently, mpox.

The quilt serves a dual purpose: it connects the artist to her mother and provides a creative outlet away from the office. Working on it helped to ground her during the long months of navigating the challenges posed by COVID-19 and brought together her professional and personal passions in a unique way.

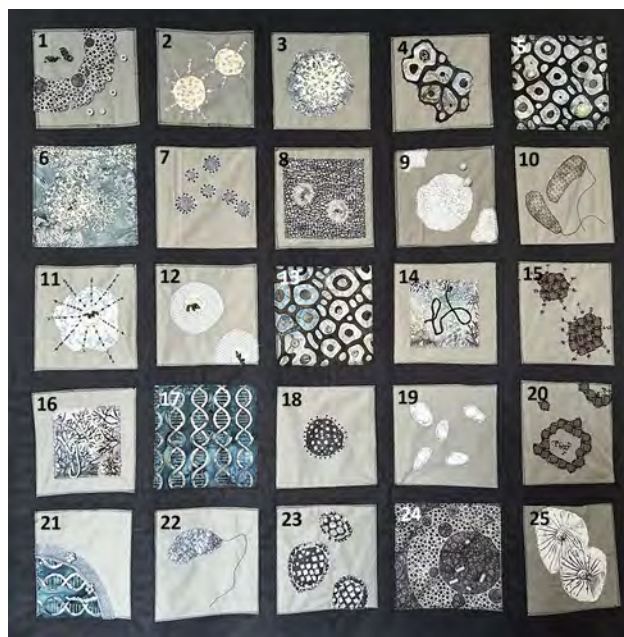


Figure. Key for quilt squares in *The Pathogen Quilt*, created by Mija-tesse Ververs. Squares 1, 8, 12, and 24, DNA and RNA viruses and replication; squares 2, 3, 6, 12, 18, and 23, pathogens with surface proteins such as glycoproteins, hemagglutinins, or neuraminidases, including HIV, SARS-CoV-2, influenza virus, Nipah virus, and monkeypox virus; squares 5 and 13, red blood cells; squares 10 and 22, *Vibrio cholerae*; squares 14 and 16, Ebola virus; squares 17 and 21, viral DNA. The remaining squares represent fantasy pathogens, designed for their visual appeal by the artist.

The intricate designs of *The Pathogen Quilt* invite the viewer's reflection on the complexities of the microbial world while celebrating the resilience that emerges from shared struggles.

Acknowledgment

Mija-tesse Ververs is now affiliated with the Johns Hopkins Bloomberg School of Public Health, Center for Humanitarian Health. Special thanks to Byron Breedlove for his careful review and comments on the manuscript.

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Article Title

Recent Systemic Antifungal Exposure and Nonsusceptible *Candida* in Hospitalized Patients, South Africa, 2012–2017

CME Questions

1. Which of the following fungal species is most likely to be sensitive to azole antifungals?

- A. *Nakaseomyces glabratus*
- B. *Candida parapsilosis*
- C. *Candida albicans*
- D. *Candida auris*

2. Which of the following statements regarding the global epidemiology and outcomes of *Candida* bloodstream infections (BSIs) is most accurate?

- A. There are nearly 100,000 cases of *Candida* BSI annually
- B. The overall mortality rate of *Candida* BSIs is 35%
- C. *C. albicans* is responsible for an increasing proportion of *Candida* BSIs
- D. The prevalence of the antifungal-resistant *Candida* species is lower in low-income countries

3. What was the percentage of nonsusceptible cases of candidemia in the current study?

- A. 16%
- B. 25%
- C. 33%
- D. 48%

4. Previous treatment with which of the following antifungal agents contributed the most to the development of nonsusceptible candidemia?

- A. Fluconazole
- B. Amphotericin B
- C. Caspofungin
- D. Micafungin

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Article Title

Reptile Exposure in Human Salmonellosis Cases and *Salmonella* Serotypes Isolated from Reptiles, Ontario, Canada, 2015–2022

CME Questions

1. Which subspecies of *Salmonella enterica* accounts for the majority of infections among adults?

- A. *salamae*
- B. *enterica*
- C. *houtenae*
- D. *indica*

2. Which of the following statements regarding factors which can increase the carriage of *Salmonella* bacteria among reptiles and amphibians is most accurate?

- A. Reptiles in captivity shed less *Salmonella* vs those in the wild
- B. Husbandry stress among snakes can promote more Gram-positive bacteria in oral flora
- C. Adult amphibians are usually omnivores
- D. Feeding sources of reptiles and amphibians, such as crickets and worms, are also natural reservoirs for *Salmonella*

3. Which of the following statements regarding the epidemiology of patients with salmonellosis in the current study is most accurate?

- A. The overall rate of recent reptile contact before illness was ≈6%
- B. Men with salmonellosis were nearly 10× more likely to have reptile contact vs women
- C. ≈10% of individuals with salmonellosis and recent reptile exposure were children
- D. The rate of reptile exposure among individuals with salmonellosis increased over time

4. Which reptile or amphibian was most associated with subsequent cases of salmonellosis in the current study?

- A. Lizards
- B. Frogs
- C. Turtles
- D. Snakes