On December 24, 2009, the New Hampshire Department of Health and Human Services (DHHS), the Centers for Disease Control and Prevention (CDC), and the Federal Bureau of Investigation (FBI) were notified of a diagnosed case of gastrointestinal anthrax in a 24-year-old woman. On December 5, the woman began experiencing minor sweating and myalgias that progressed to back pain. Within a week, symptoms of nausea and vomiting had developed, and by December 15, she was admitted to a local NH hospital. Blood was drawn for culture and the patient underwent an exploratory laparotomy where necrosis of the terminal ileum was noted. She was transferred to a Massachusetts hospital for further evaluation.

A gram-positive, spore-forming bacilli in chains, was isolated from blood cultures obtained at the NH hospital. The isolate was non-hemolytic and non-motile, and originally thought to be a possible contaminant. As described by the Gastrointestinal Anthrax in New Hampshire: A 2009 Case Report

Wendy D. Lamothe, MPH, M(ASCP)CM
(New Hampshire Public Health Laboratories, Concord, NH)
DOI: 10.1309/LMD56CC000UEFQID

Abstract
The isolation of Bacillus anthracis (B. anthracis) from a clinical specimen has the ability to stir a state public health department and all of its counterparts into a colossal windstorm of activity. The organism has been used as an agent of bioterrorism and has the ability to cause severe illness and death. Although neither difficult to grow nor requiring special media, anthrax does require specific methods for confirmation and identification, distinguishing it from other Bacillus species. This article describes the presence of B. anthracis in the United States, the type of infections it causes, and what occurred when a single case of gastrointestinal anthrax was identified in New Hampshire.

Keywords: microbiology, anthrax, gastrointestinal

After reading this article, readers should be able to describe B. anthracis, discuss infections caused by anthrax, the methods used to detect anthrax, and the importance of submitting isolates for confirmation to a Laboratory Response Network reference laboratory.

Microbiology exam 71102 questions and corresponding answer form are located after this CE Update on page 369

Corresponding Author
Wendy D. Lamothe, MPH, M(ASCP)CM
wdlamothe@dhhs.state.nh.us

Abbreviations
DHHS, Department of Health and Human Services; CDC, Centers for Disease Control and Prevention; FBI, Federal Bureau of Investigation; ASM, American Society for Microbiology; LRN, Laboratory Response Network; PHL, Public Health Laboratories; CST, Civil Support Team; DES, Department of Environmental Services; OSHA, Occupational Safety and Health Administration; EPA, Environmental Protection Agency; NIOSH, National Institute for Occupational Safety and Health; MLVA, multiple-locus variable-number tandem repeat analysis; PCR, polymerase chain reaction; CAP, College of American Pathologists; HAN, Health Alert Network
American Society for Microbiology (ASM), the conditions for sending isolates to a Laboratory Response Network (LRN) reference laboratory to rule out or confirm *Bacillus anthracis* are catalase positive, non-hemolytic, non-motile, gram-positive spore-forming bacilli. Based on the above conditions, the isolate should have been submitted to the NH Public Health Laboratories (PHL).

A laboratory technician who routinely rotates through the microbiology department in the supervisor’s absence performed the work. Consultation with the NH PHL about the isolate was never initiated. By the time the isolate was received, evaluated, referred, and confirmed as *B. anthracis*, more than 1 week had passed, and the patient remained in critical condition.

Following notification of the diagnosis, an epidemiological investigation immediately began on December 24, 2009. To aid in the investigation, additional partners were brought in, including the NH National Guard’s 12th Civil Support Team (CST), NH Department of Safety, Occupational Safety and Health Administration (OSHA), Environmental Protection Agency (EPA), and the National Institute for Occupational Safety and Health (NIOSH).

The NH DHHS investigation team interviewed friends and family to obtain information regarding possible risk factors to the case patient. The case patient was a vegan, worked on an organic farm 3 months prior, and had participated in an African drumming event held on December 4 at the United Campus Ministry House located at the University of New Hampshire in Durham. The NH DHHS also used statewide surveillance including the Automated Hospital Emergency Department Data System and Vital Records Death Data to attempt to identify possible cases by querying for clinical presentations similar to those of anthrax. Hospital laboratories, as members of the NH LRN, were asked to review 3 months of bacterial culture records and report any gram-positive rods that were not identified. No additional anthrax cases were identified.

The CST collected environmental samples within the United Campus Ministry House and the case patient’s residence to determine the source of infection. Forty drums and 6 environmental samples were collected. Using LRN protocols, the samples were tested by the NH PHL for the presence of *B. anthracis*. Of the 46 samples tested, *B. anthracis* was isolated from 3 (2 drums and 1 pooled sample of electrical outlets). Isolates from the case patient and the drums were submitted to the CDC for multiple-locus variable-number tandem repeat analysis (MLVA), a method used to discriminate or compare different *B. anthracis* isolates. This method employs the amplification of 8 different variable-number tandem repeat loci by polymerase chain reaction (PCR). The markers are then detected and measured using a DNA sequencer. Results from the isolates indicated that the loci were identical by MLVA-8. As a result, the building was quarantined and 84 individuals who were associated with the facility and/or participated in the drumming event were considered exposed. See Figure 1 for a timeline of events.

New Hampshire DES, EPA, NIOSH, CDC, and DHHS developed a second sampling plan designed to better characterize exposure pathways to the case patient. On January 7, 2010, and January 8, 2010, the second phase of samples were obtained for semi-quantitative analysis. Due to the volume

| Table 1 United Campus Ministry House Summary of Testing |
|----------------|----------------|----------------|----------------|
| Testing Phase | Date            | Total Samples Tested | Positive Samples | Total CFUs* |
| Phase 1 qualitative | 12/26/2009 | 40 drums from United Campus Ministry House 6 environmental | 2 drums | NA |
|                  | 12/28/2009 | 10 drums from drum teacher | 1 composite of 3 electrical outlets | NA |
| Phase 2 semi-quantitative | 1/7/2010 | 2 drums from United Campus Ministry House (previous positive) 72 environmental | 2 drums | 300 and 171 CFUs |
|                  | 1/8/2010 | 11 drums from community brought to event | Baseboard heater of event room | 44 |
|                  |          |                           Upper surface of cabinet in adjacent kitchen | 20 |
|                  |          |                           Computer screen in community area | 20 |
|                  |          |                           Computer tower in community area | 20 |
|                  |          |                           None | NA |

NA, not applicable.

*CFU, colony forming units.

20 CFU/sample is at the limit of detection for this test.
of samples and the extensive processing time, the NH PHL requested assistance through the LRN from other state PHL, including New York City, Connecticut, Virginia, and Tennessee. Of the 86 additional samples processed, *B. anthracis* was isolated from 6, including 2 drums that had previously tested positive, a baseboard heater, the top surface of a kitchen cabinet, a computer screen, and desktop computer. Table 1 reflects results from both phases of testing.

### Anthrax in the United States

*Bacillus anthracis*, the cause of anthrax, is a zoonotic disease in domestic animals and was first isolated by Dr. Robert Koch in 1877. The name “anthrax” comes from the Greek word for “coal,” because of the black skin lesions developed by victims with a cutaneous anthrax infection. Goats, cattle, sheep, and other grass-grazing animals become infected when they ingest soil-borne spores. In turn, humans may become infected through contact with infected animals or by handling contaminated animal products such as wool, hair, and hides. In August 2000, a case of cutaneous anthrax was linked to an outbreak among livestock in North Dakota resulting in the quarantine of 32 farms and the death of 157 animals.

The incidence of human infection with *B. anthracis* cannot be accurately determined due to unreliable reporting; however, it is more common in regions where agriculture is predominant and the control of anthrax among animals is poor. Regions where disease is more common include South and Central America, South and Eastern Europe, Asia, Africa, the Caribbean, and the Middle East. Within the United States, anthrax is rare, with an average of 1 to 2 cases of cutaneous disease a year.

During the late 19th and 20th centuries, the flourishing U.S. wool industry generated anthrax-based health concerns. In 1925 the American Public Health Association’s Committee on Anthrax began reporting the number of anthrax cases, as states would report to them. By 1929 it was noted that the number of fatalities associated with tanneries had decreased due to increased awareness and prompt treatment. However, cases of industrial anthrax persisted. One of the first and most deadly outbreaks associated with occupational exposure in the United States occurred in 1957. A total of 9 individuals became ill while working with black goat hair at the Arms Textile Mill located in Manchester, NH. Four out of 5 individuals who were exposed by inhalation developed “anthrax pneumonia” and died thereafter. During this time the mill along with 3 other facilities processing raw imported goat hair were participating in the first study to evaluate the effectiveness of a vaccine. None of the 9 individuals who became ill in the Manchester mill received vaccine. The vaccine was first developed for use in humans in 1954 and has since been improved, increasing protective antigenicity and the stability of the vaccine.

October 2001 opened our eyes to the true dangers of *B. anthracis* and how the organism could be used to threaten and harm. Anthrax spores were mailed to 4 public figures, and their paths were traced. Two of the envelopes were postmarked September 18, 2001. One was addressed to Tom Brokaw, NBC news anchor; the other to the editor of the *New York Post*. The remaining 2 letters were postmarked October 9, 2001, and addressed to Senators Tom Daschle...
and Patrick Leahy. Twenty-two individuals exposed to these letters became infected with anthrax. Of the 22, 11 were confirmed with inhalation anthrax and the remaining 11 were identified with cutaneous anthrax (7 confirmed). Five of the inhalation cases resulted in death. Thirty-two thousand individuals began antimicrobial prophylaxis as a result of the investigation, and 10,300 were recommended to complete a 60-day course. During this bioterrorism threat, PHL from around the country, as part of the LRN, tested more than 125,000 clinical specimens and an estimated 1 million environmental samples.

Since the 2001 bioterrorism event, additional cases of non-bioterrorism-related anthrax continue to emerge sporadically. A case of inhalation anthrax was reported by the Pennsylvania Department of Health in February 2006. Upon investigation, the source of infection in a New York City man was traced to dried goat and cowhides that were imported from Africa. He would scrape the hair off the hides before using them to make traditional African drums. In August 2007, the Connecticut Department of Health reported cutaneous anthrax cases in a drum maker and his 8-year-old child. The drum maker was reported to have been working with untreated African goat hides from Guinea.

**Infection Caused by *B. anthracis***

Human infection can occur via 3 routes: 1) abrasions in the skin; 2) inhaling airborne spores; or 3) ingesting contaminated food. Cutaneous anthrax causes black necrotizing lesions primarily located on the hands, forearms, and head. During the industrial age it was known as “woolsorter’s disease” because of its association with individuals who worked with wool or animal hair in textile mills.

Inhalation anthrax occurs when spores are inhaled and lodge into the alveoli. Phagocytes then transport the spores to the thoracic lymph nodes, where they germinate, multiply, and begin producing edema and lethal toxin. These toxins are responsible for hemorrhagic inflammation of the thoracic lymph nodes, edema, and necrosis.

Deaths due to gastrointestinal anthrax have been reported in Thailand, Uganda, India, and Gambia. Causes of gastrointestinal anthrax are often attributed to the ingestion of contaminated food, primarily meat from an infected animal. The spectrum of disease ranges from no symptoms to death by shock or sepsis.

There are 2 clinical manifestations of gastrointestinal anthrax: oropharyngeal and intestinal. Characteristics of oropharyngeal anthrax are body temperature greater than 39°C, ulcers in the oropharynx, sore throat, difficulty swallowing, and regional swelling. Symptoms of intestinal anthrax are progressive and usually begin with nausea, vomiting, anorexia, a temperature greater than 39°C, and can advance to severe abdominal pain, vomiting of blood, and bloody diarrhea. Spores may produce lesions in the stomach, esophagus, jejunum, ileum, and cecum, and these lesions may hemorrhage. The mucosa and regional lymph nodes of the intestinal tract are always implicated, and other complications such as the accumulation of serous fluid in the peritoneal cavity, shock, and sepsis may occur. Severe gastrointestinal and pulmonary infections are more often fatal. However, any form can lead to death if not identified and treated promptly.
Lessons Learned

Everyone learns from an experience such as this. The NH hospital that was directly involved graciously shared details of its experience with other clinical laboratories during an NH LRN meeting held in early April 2010. Recommendations resulting from their review of the incident included changes to procedures that would require all suspect isolates to be immediately sent to NH PHL for rule out or confirmation. Staff working on the bench would have clear algorithms and charts, listing biochemicals for ruling out select agents. Policy changes requiring all staff rotating through the microbiology department to attend wet lab trainings, as well as a requirement for staff members to participate in the College of American Pathologists’ (CAP) Laboratory Preparedness Surveys, were considered. Concerns including the lack of staffing and funding to send individuals to trainings were raised.

As a PHL, the NH PHL recognized these concerns and will look at ways to make training sessions more accessible. This will include evaluating the training format and exploring ideas such as making training sessions shorter and/or having “train the trainer” sessions. Trained staff could then bring what they learned back to their laboratories to conduct training sessions. Recognizing the current workforce shortage and the impact the economy has had on staffing, the NH PHL will look at possible ways to help fund attendance to training sessions. As Louis Pasteur once said, “Chance favors only the mind that is prepared.”

Communication was a challenge due to the numerous agencies involved and the constant flow of new information. The NH Health Alert Network (HAN) and LRN list serve were used to communicate with NH LRN laboratories, hospitals, and health care providers via e-mail messages and faxes. A technician associated with the NH hospital involved in the event was comfortable with various individuals from different agencies and was able to obtain assistance as needed. Since communication is crucial in any event, the NH PHL hopes to keep channels of communication open and continue to build on relationships, so providers can feel comfortable contacting the NH PHL when needed.

Acknowledgments: I would like to thank my colleagues at the NH PHL for their hard work and dedication to protecting the public’s health in NH.