# Questions Linger over Science behind Anthrax Letters

Despite the outcome in mid-2008 of the Federal Bureau of Investigation (FBI) probe into the deadly and disruptive anthrax attacks of 2001, the FBI in May arranged for the National Academy of Sciences (NAS) to review the microbial and other forensic efforts that bureau officials coordinated as part of its broader investigation. It led FBI officials to conclude that microbiologist Bruce Ivins of the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) at Fort Detrick in Frederick, Md., was the sole culprit behind the letter-based attacks (*Microbe*, October 2008, p. 453).

Nonetheless, skepticism persists, as is evident not only from the forthcoming NAS review but also during the plenary session, "The Science behind the 'Anthrax Letter' Attack Investigation," convened as part of the 7th ASM Biodefense & Emerging Diseases Research Meeting, held in Baltimore, Md., last February, and during the news conference that followed. "Everybody is frustrated by the lack of closure," says plenary session participant Paul Keim of Northern Arizona University (NAU) in Flagstaff. Soon after the World Trade Center in New York, N.Y., and the Pentagon outside Washington, D.C., were attacked on September 11, 2001, letters containing spores of *Bacillus anthracis* were sent to members of the news media and Congress. Contact with those letters led to 22 cases of anthrax, including five deaths, along with cleanup measures that, for example, cost the U.S. Postal service $1.2 billion to decontaminate several of its facilities, according to Jason Bannan of the FBI Chemical-Biological Sciences Unit in Quantico, Va., and a participant in the ASM plenary session. "FDA never had a case like this before," he says.

No spore-containing letter was recovered from the first attack that led to the death of a photojournalist in Boca Raton, Fla. However, investigators recovered spores as part of a granular, white powdery material from an envelope involved in the second incident. Bannan describes it as a "crude prep," in part because it also contained *Bacillus subtilis.* Additional material from other letters to then- Senator Tom Daschle (D-SD) and to Senator Patrick Leahy (D-VT) in October 2001 appeared "more refined," was "beige" instead of white, and contained no spores other than *B. anthracis*.

The FBI quickly requested outside microbiologists to help in analyzing those materials. The available "research assays . . . didn't meet forensic standards," says Keim who, with his collaborators at NAU, worked closely with the FBI, as did other outside groups of microbiologists and investigators with other expertise. Moreover, efforts to develop such assays were complicated by the strictly clonal biology that *B. anthracis* follows during replication.

Those facts soon led microbial and molecular forensics investigators into conducting genomics-level analyses, according to Jacques Ravel, now at the University of Maryland School of Medicine and Institute for Genome Sciences in Baltimore, Md. A more conventional phenotypic analysis supplemented those genomic-level efforts, leading another group of microbiologists at

USAMRIID, who were working with the FBI and others on the anthrax investigation, to take advantage of distinct *B. anthracis* "morphotypes" that could be observed on growth plates. Those morphotypes vary not only by colony appearance but also in sporulation efficiencies and in telltale mutations at a rare "hot spot" within the otherwise stable genome of this species.

That information became the basis for a PCR screening assay for *B. anthracis* specimens that then was validated at Commonwealth Biotechnologies (CB) in Richmond, Va., and the Midwest Research Institute in Palm Bay, Fla., to ensure that such testing could meet forensics standards applied by U.S. courts. By 2007, the "highly specific" PCR assay identified several samples during a "blinded" analysis that included "seized materials," Bannan says. Ultimately, the PCR-based analysis along with other information from the criminal side of the investigation indicated that the anthrax- causing specimens from the 2001 letters derived from stocks produced several years earlier at USAMRIID for an aerosol challenge in anthrax vaccine studies, he says.

Based on that and other information from more conventional lines of evidence, FBI investigators concluded that Ivins, who died following a drug overdose in July 2008, produced spores from those stocks for the 2001 anthrax attacks.

Despite that painstaking analysis and the unequivocal conclusions put forth by FBI officials, doubts linger over some matters that are mainly scientific as well as others that intersect with the broader thrust of the investigation. For instance, none of the microbiologists, including Bannan and similar specialists at FBI, was privy to other evidence, including lab records from USAMRIID, that their FBI colleagues collected. "I know nothing of that information," he says. "I'm a microbiologist, and was not involved in the seizure of evidence."

Other lingering questions focus on more purely scientific issues, some of them pertaining to how the lethal bacteria were handled. For example, USAMRIID held *B. anthracis* in aqueous suspensions, not as spores. Presumably, the spores sent via letters were produced in at least two separate batches, contaminated with *B. subtilis* at least once, but when and how remain unknown. "We don't know the process used," Bannan says. "We never found the equivalent *B. subtilis* at USAMRIID in any of the evidence that we had." Efforts to trace the source of that bacterial contaminant "didn't lead anywhere," adds Keim.

Early reports suggested that the spores were "weaponized," possibly with "silica." However, later analysis determined that the spores were not coated with silica, although silicon was found within-not outside-the coat of spores used in the attacks, according to Joseph Michael of Sandia National Laboratory in Albuquerque, N.M. About two-thirds of the spores contain that silicon "signature," he says. Attempts to grow fresh spores with silicon to determine whether it also would locate within the spore coat led to "variable" results, Bannan adds. "We don't understand why there is a varying degree of silicon from one batch to another."

Other questions regarding physical properties of the spores similarly remain unexplained. Asked whether the spores were milled, Bannan points out that *B. anthracis* spores in letters went through rollers in automated postal sorting equipment that subjected them to high pressures. "It's a high-energy process, and [spore] plumes went up 30 feet [about 10 m] from the mail sorters," he says. How those spores looked beforehand or whether they were

pulverized after being dried and before being inserted into envelopes is not known.

**Jeffrey L. Fox**
Jeffrey L. Fox is the *Microbe* Current Topics and Features Editor.



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