

Injectional Anthrax in Heroin Users, Europe, 2000–2012

To the Editor: Anthrax is a global zoonotic disease, but human infections are rare in countries of Western Europe. During 2009–2010, a total of 119 (47 laboratory-confirmed) drug-abuse-related cases of anthrax were reported in the United Kingdom and Germany (1). In these patients, the disease had an unusual manifestation. In contrast to acquiring the disease through typical routes of infection, leading to cutaneous, inhalation, or gastrointestinal anthrax, these patients became infected by injecting heroin (1–3). The term injectional anthrax was then coined to describe this new mode of infection. Patients with injectional anthrax show severe symptoms, and death rates are high. Of the 47 patients with confirmed cases of injectional anthrax acquired during the 2009–2010 outbreak, 19 died from the disease (1). However, this outbreak was not the first report of death caused by *B. anthracis* in an injectional drug (heroin) user; the disease was described in 1 person who died in Norway in 2000 (3).

Attempts to directly connect the United Kingdom cases to batches of anthrax-contaminated heroin were unsuccessful. No viable *B. anthracis* or DNA could be retrieved from the investigation's drug samples. However, PCR- or culture-positive samples were obtained from some patients and used for genotyping (2). Later, researchers from Arizona and the United Kingdom worked together to use a high-resolution molecular approach to genotype the 2009–2010 outbreak strains. This information was used to obtain insight into the epidemiology and likely geographic origin of the European outbreak strains.

All patients were infected by a single *B. anthracis* strain type (2,4) that belonged to the large

Trans-Eurasian clade of *B. anthracis*. The whole genome of a representative strain of this type, Ba4599, had previously been sequenced (2). Strains related to strains associated with those isolated from European drug users, which belong to the A.Br. 008/011 canSNP cluster but are still genetically distinct, have so far only been identified from Turkey (2). However, more isolates from other relevant regions need to be investigated to confirm the likely geographic source.

In June 2012, after a 20-month gap, 2 new cases of injectional anthrax in heroin consumers were reported in Bavaria (5,6). Additional cases have been reported since then from Germany, Denmark, the United Kingdom, and France, leading to 26 deaths as of August 2013 (7).

Molecular phylogenetic methods were used to determine the genetic relatedness of these strains with Ba4599 (8,9). Genotyping results using canonical single nucleotide polymorphisms (SNPs) (Figure) (2,4) placed all of these strains along branch A.Br.008 within the Trans-Eurasian group of

B. anthracis (10). Further hierarchical fine-scale typing of the isolates by interrogating SNPs that were discovered from the heroin-associated strain Ba4599 (2) indicated that all isolates are identical at these SNPs (Figure) (7). The initial strain isolated in Norway in 2000 also falls into this group (7). In addition, analysis by multiple locus variable number tandem repeats suggested that all investigated strains are closely related, differing at just 2 markers (7). Thus, we conclude that all injectional anthrax isolates likely came from the same source.

This reemergence of drug-related anthrax in Europe strengthens the view that heroin may provide a continuing route of entry for *B. anthracis* into Western Europe (2). Ideally, this unfortunate deadly incident could offer an opportunity to sensitize heroin users to the risks for severe infection and to educate public health officials to be vigilant for this rare disease. This study also shows the power of molecular genotyping approaches for trace-back analysis of infectious disease agents.

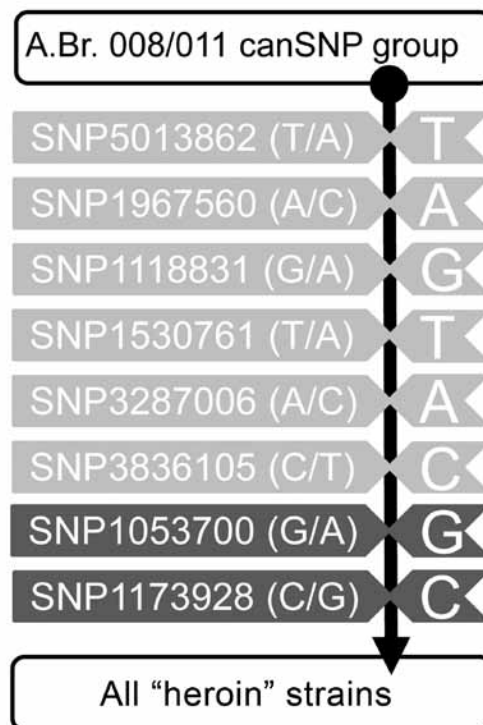


Figure. Diagram of single nucleotide polymorphism (SNP) assays used for bioforensic genotyping of heroin-associated *Bacillus anthracis* strains. Shown are the results of PCR-based SNP assays performed to elucidate the phylogenetic position of strains. Indicated at the top of the column is the whole strain pool of *B. anthracis* genotype A.Br. 008/011; the vertical black line indicates the assays in a direction of revealing increasing proximity to the heroin-associated strains. SNPs common to those of some strains from Turkey (2) are shown in light gray, and SNPs unique for the heroin-associated strains (2), including the isolate of 2000 from Norway, are depicted in dark gray (SNP designations and alleles are indicated).

Acknowledgments

We thank Daniela Horenkamp and Claudia Kahlhofer for skillful technical assistance and Holger C. Scholz, Roland Grunow, and Dawn Birdsell for helpful discussions.

Collaboration between the Bundeswehr Institute of Microbiology and Northern Arizona University is part of a project funded by the US Department of Homeland Security Science and Technology Directorate through award 2010-ST-108-000015. This work was also supported in part by the US Department of Homeland Security Science and Technology Directorate award HSHQDC-10-C-00139.

**Matthias Hanczaruk,
Udo Reischl,
Thomas Holzmann,
Dimitrios Frangoulidis,
David M. Wagner, Paul S. Keim,
Markus H. Antwerpen,
Hermann Meyer,
and Gregor Grass**

Author affiliations: Bundeswehr Institute of Microbiology, Munich, Germany (M. Hanczaruk, D. Frangoulidis, M.H. Antwerpen, H. Meyer, G. Grass); University Hospital Regensburg, Regensburg, Germany (U. Reischl, T. Holzmann); and Northern Arizona University, Flagstaff, Arizona, USA (D. Wagner, P.S. Keim)

DOI: <http://dx.doi.org/10.3201/eid2002.120921>

References

1. Health Protection Scotland. An outbreak of anthrax among drug users in Scotland, December 2009 to December 2010. A report on behalf of the national Anthrax Outbreak Control Team. 2011 [cited 2013 Aug 20]. <http://www.documents.hps.scot.nhs.uk/giz/anthrax-outbreak/anthrax-outbreak-report-2011-12.pdf>
2. Price EP, Seymour ML, Sarovich DS, Latham J, Wolken SR, Mason J, et al. The molecular epidemiologic investigation of an anthrax outbreak in European heroin users. *Emerg Infect Dis.* 2012;18:1307–13. <http://dx.doi.org/10.3201/eid1808.111343>
3. Ringertz SH, Hoiby EA, Jensenius M, Maehlen J, Caugant DA, Myklebust A, et al. Injectional anthrax in a heroin

- skin-popper. *Lancet.* 2000;356:1574–5. [http://dx.doi.org/10.1016/S0140-6736\(00\)03133-0](http://dx.doi.org/10.1016/S0140-6736(00)03133-0)
4. Van Ert MN, Easterday WR, Huynh LY, Okinaka RT, Hugh-Jones ME, Ravel J, et al. Global genetic population structure of *Bacillus anthracis*. *PLoS One.* 2007;2:e461. <http://dx.doi.org/10.1371/journal.pone.0000461>
 5. Holzmann T, Frangoulidis D, Simon M, Noll P, Schmoltdt S, Hanczaruk M, et al. Fatal anthrax infection in a heroin user from southern Germany, June 2012. *Euro Surveill.* 2012;17:pii=20204.
 6. Rückert C, Licht K, Kalinowski J, Espirito Santo C, Antwerpen M, Hanczaruk M, et al. Draft genome sequence of *Bacillus anthracis* UR-1, isolated from a German heroin user. *J Bacteriol.* 2012;194:5997–8. <http://dx.doi.org/10.1128/JB.01410-12>
 7. Grunow R, Klee SR, Beyer W, George M, Grunow D, Barduhn A, et al. Anthrax among heroin users in Europe possibly caused by same *Bacillus anthracis* strain since 2000. *Euro Surveill.* 2013;18:pii=20204.
 8. Antwerpen MH, Zimmermann P, Bewley K, Frangoulidis D, Meyer H. Real-time PCR system targeting a chromosomal marker specific for *Bacillus anthracis*. *Mol Cell Probes.* 2008;22:313–5. <http://dx.doi.org/10.1016/j.mcp.2008.06.001>
 9. Ellerbrok H, Nattermann H, Ozel M, Beutin L, Appel B, Pauli G. Rapid and sensitive identification of pathogenic and apathogenic *Bacillus anthracis* by real-time PCR. *FEMS Microbiol Lett.* 2002;214:51–9. <http://dx.doi.org/10.1111/j.1574-6968.2002.tb11324.x>
 10. Price EP, Seymour ML, Sarovich DS, Latham J, Wolken SR, Mason J, et al. Molecular epidemiologic investigation of an anthrax outbreak among heroin users, Europe. *Emerg Infect Dis.* 2012;18:1307–13. <http://dx.doi.org/10.3201/eid1808.111343>

Address for correspondence: Gregor Grass, Bundeswehr Institute of Microbiology, Neuherbergstrasse 11, 80937 Munich, Germany; email: gregorgrass@bundeswehr.org

EMERGING INFECTIOUS DISEASES

Free Online RSS Feed

in PubMed Central

Ahead of print

CME Peer-Review

podcasts

GovDelivery

Nasopharyngeal Bacterial Interactions in Children

To the Editor: Xu and colleagues (1) examined the nasopharyngeal bacterial colonization rates in children with acute otitis media (AOM) and in healthy children. They found that *Haemophilus influenzae* colonization was competitively associated with *Streptococcus pneumoniae* and *Morexella catarrhalis* colonization in children with AOM but was not associated with *S. pneumoniae* and *M. catarrhalis* colonization in healthy children. We have a serious concern regarding their analysis.

The authors calculated odds ratios (ORs) by considering a bacterial colonization as an outcome variable and another bacterial colonization as an exposure variable. They considered an OR >1 as the presence of synergistic associations between bacteria (i.e., co-colonization is more likely to occur than it would by chance) and OR <1 as the presence of competitive associations (i.e., co-colonization is less likely to occur than it would by chance). This inference may be justified in a population-based cross-sectional study. If 2 bacterial colonizations occur independently in a stationary population, the prevalence of co-colonization will be the product (multiplication) of each prevalence, and the OR between 2 bacterial colonizations in the population (OR_{pop}) will be 1 (online Technical Appendix, wwwnc.cdc.gov/EID/article/20/2/12-1724-Techapp1.pdf).

However, the authors enrolled their case-patients according to clinical signs (i.e., AOM or healthy). Let us assume that case-patients are enrolled from a population of children during a time period of t . Let r_c be the risk for enrollment (that is, of developing the disease) among colonized children and r_n be the risk for enrollment