

Researchers publish scientific paper on 2001 anthrax attacks

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Researchers at the Institute for Genome Sciences at the University of Maryland School of Medicine and collaborators at the FBI, the U.S. Army Medical Research Institute of Infectious Diseases and Northern Arizona University have published the first scientific paper based on their investigation into the anthrax attacks of 2001. The case was groundbreaking in its use of genomics and microbiology in a criminal investigation. More than 20 people contracted anthrax from *Bacillus anthracis* spores mailed through the U.S. Postal Service in 2001, and five people died as a result of the attacks. Research scientists from the Institute for Genome Sciences played a key role in the investigation known as Amerithrax. The work is a pioneering advance in the new field known as microbial forensics, a science that would likely play a key role in the investigations of any future bioterror attacks. The paper was published online today in the *Proceedings of the National Academy of Sciences*.

The paper describes how the Institute for Genome Sciences faculty and collaborators from the FBI found that the anthrax samples used in all the attacks were genetically identical. Later, another group of scientists – also including Institute for Genome Sciences faculty – would trace the anthrax spore used in the letters back to a flask of [Bacillus anthracis](#) and several samples taken from that flask. The primary custodian of the flask was Bruce Ivins, Ph.D., a scientist at a U.S. Army biodefense laboratory in Maryland. With this key investigative lead from the scientific team, the FBI used additional police work to conclude that Dr. Ivins was the perpetrator of the mail attacks. Dr. Ivins killed himself before the case

could go to court. The FBI has since closed the Amerithrax investigation.

"This paper and the Amerithrax investigation really marked the beginning of a new approach for the science we call forensic genomics," says senior author Jacques Ravel, Ph.D., associate professor of microbiology and immunology at the University of Maryland School of Medicine and associate director for genomics at the Institute for Genome Sciences. "The science was a critical component of the Amerithrax case. Without genomics, it would have been extremely difficult to narrow the pool of potential suspects."

"Before Amerithrax, no one appreciated the precision, accuracy and reliability that this type of genomics can offer as a microbial forensic technique," says first author David Rasko, Ph.D., assistant professor for microbiology and immunology at the School of Medicine and a research scientist at the Institute for Genome Sciences. "To this day, this is still the only case in which microbiology and genomics have been used in a [criminal investigation](#). Microbial forensics would be a critical investigative tool if another bioterror attack were ever to strike the U.S."

The newly published paper describes the work that the FBI assigned to Institute for Genome Sciences faculty members including Drs. Ravel and Rasko as well as the institute's director, Claire Fraser-Liggett, Ph.D., professor of medicine and microbiology and immunology at the School of Medicine, from 2001 through 2003. The scientists worked with a team of investigators including Paul Keim, Ph.D., regents professor and division director at Northern Arizona University and the Translational Genomics Research Institute, as well as military and FBI investigators.

"We have assembled a world-class team of genomics researchers at the Institute for Genome Sciences," says E. Albert Reece, M.D., Ph.D., M.B.A, vice president for medical affairs, University of Maryland, and John Z. and Akiko K. Bowers Distinguished Professor and dean,

University of Maryland School of Medicine. "Their pioneering work in the field of microbial forensics is typical of their cutting-edge research. We are proud to have them on our team, leading us into a new age of science."

The scientific investigation began by asking if the anthrax used in all the letters had come from the same source. The spores in each letter had been prepared differently, making them look different from one another to the naked eye. Military scientists at the U.S. Army Medical Research Institute of [Infectious Diseases](#) (USAMRIID) at Fort Detrick, Md., took spores from each letter and grew them in the laboratory. Looking with an expert eye at the samples they had grown, the scientists could see that a small number of the bacterial colonies looked very different from the ordinary appearance of most of the anthrax bacteria. The scientists isolated those unusual spores and grew them alone. As the spores replicated, the scientists saw that the differences or variations persisted, indicating that they were not some kind of aberration.

"Samples from the letters had the same combination of variants in the spores," said Dr. Ravel. "That was one of the first things that began to link the letters."

Next, Institute for Genome Sciences researchers were charged with sequencing the genome of those populations of variant bacterial colonies — just those [spores](#) that looked unusual. They wanted to find out if there were genetic differences that were making the colonies of bacteria look unique. There were, and those same genetic differences were found the spore preparations from all the letters, conclusively linking them to the same source. There were four types of these variations found in the anthrax that came in the letters. Scientists eventually discovered that the anthrax used in the attacks was the product of at least two different production batches of [anthrax](#) that had been mixed together, each with its own unique distribution of variants. Mixing the batches created a

unique combination of genetic signatures that later helped them track the spore preparations back to the source flask in the lab of Dr. Ivins.

"This data we uncovered acted like a genetic fingerprint," says Dr. Keim, of Northern Arizona University. "It could link microbial evidence to its potential source."

"The science was one technique used to generate leads as part of a larger FBI investigation," says Dr. Ravel. "Science tells us the spore came from that particular flask, but it's important to note that the science never pointed to Bruce Ivins. It was police work that did that."

As one of the first and most high-profile investigations of its kind, Amerithrax has helped to shape the emerging field of microbial forensics. Since the case, Dr. Ravel, Dr. Rasko and their colleagues at the Institute for Genome Sciences have been leaders in the scientific community's effort to expand the field by contributing to the development of standards and guidelines for future investigations.

"We were figuring this out as we went along," says Dr. Rasko. "For example, to produce evidence that will hold up in criminal court, you need a very high standard of accuracy with well validated methodologies. It is a much higher standard than our own academic research. Your results need to be completely foolproof and stand in a court of law. Those are the kinds of standards and guidelines we're developing now, so that microbial forensic scientists can be prepared in the event of another biological attack."

Provided by University of Maryland Medical Center

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