

Zoonotic Transmission of Diphtheria from Domestic Animal Reservoir, Spain

Andreas Hoefler,¹ Silvia Herrera-León,¹ Lucas Domínguez, María Ordobás Gavín, Beatriz Romero, Ximena Belen Araujo Piedra, Cristina Sobrino Calzada, María José Uría González, Laura Herrera-León, Case Study Investigation Group²

Toxigenic *Corynebacterium ulcerans* is as an emerging zoonotic agent of diphtheria. We describe the zoonotic transmission of diphtheria caused by toxigenic *C. ulcerans* from domestic animals in Spain, confirmed by core-genome multilocus sequence typing. Alongside an increasing number of recent publications, our findings highlight the public health threat posed by diphtheria reemergence.

Diphtheria has been increasing in relevance because of increasing individual travel and surges in mass relocation events of refugees, asylum seekers, and immigrants from countries where diphtheria remains endemic (1–3). These importation events, in combination with growing vaccine hesitancy in non-endemic countries, give diphtheria a high potential for reemergence.

Toxigenic *Corynebacterium ulcerans*, an agent of diphtheria, has frequently been identified in domesticated animals such as cats, dogs, and pigs in which zoonotic transmission has been demonstrated (4–7). Toxigenic *C. ulcerans* has also been identified in wild animals such as ferrets, boars, and deer (8). This natural reservoir of *C. ulcerans*, in both wild and domesticated animals, constitutes a major public health threat.

The Case Report

On February 23, 2019, a 60-year-old man visited the emergency department of the Hospital Universita-

Author affiliations: European Public Health Microbiology Training Programme, Stockholm, Sweden (A. Hoefler, S. Herrera-León); Instituto de Salud Carlos III, Madrid, Spain (A. Hoefler, S. Herrera-León, L. Herrera-León); Universidad Complutense de Madrid, Madrid (L. Domínguez, B. Romero); Dirección General de Salud Pública, Madrid (M. Ordobás Gavín); Hospital Universitario del Sureste, Madrid (X.B. Araujo Piedra, C. Sobrino Calzada); Laboratorio Br Salud Ut, Madrid (M.J. Uría González)

DOI: <https://doi.org/10.3201/eid2806.211956>

rio del Sureste in Madrid, Spain. At examination, he was found to have odynophagia, dysphonia, and a whitish membrane in the oropharynx. He visited this emergency department several times during February 26–March 5. On March 5, an emergency department doctor took a pharyngeal exudate sample and sent it to Spain's National Centre of Microbiology (CNM), and the patient was started on a course of clarithromycin on March 6.

CNM received the swab on March 7. On March 10, laboratory cultures confirmed that the throat swab contained *C. ulcerans* and was positive for the *tox* gene on PCR (9). The hospital then contacted the patient, who was recovering at home, and requested his immediate hospitalization for treatment and isolation. The patient complied and was started on a 12-hour course of intravenous clarithromycin. Administering antitoxin was ruled out because the patient responded well to antibiotics.

On March 11, the National Directorate of Epidemiologic Services initiated an outbreak investigation. CNM sent a culture to the Centre for Reference on Diphtheria and Streptococcal Infections (part of the United Kingdom's Health Security Agency) for toxigenicity testing, where the sample was confirmed as toxigenic by ELEK test on March 18 (10). Subsequent samples, taken on March 21 and 22, were negative for *C. ulcerans*, and the patient was released. While in the hospital, the patient also was revaccinated for diphtheria.

The Regional Epidemiologic Services of Madrid (RESM) conducted a survey, which confirmed that no exposure to conventional sources of infection or recent overseas travel had occurred. The case-patient

¹These first authors contributed equally to this article.

²Authors in the Case Study Investigation Group are listed at the end of this article.

and his partner live in relative isolation ≈ 8 km from a small urban center and are not associated with any agriculture activities. No record of a recent diphtheria booster dose was found for the case-patient or his partner. The case-patient owns 2 cats and 3 dogs, and he is known to regularly feed stray cats that frequent his estate.

RESM conducted contact tracing as indicated by national guidelines (11). A risk assessment identified 2 close contacts (considered high-risk) and 20 further contacts (considered moderate-risk). All 22 contacts were tested, and no *C. ulcerans* was identified. The asymptomatic household contact, the case-patient's partner, received prophylactic azithromycin, and diphtheria vaccine was administered. The second high-risk contact was the attending physician who performed the physical examination without the personal protective equipment required when treating a patient with an active case of diphtheria. The physician's vaccination history was confirmed, and prophylactic azithromycin was administered. The 20 moderate-risk contacts (including 1 domestic assistant and 19 hospital staff members) all had their vaccination coverage confirmed and were briefed on recognizing potential symptoms.

In adherence with World Health Organization and national protocols, RESM requested Animal Health Services of Madrid to investigate the animals in contact with the human case (12). This investigation was performed by the VISAVET Health Surveillance Centre. On March 18–19, nasal, pharyngeal, and conjunctival swabs were collected from the 2 cats (CAT1 and CAT2) and 3 dogs (DOG1, DOG2, and DOG3) that lived with the human case-patient. Three isolates from nasal swab specimens were obtained during

selective culturing, 1 from CAT1, 1 from CAT2, and 1 from DOG1 (European Nucleotide Archive accession nos. ERR6177889, ERR6177890, and ERR6177890, respectively); the 2 other dogs tested negative. All isolates were identified as *tox* gene-bearing *C. ulcerans* by PCR and whole-genome sequencing. All 3 animals that had tested positive were placed in isolation by the Central Animal Shelter of the Madrid Community and treated with amoxicillin for 15 days. The animals were retested, and all the swab specimens collected were negative, at which point, the animals were returned to their owner. Workers at the animal shelter were briefed on biosafety measures and management of the infected animals. Contact tracing of the cats was performed, and 4 stray cats were captured and tested; all were negative for *C. ulcerans*. All domestic and stray animals tested were asymptomatic.

All microbiologic procedures were conducted by CNM in accordance with World Health Organization guidelines (13). VISAVET identified the isolates by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

CNM purified the genomic DNA from *C. ulcerans* isolates with the DNeasy Blood and Tissue Kit (QIAGEN, <https://www.qiagen.com>). Libraries were prepared by using Nextera XT DNA Library Preparation Kit and sequenced on a MiSeq platform by using version 3 reagents for 2×300 paired-end libraries (both from Illumina, <https://www.illumina.com>). Multilocus sequence typing (MLST) based on 7 housekeeping *loci* extracted from the next-generation sequencing data identified the human and animals isolates as sequence type (ST) 514 (14). Next-generation sequencing-derived core-genome MLST comprising 2,170 target loci revealed no allelic differences

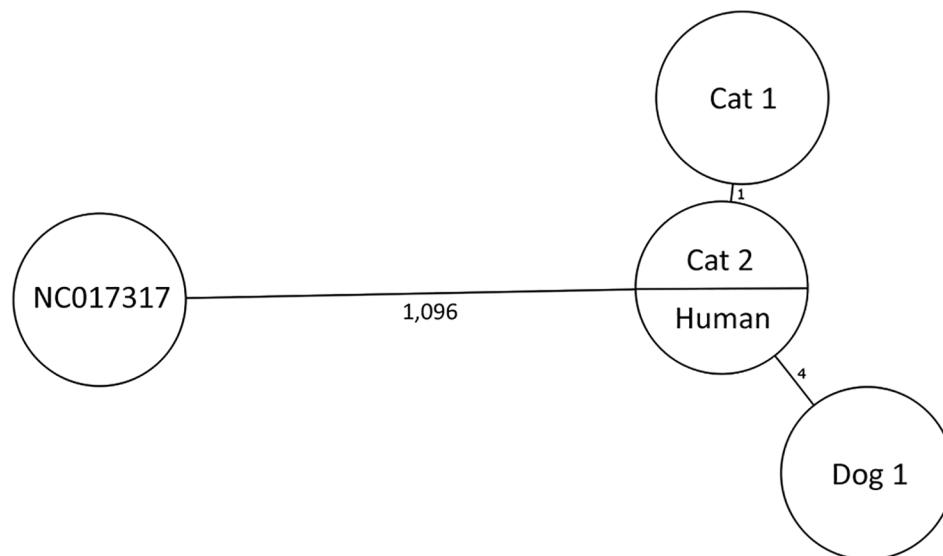


Figure. Core-genome multilocus sequence typing-based minimum spanning tree of all toxigenic *Corynebacterium ulcerans* strains associated with the zoonotic transmission of diphtheria in Spain in 2019 compared with reference strain NCTC_NC017317 from GenBank. Branches are labeled with the number of allelic differences between strains. European Nucleotide Archive accession numbers: human strain, ERR4880084; CAT1 strain, ERR6177889; CAT2 strain, ERR6177890; DOG1 strain, ERR6177891.

between the human (European Nucleotide Archive accession no. ERR4880084) and CAT2 strains, whereas CAT1 harbored 1 allelic difference (4). The strain from DOG1 had 4 allelic differences from the human and CAT2 strains, indicating a close relationship (4) (Figure). Zoonotic *C. ulcerans* collected from the human, dog, and cat exhibited a high degree of similarity, whereas epidemiologically nonrelated strains differed by thousands of single-nucleotide polymorphisms from each other (data not shown) (6,8).

Conclusions

The number of cases of diphtheria caused by toxigenic *C. ulcerans* with an epidemiologic link to domestic animals is small but rising (4). Under the scope of the One Health initiative, the collaboration between human and animal public health authorities was essential to identify the origin of this case. This case report highlights the sustained risk posed by zoonotic toxigenic *C. ulcerans* reservoirs in peridomestic and domestic animals. Given the high degree of conservation between the human and animal strains, a zoonotic transmission has certainly occurred in this instance. Although the captured stray cats tested negative for *C. ulcerans*, only a small portion of the stray cats in contact with the domesticated animals could be tested. The actual number of cats that were in direct contact with the domesticated animals is unknown. Currently, *C. ulcerans* is not a notifiable organism if it is detected in animals (11). To mitigate the future public health burden of toxigenic *C. ulcerans* from animal reservoirs, its declaration should be considered as part of the national surveillance guidelines.

Feline, canine, and porcine zoonotic transmission of toxigenic *C. ulcerans* has been previously supported by findings of the same ST (derived from MLST) in the suspected animal and the epidemiologically linked human case or cases (7). The ST identified in this study (ST514) was previously described in an isolate from a 59-year-old man with cutaneous lesions in France in 2005 (14).

Vaccinations against diphtheria are offered at 2, 4, and 11 months of age in Spain, with booster doses at 6, 14, and >65 years of age. Earlier administration of boosters may need to be considered because levels of antibodies may not be sufficient to prevent the disease in older persons who are <65 years of age (15).

Authors from the Case Study Investigation Group (listed alphabetically): Carmen Bárcena, Jesús Carpintero Hervás, Esther Córdoba Deorador, Fernando Fuster

Loran, Marta Hernández, Beatriz Isidoro Fernández, Marta Pérez-Sancho, Elena Rodríguez Baena, Lourdes Sainz de los Terreros Soler

Acknowledgments

We thank all persons and agencies directly and indirectly involved in the effective management of this case study.

This work was partially funded by Área de Ganadería de la Dirección General de Agricultura, Ganadería y Alimentación de la Comunidad de Madrid.

About the Author

Dr. Hoefer is currently completing the European Centre for disease prevention and control Microbiology Training Programme fellowship at the National Center of Microbiology in Spain. His background is in vaccine-preventable diseases, diphtheria, and antimicrobial resistance.

References

1. Wagner KS, White JM, Lucenko I, Mercer D, Crowcroft NS, Neal S, et al.; Diphtheria Surveillance Network. Diphtheria in the postepidemic period, Europe, 2000–2009. *Emerg Infect Dis*. 2012;18:217–25. <https://doi.org/10.3201/eid1802.110987>
2. Nelson TG, Mitchell CD, Sega-Hall GM, Porter RJ. Cutaneous ulcers in a returning traveller: a rare case of imported diphtheria in the UK. *Clin Exp Dermatol*. 2016;41:57–9. <https://doi.org/10.1111/ced.12763>
3. Rahman MR, Islam K. Massive diphtheria outbreak among Rohingya refugees: lessons learnt. *J Travel Med*. 2019;26:26. <https://doi.org/10.1093/jtm/tay122>
4. Hoefer A, Pampaka D, Herrera-León S, Peiró S, Varona S, López-Perea N, et al. Molecular and epidemiological characterization of toxigenic and nontoxigenic *Corynebacterium diphtheriae*, *Corynebacterium belfantii*, *Corynebacterium rouxii*, and *Corynebacterium ulcerans* isolates identified in Spain from 2014 to 2019. *J Clin Microbiol*. 2021;59:59. <https://doi.org/10.1128/JCM.02410-20>
5. Marosevic DV, Berger A, Kahlmeter G, Payer SK, Hörmansdorfer S, Sing A. Antimicrobial susceptibility of *Corynebacterium diphtheriae* and *Corynebacterium ulcerans* in Germany 2011–17. *J Antimicrob Chemother*. 2020;75:2885–93. <https://doi.org/10.1093/jac/dkaa280>
6. Meinel DM, Margos G, Konrad R, Krebs S, Blum H, Sing A. Next generation sequencing analysis of nine *Corynebacterium ulcerans* isolates reveals zoonotic transmission and a novel putative diphtheria toxin-encoding pathogenicity island. *Genome Med*. 2014;6:113. <https://doi.org/10.1186/s13073-014-0113-3>
7. König C, Meinel DM, Margos G, Konrad R, Sing A. Multilocus sequence typing of *Corynebacterium ulcerans* provides evidence for zoonotic transmission and for increased prevalence of certain sequence types among toxigenic strains. *J Clin Microbiol*. 2014;52:4318–24. <https://doi.org/10.1128/JCM.02291-14>
8. Berger A, Dangel A, Peters M, Mühlendorfer K, Braune S, Eisenberg T, et al. *Tox*-positive *Corynebacterium ulcerans* in

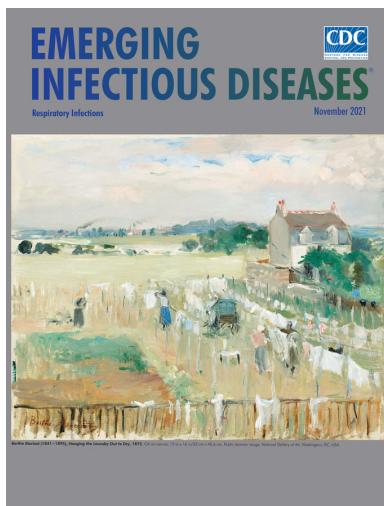
- hedgehogs, Germany. *Emerg Microbes Infect.* 2019;8:211–7. <https://doi.org/10.1080/22221751.2018.1562312>
9. Pacheco LGC, Pena RR, Castro TLP, Dorella FA, Bahia RC, Carminati R, et al. Multiplex PCR assay for identification of *Corynebacterium pseudotuberculosis* from pure cultures and for rapid detection of this pathogen in clinical samples. *J Med Microbiol.* 2007;56:480–6. <https://doi.org/10.1099/jmm.0.46997-0>
 10. Public Health England. UK standards for microbiology investigations: identification of *Corynebacterium* species. 2014 Oct 29 [cited 2021 Sep 1]. <https://www.gov.uk/government/publications/smi-id-2-identification-of-corynebacterium-species>
 11. Centro Nacional de Epidemiología. Protocolos de la Red Nacional de Vigilancia Epidemiológica. 2015 May [cited 2021 Sep 1]. https://www.isciii.es/QueHacemos/Servicios/VigilanciaSaludPublicaRENAVE/EnfermedadesTransmisibles/Documents/PROTOCOLOS/PROTOCOLOS%20EN%20BLOQUE/PROTOCOLOS_RENAVE-ciber.pdf
 12. World Health Organization. Surveillance standards for vaccine-preventable diseases, second edition. 2018 [cited 2021 Sep 1]. <https://apps.who.int/iris/handle/10665/275754>
 13. Begg N. Manual for the management and control of diphtheria in the European region 1994 [cited 2021 Sep 1]. <https://apps.who.int/iris/handle/10665/108107>
 14. Bolt F, Cassidy P, Tondella ML, Dezoysa A, Efstratiou A, Sing A, et al. Multilocus sequence typing identifies evidence for recombination and two distinct lineages of *Corynebacterium diphtheriae*. *J Clin Microbiol.* 2010;48:4177–85. <https://doi.org/10.1128/JCM.00274-10>
 15. Limia Sánchez A, Olmedo Lucerón C. Second seroprevalence study in Spain, 2017–2018 [in Spanish]. *Rev Esp Salud Publica.* 2021;95:95.

Address for correspondence: Laura Herrera León, Instituto de Salud Carlos III, Ctra. de Pozuelo 28, 28222 Majadahonda, Madrid; email: lherrera@isciii.es

November 2021

Respiratory Infections

- Policy Review and Modeling Analysis of Mitigation Measures for Coronavirus Disease Epidemic Control, Health System, and Disease Burden, South Korea
- Effectiveness of Abbott BinaxNOW Rapid Antigen Test for Detection of SARS-CoV-2 Infections in Outbreak among Horse Racetrack Workers, California, USA
- Ehrlichiosis and Anaplasmosis among Transfusion and Transplant Recipients in the United States
- Interventions to Disrupt Coronavirus Disease Transmission at a University, Wisconsin, USA, August–October 2020
- Probability-Based Estimates of Severe Acute Respiratory Syndrome Coronavirus 2 Seroprevalence and Detection Fraction, Utah, USA
- Seroprevalence of SARS-CoV-2–Specific Antibodies among Quarantined Close Contacts of COVID-19 Patients, Faroe Islands, 2020
- Rapid Increase in SARS-CoV-2 P.1 Lineage Leading to Codominance with B.1.1.7 Lineage, British Columbia, Canada, January–April 2021
- Multidrug-Resistant Methicillin-Resistant *Staphylococcus aureus* Associated with Bacteremia and Monocyte Evasion, Rio de Janeiro, Brazil



- Genomic Profiling of *Mycobacterium tuberculosis* Strains, Myanmar
- Hepatitis A Virus Incidence Rates and Biomarker Dynamics for Plasma Donors, United States
- Population Genomics and Inference of *Mycobacterium avium* Complex Clusters in Cystic Fibrosis Care Centers, United States
- Changing Patterns of Disease Severity in *Blastomyces dermatitidis* Infection, Quebec, Canada
- Encephalitis and Death in Wild Mammals at a Rehabilitation Center after Infection with Highly Pathogenic Avian Influenza A(H5N8) Virus, United Kingdom
- Co-infection with *Legionella* and SARS-CoV-2, France, March 2020
- Epidemiologic Analysis of Efforts to Achieve and Sustain Malaria Elimination along the China–Myanmar Border
- Socioeconomic Patterns of COVID-19 Clusters in Low-Incidence City, Hong Kong
- Prevalence of SARS-CoV-2 Antibodies after First 6 Months of COVID-19 Pandemic, Portugal
- Association of Shared Living Spaces and COVID-19 in University Students, Wisconsin, USA, 2020
- Correlation of SARS-CoV-2 Subgenomic RNA with Antigen Detection in Nasal Midturbinate Swab Specimens
- Mutations Associated with SARS-CoV-2 Variants of Concern, Benin, Early 2021
- Multinational Observational Cohort Study of COVID-19–Associated Pulmonary Aspergillosis
- *Bordetella hinzii* Pneumonia and Bacteremia in a Patient with SARS-CoV-2 Infection

**EMERGING
INFECTIOUS DISEASES**

To revisit the November 2021 issue, go to:
<https://wwwnc.cdc.gov/eid/articles/issue/27/11/table-of-contents>