

The precision of subtyping assays is a matter of importance and debate for epidemiologic and, recently, forensic investigations. Strain identity is commonly used to infer a common source even when spatial and temporal data are not congruent. Moreover, the definition of a strain is somewhat unclear and relies on analytical methods that vary widely. Therefore, isolates may be erroneously excluded or included into a strain definition and disease outbreak as illustrated with the Ames strain and 2 contrasting approaches to identification. MLVA15 ties naturally occurring isolates to bioterrorism-associated attacks, while specific SNP assays can distinguish among them.

MLVA is an unbiased approach and can be used on any set of *B. anthracis* strains, although, as in the 2006 and 2007 Texas outbreaks, it can be limited in resolving power. In contrast, our SNP assays have great resolving power but are useful only for differentiating the Ames strain, thus limiting their value to categorical inclusion or exclusion in outbreaks. Future rational use of a battery of different molecular signatures will yield far greater insights into strain identity than the application of 1 specific signature.

Funding for this project was provided to Northern Arizona University by the Department of Homeland Security Science and Technology Directorate (contract no. NBCH2070001) and by the Cowden Endowment for Microbiology at Northern Arizona University.

**Leo J. Kenefic, Talima Pearson,
Richard T. Okinaka,
Wai-Kwan Chung, Tamara Max,
Matthew N. Van Ert,
Chung K. Marston,
Kathy Gutierrez,
Amy K. Swinford,
Alex R. Hoffmaster,
and Paul Keim**

Author affiliations: Northern Arizona University, Flagstaff, Arizona, USA (L.J. Kenefic, T. Pearson, R.T. Okinaka, W.-K. Chung, T. Max, M.N. Van Ert, P. Keim); Los Alamos National Laboratory, Los Alamos, New Mexico, USA (R.T. Okinaka); Centers for Disease Control and Prevention, Atlanta, Georgia, USA (C.K. Marston, A. R. Hoffmaster); Texas Veterinary Medical Diagnostic Laboratory, College Station, Texas, USA (K. Gutierrez, A.K. Swinford); and Translational Genomics Research Institute, Phoenix, Arizona, USA (P. Keim)

DOI: 10.3201/eid1409.080076

References

- Hoffmaster AR, Fitzgerald CC, Ribot E, Mayer LW, Popovic T. Molecular subtyping of *Bacillus anthracis* and the 2001 bioterrorism-associated anthrax outbreak, United States. *Emerg Infect Dis*. 2002;8:1111–6.
- Read TD, Salzberg SL, Pop M, Shumway M, Umayam L, Jiang L, et al. Comparative genome sequencing for discovery of novel polymorphisms in *Bacillus anthracis*. *Science*. 2002;296:2028–33. DOI: 10.1126/science.1071837
- Van Ert MN, Easterday WR, Simonson TS, U'ren JM, Pearson T, Kenefic LJ, et al. Strain-specific single-nucleotide polymorphism assays for the *Bacillus anthracis* Ames strain. *J Clin Microbiol*. 2007;45:47–53. DOI: 10.1128/JCM.01233-06
- Pearson, T, Busch JD, Ravel J, Read TD, Rhoton SD, U'Ren JM, et al. Phylogenetic discovery bias in *Bacillus anthracis* using single-nucleotide polymorphisms from whole genome sequencing. *Proc Natl Acad Sci U S A*. 2004;101:13536–41. DOI: 10.1073/pnas.0403844101
- 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. DOI: 10.1371/journal.pone.0000461
- US Department of Agriculture. Epizootiology and ecology of anthrax; 2006 [cited 2008 Jan 1]. Available from http://www.aphis.usda.gov/vs/ceah/cei/taf/emerging_animalhealthissues_files/anthrax.pdf
- Keim P, Price LB, Klevytska AM, Smith KL, Schupp JM, Okinaka R, et al. Multiple-locus variable-number tandem repeat analysis reveals genetic relationships within *Bacillus anthracis*. *J Bacteriol*. 2000;182:2928–36. DOI: 10.1128/JB.182.10.2928-2936.2000
- Lista F, Faggioni G, Valjevac S, Ciaramarconi A, Vaissaire J, Doujet C, et al. Genotyping of *Bacillus anthracis* strains based on automated capillary 25-loci multiple locus variable-number tandem repeats analysis. *BMC Microbiol*. 2006;6:33. DOI: 10.1186/1471-2180-6-33
- Kenefic LJ, Beaudry J, Trim C, Daly R, Parmar R, Zanecki S, et al. High resolution genotyping of *Bacillus anthracis* outbreak strains using four highly mutable single nucleotide repeat (SNR) markers. *Lett Appl Microbiol*. 2008;46:600–3. DOI: 10.1111/j.1472-765X.2008.02353.x
- Keim P, Van Ert MN, Pearson T, Vogler AJ, Huynh LY, Wagner DM. Anthrax molecular epidemiology and forensics: using the appropriate marker for different evolutionary scales. *Infect Genet Evol*. 2004;4:205–13. DOI: 10.1016/j.meegid.2004.02.005

Address for correspondence: Paul Keim, Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011-5640, USA; email: paul.keim@nau.edu

Bluetongue in Eurasian Lynx

To the Editor: Bluetongue is an infectious disease of ruminants; it is caused by bluetongue virus (BTV), has 24 known serotypes, and is transmitted by several species of *Culicoides* biting midges. The disease mainly affects sheep and occurs when susceptible animals are introduced to areas where BTV circulates or when BTV is introduced to naive ruminant populations. The natural host range is strictly limited to ruminants, although seroconversion without disease has been reported in carnivores (*1*). We report BTV infection, disease, and death in 2 Eurasian lynx (*Lynx lynx*) and the isolation of BTV serotype 8 (BTV-8) from this carnivorous species.

The 2 Eurasian lynx, held in the same cage in a zoo in Belgium, became lethargic in September 2007; animal 1 died after 2 days, and animal 2 died in February 2008. Both had been fed ruminant fetuses and stillborns from sur-

rounding farms in an area where many bluetongue cases had been confirmed (2). Necropsy findings for animal 1 were anemia, subcutaneous hematomas, petechial hemorrhages, and lung congestion with edema. Necropsy findings for animal 2 were emaciation, anemia, enlarged and gelatinous lymph nodes, petechial hemorrhages, and pneumonia. For each animal, microscopic examination showed edematous vascular walls; enlarged endothelial cells; and evidence of acute to subacute vasculitis in muscle, myocardium, peritoneum, and lung. Tissue samples (spleen, lung, intestine) were analyzed by using 2 real-time reverse transcriptase–quantitative PCR techniques targeting BTV segment 5 and host β -actin mRNA as a control. BTV RNA was found in all samples from animal 1; cycle threshold values (3) ranged from 28.6 to 36.2. Tissues from animal 2 were negative for BTV RNA. Although the internal control was originally designed to detect β -actin mRNA of bovine or ovine species, clear positive signals were noted in all lynx samples, which indicated that this was a reliable control procedure. Infectious virus was subsequently isolated from the lung sample of animal 1 after inoculation of embryonated chicken eggs and amplification in baby hamster kidney–21 cell cultures (4). The specificity of the cytopathic effect, observed 48 hours after passage on baby hamster kidney–21 cells, was confirmed by real-time reverse transcriptase–quantitative PCR. Virus neutralization using specific reference serum (5) proved that the isolated virus was BTV-8. Anti-BTV antibodies were detected in lung tissue fluid from animal 2 (ID Screen Bluetongue Competition assay, ID VET, Montpellier, France) (6).

We describe a natural, wild-type infection of a carnivorous species. Although deaths have been documented in dogs accidentally infected with a BTV-contaminated vaccine (7), the 2 lynx in this report were neither vac-

inated nor medically treated by injection. BTV-8 was first introduced to northern Europe in 2006 and has subsequently spread rapidly to many countries on that continent. During 2007, a total of 6,870 bluetongue cases were reported in Belgium (2); animal 1 died in September 2007, which corresponded to the peak of bluetongue outbreaks in that region. No deaths were reported during that period among other animals, including ruminants, held in the same zoo as the 2 lynx reported here. The time lapse between initial clinical signs and death could explain the failure to detect BTV-8 RNA in animal 2. Although speculative, the suspicion of bluetongue in this animal is based on the presence of anti-BTV-8 antibodies, vasculitis, and pneumonia, which have been found in dogs accidentally infected with BTV (7).

This report raises questions about the current knowledge of the epidemiology of bluetongue. Bluetongue in lynx indicates that the list of known susceptible species must be widened, at least for serotype 8. Although infection of a susceptible host by an insect vector is the only proven natural transmission mechanism for wild-type BTV, transplacental transmission of BTV-8, resulting in the birth of seropositive (8) or virus-positive calves (9), has recently been described in cattle. Although infection by an insect vector cannot be excluded, transmission by the oral route must be strongly suspected because the lynx described in this report had been fed ruminant fetuses and stillborn animals from surrounding farms. This possibility is supported by a previous suspicion that seroconversion to BTV in carnivores was a result of oral infection (1). The possibility of oral transmission is also supported by evidence of lateral transmission of BTV infection to cattle having occurred, in the absence of insect vectors, as a result of direct contact with newborn viremic calves born to infected dams that had been imported to Northern Ireland from a bluetongue-infected region of continental Europe

(S. Kennedy, unpub. data). The role of wildlife, especially carnivores, in the epidemiology of bluetongue deserves further study to elucidate their role as either dead-end hosts or new sources of infection for livestock and to help determine the risks for wildlife populations.

Our findings clearly indicate that a novel transmission pathway enables the virus to cross species. Consequently, transmission to other species, including domestic animals, can no longer be excluded. Moreover, oral transmission is likely to have considerable implications for disease control, including vaccination, because BTV-8 is a fast-emerging virus with major financial consequences.

Acknowledgment

We thank M. Sarlet for technical help.

**Thierry P. Jauniaux,
Kris E. De Clercq,
Dominique E. Cassart,
Seamus Kennedy,
Frank E. Vandebussche,
Elise L. Vandemeulebroucke,
Tine M. Vanbinst,
Bart I. Verheyden,
Nesya E. Goris,
and Freddy L. Coignoul**

Author affiliations: Royal Belgian Institute of Natural Sciences, Brussels, Belgium (T.P. Jauniaux); University of Liege, Liege, Belgium (T.P. Jauniaux, D.E. Cassart, F.L. Coignoul); Veterinary and Agrochemical Research Centre, Brussels (K.E. De Clercq, F.E. Vandebussche, E.L. Vandemeulebroucke, T.M. Vanbinst, B.I. Verheyden, N.E. Goris); and Agri-Food and Biosciences Institute, Belfast, Northern Ireland, UK (S. Kennedy)

DOI: 10.3201/eid1409.080434

References

- Alexander KA, MacLachlan NJ, Kat PW, House C, O'Brien SJ, Lerche NW, et al. Evidence of natural bluetongue virus infection among African carnivores. *Am J Trop Med Hyg.* 1994;51:568–76.

2. Federal Agency for the Safety of the Food Chain. Epidemiological situation of the bluetongue in Belgium [cited 2008 Jan 25]. Available from http://www.afsca.be/crisis/sa-blueT/doc07/2008-01-25_KAART_v65_bis.pdf
3. Toussaint JF, Sailleau C, Bréard E, Zientara S, De Clercq K. Bluetongue virus detection by two real-time RT-qPCRs targeting two different genomic segments. *J Virol Methods*. 2007;140:115–23. DOI: 10.1016/j.jviromet.2006.11.007
4. Bréard E, Sailleau C, Coupier H, Mure-Ravaud K, Hammoui S, Gicquel B, et al. Comparison of genome segments 2, 7 and 10 of bluetongue viruses serotype 2 for differentiation between field isolates and the vaccine strain. *Vet Res*. 2003;34:777–89. DOI: 10.1051/vetres:2003036
5. Toussaint JF, Sailleau C, Mast J, Houdart P, Czaplinski G, Demeestere L, et al. Bluetongue in Belgium, 2006. *Emerg Infect Dis*. 2007;13:614–6 [cited 2008 Jan 25]. Available from <http://www.cdc.gov/eid/content/13/4/614.htm>
6. Vandenbussche F, Vanbinst T, Verheyden B, van Dessel W, Demeestere L, Houdart P, et al. Evaluation of antibody-ELISA and real-time RT-PCR for the diagnosis and profiling of bluetongue virus serotype 8 during the epidemic in Belgium in 2006. *Vet Microbiol*. 2008;129:15–27. DOI: 10.1016/j.vetmic.2007.10.029
7. Evermann JF, McKeiman AJ, Wilbur LA, Levings RL, Trueblood ES, Baldwin TJ, et al. Canine facilities associated with the use of a modified live vaccine administered during late stages of pregnancy. *J Vet Diagn Invest*. 1994;6:353–7.
8. Desmecht D, Bergh RV, Sartelet A, Leclerc M, Mignot C, Misse F, et al. Evidence for transplacental transmission of the current wild-type strain of bluetongue virus serotype 8 in cattle. *Vet Rec*. 2008;163:50–2.
9. Wouda W, Roumen M, Peperkamp N, Vos J, van Garderen E, Muskens J. Hydranencephaly in calves following the bluetongue serotype 8 epidemic in the Netherlands. *Vet Rec*. 2008;162:422–3.

Address for correspondence: Thierry P. Jauniaux, Department of Pathology, Veterinary College, University of Liege, Sart Tilman B43, 4000 Liege, Belgium; email: t.jauniaux@ulg.ac.be

The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the Centers for Disease Control and Prevention or the institutions with which the authors are affiliated.

Indigenous Dengue Fever, Buenos Aires, Argentina

To the Editor: For 2 decades dengue has increased in the Americas, with epidemic peaks every 3 to 5 years (1). The disease has reemerged in 3 South American countries bordering Argentina, namely, Bolivia, Brazil, and Paraguay.

Argentina had remained free from dengue for >80 years before the disease was reintroduced in 1998 (2) as a consequence of insufficient mosquito control and importation of cases from disease-epidemic areas. Since then, indigenous dengue circulation has only been reported in the northern provinces of the country, which are close to endemoepidemic countries. However, the principal dengue vector, the *Aedes aegypti* mosquito, has spread southward to latitude 35°S near Buenos Aires (3).

We describe what might be the southernmost indigenous case of dengue fever documented in South America; this case occurred in 2007, an epidemic peak year for the disease on this continent (1). The patient was a pneumonologist who worked part-time at Muñiz Hospital, a referral infectious diseases treatment center in the Buenos Aires Federal District. She also provided healthcare at an outpatient clinic in Lanus, her town of residence, a suburb 6 km south of the Federal District. Febrile illness started suddenly in February 2007, midsummer season in Argentina. On day 5 of illness, fever was replaced by a short-lived rash and itching followed by asthenia and nausea that persisted for 2 days. The patient had not traveled or been accidentally exposed to patients' blood during the previous weeks. She had never been vaccinated against yellow fever. Dengue fever was only suspected retrospectively.

Serologic results provided supportive evidence of a recent dengue

infection i.e., presence of immunoglobulin M, as determined by antibody-capture enzyme immunoassay, and immunoglobulin G seroconversion by 90% plaque reduction neutralization test on Vero cells (4). As shown in the Table, dengue virus serotype 3 was identified, and antibody results were negative for 3 other flaviviruses. Thus, this case fulfills Pan American Health Organization criteria for the diagnosis of dengue fever (5). Household contacts were seronegative.

For several years, conditions have been set for dengue virus circulation in Buenos Aires' urban and suburban areas because of the abundance of mosquitoes and disease in persons recently returning from neighboring countries. Risk for vector transmission is highest in the peripheral quarters of the city and towards late summer (6). Besides, Buenos Aires, like other Latin American metropolitan areas, is undergoing demographic changes that convey further risk for mosquito-borne disease transmission, namely, accelerated population growth mainly caused by informal settlements, deficient public health infrastructure and basic services, unregulated immigration from neighboring countries, and increased international mobility especially in or from neighboring countries (1).

Only imported dengue cases have been previously documented in Buenos Aires (2). According to official information, all 158 cases confirmed by antibody conversion in Buenos Aires Federal District and Province during 2007 were also imported (7). Of these, 50 occurred in the southern suburban district where our patient lives and works. In the summer of 2007, dengue infection was mainly introduced into the area by Paraguayan natives living in Buenos Aires who had recently visited their homeland. Dengue 3 serotype conversion was demonstrated in most of the cases investigated by plaque reduction neutralization assay, except for a few cases imported from Brazil, in which dengue 1 serotype was detected.