

Bacterial Bioterror

New method can rapidly detect potential bioterror agent and pinpoint bacterial strain.

BY JANE M. SANDERS

UPPER RIGHT:

Research by Georgia Tech and the CDC has yielded a rapid testing method that is 95.2 percent accurate in identifying and classifying the potential bioterrorism agent *Coxiella burnetii*. The method combines mass spectrometry, being done here by Ph.D. student Carrie Young, and mathematical data analysis techniques.

FAR RIGHT:

Georgia Tech Assistant Professor of Chemistry and Biochemistry Facundo Fernandez, center, explains a mass spectrometry procedure to graduate students in his lab.

BACKGROUND

& INSET: This micrograph shows the bacterium *Coxiella burnetii* up close.

A new combination of analytical chemistry and mathematical data analysis techniques allows the rapid identification of the species, strain and infectious phase of the potential biological terrorism agent *Coxiella burnetii*. The bacterium causes the human disease Q fever, which can cause serious illness and even death.

Research by the Georgia Institute of Technology and the Centers for Disease Control and Prevention (CDC) has yielded a method that proved to be 95.2 percent accurate in identifying and classifying *Coxiella burnetii*. The laboratory test delivers results in about five minutes compared to about two hours for the lab technique currently used to detect this bacterium.

"Because of its potential use as a bioweapon, we needed a method to detect *Coxiella burnetii* at an early stage, and we needed to be able to determine which strain is present so authorities can determine the geographic area from which it came," says Facundo Fernandez, an assistant professor of chemistry and biochemistry at Georgia Tech. He presented the research team's findings at the 230th American Chemical Society National Meeting in early September 2005.

Fernandez and his Ph.D. student Carrie Young, a chemist in the CDC's Environmental Health Lab, collaborated with CDC researchers in the National Center for Environmental Health and the National Center for Infectious Diseases. They

combined mass spectrometry — an analytical technique to study ionized molecules in the gas phase — and a mathematical data analysis technique called partial least squares analysis.

Mass spectrometry allows researchers to look at the

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profiles of different proteins expressed in a microorganism. Partial least squares analysis lets researchers separate important information from "noise" — or biological baseline shifts caused by sample preparation variations — that could corrupt a predictive model.

Not only is the combination of these techniques into one method a novel concept, this research also represents the first time that *Coxiella burnetii* has been detected at the strain level with a rapid detection process, Fernandez noted. Such classification is a challenging task with bacteria, he added. Researchers believe the technique also will work with other pathogens, which they began studying this fall.

Coxiella burnetii is a species of concern because it causes the highly infective human disease Q fever, which is transmitted primarily by cattle, sheep and goats. A human can be infected by as few as one bacterium. The disease can be manifested as a chronic or acute case, depending on the strain. Symptoms can include high fever, severe headache, vomiting, diarrhea, abdominal pain and chest pain. Q fever can also lead to pneumonia and hepatitis. The chronic form of the disease can cause endocarditis, an infection of a heart valve, and even lead to death.

In addition to being a public health threat, *Coxiella burnetii* is listed as a Category B bioterrorism agent because of its long-term environmental stability, resistance to heat and drying, extremely low infectious dose, aerosol infectious route and history of weaponization by various countries, according to the CDC.

To date, Georgia Tech and CDC researchers can differentiate between seven *Coxiella burnetii* strains, which come from Australia, the United States and Europe. Some strains are more infective than others, and the researchers' method determines not only the strain, but whether it's a Phase I or II strain depending on its ability to infect, Fernandez explains.

IMAGE COURTESY ROCKY MOUNTAIN LABORATORIES, NATIONAL INSTITUTES OF HEALTH



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"The next step is to fine tune our model and increase the number of strains we can identify," Fernandez says. "There is a library of strain samples available to us, though the samples are sanitized with gamma radiation and rendered inactive before analysis."

To identify strains, researchers examine the appearance of biomarker proteins in samples. "In some cases, we classify a strain by the presence or absence of a biomarker. And sometimes we see the same biomarker proteins, but at varying levels, in different strains," Fernandez notes.

The researchers' detection technique is highly sensitive, meaning it can detect *Coxiella burnetii* strains at very low concentrations — specifically at the attomole level, which is equivalent to 1×10^{-17} moles. (Moles measure the actual number of atoms or molecules in a sample.)

Until now, the best method to differentiate between strains of *Coxiella burnetii* was a laboratory technique called polymerase chain reaction (PCR), which analyzes the genes of a bacterium and yields results in one to two hours. The new method, which analyzes the proteins of a bacterium, can yield results in five minutes. For now, it is also a laboratory test, though separate research involving Fernandez and other Georgia Tech researchers is pursuing development of a field-testing instrument.

"In a bioterrorism event, you want more than one method to determine the strain you are dealing with," Fernandez notes. "So you would use our technique first and then use PCR as a second method to independently confirm your results. Also, our method using mass spectrometry, allows you to quickly replicate your analysis — even 10 times if you want to. That gives you an added degree of statistical significance."

Fernandez and his colleagues began the

research in June 2004 with funding from the CDC and a Georgia Tech Research Corporation seed grant. With their encouraging results about the method's capability, they plan to apply for additional federal funds in the near future.

Working with Fernandez and Young are John Barr and his colleagues Adrian Woolfitt and Hercules Moura of the National Center for Environmental Health, and Edward Shaw (now at Oklahoma State University) and Herbert Thompson of the National Center for Infectious Diseases.

@ Read more at: gtresearchnews.gatech.edu/newsrelease/q-fever.htm

