and coordination of information could probably have been conducted in the offices of NSW Health, from data provided directly from the Police Forensics Services Group and the laboratories of the ICPMR.

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LABORATORY INVESTIGATION OF SUSPECTED BIOTERRORISM INCIDENTS, NSW, OCTOBER 2001 TO FEBRUARY 2002

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In October 2001, the threat of bioterrorism became a reality, following identification of a case of inhalational anthrax in the United States. This was the first case in a bioterrorism-related outbreak, caused by exposure to mail contaminated with spores of *Bacillus anthracis*. This provoked a worldwide spate of hoaxes and scares related to suspicious 'white powders'. In New South Wales, between October 2001 and February 2002, more than 1,000 incidents were investigated and 594 samples of suspicious substances were submitted for microbiological examination to the Centre for Infectious Diseases and Microbiology, Institute of Clinical Pathology and Medical Research, Westmead. This article describes the laboratory investigation of those suspicious substances.

BACKGROUND

In preparation for the Sydney Olympic Games in 2000, the Centre for Infectious Diseases and Microbiology Laboratory Services (CIDMLS) developed procedures for the culture and identification of infectious bacterial agents known to be potential weapons of bioterrorism. These include *Bacillus anthracis* (anthrax), *Brucella melitensis* (brucellosis), *Yersinia pestis* (plague), *Francisella tularensis* (tularemia), and *Burkholderia pseudomallei* (melioidosis). Procedures were established for the management of a bioterrorism-related outbreak, and for communication between relevant agencies including: the Australian Defence Force, the Defence Science and Technology Organisation, the NSW Police Forensic Services, the NSW Fire Brigade, and the NSW Department of Health.

These procedures were reactivated in mid-October 2001, following the report of a case of inhalational anthrax in the United States on 4 October and of laboratory confirmation of a second case on 12 October. These cases were the first in a bioterrorism-related outbreak of anthrax in the United States that eventually involved 22 cases and five deaths due to exposure to finely milled spores of *Bacillus anthracis* sent through the mail.[1] This outbreak led to widespread laboratory testing for environmental contamination in the United States, and a worldwide spate of hoaxes and perceived threats of bioterrorism involving possible exposure to suspicious 'white powders' and other suspicious substances.[2,3]

METHODS

Organisation of the NSW response

The CIDMLS received the first specimens of suspicious substances for analysis on 12 October 2001. Procedures for the handling of suspicious substances, and identification of agents of bioterrorism (that is, the bacteria isolated from suspicious substances), established before the Sydney Olympic Games in 2000, were reactivated. A team of staff was formed to deal with large numbers of specimens as rapidly as possible.

Initially, the CIDMLS received a number of large objects—such as mailbags, parcels, other potentially contaminated articles, and quantities of suspicious powders—in

‘hazardous material’ (hazmat) containers, with a request to exclude the presence of infectious material prior to forensic examination. Procedures were developed to maintain integrity of evidence, in case of subsequent criminal prosecution. All samples submitted to the CIDMLS remained in the custody of the Police Forensic Services Group, which reported the results of laboratory testing; this relieved laboratory staff from constant telephone enquiries.

Within a few days, a NSW Police Operations Centre was opened to coordinate communication between all the agencies involved in the investigation and control of incidents, including the police, the fire brigade, and NSW Health. ‘Hazardous material’ teams were responsible for management of incident sites, and for collection and packaging of articles for testing using standard operating procedures. Each sample for laboratory investigation was given a unique ‘event number’ that allowed tracking, collation of results, and follow-up of people exposed in the incident. All laboratory results were sent to both the Police Operations Centre and Police Forensic Services Group via secure fax. Public health units were responsible for follow-up, for communicating results of laboratory testing, and for reassuring people potentially exposed to suspicious substances. This responsibility is described in an accompanying article by Leask, Delpech, and McAnulty in this issue of the *NSW Public Health Bulletin*.[4]

Meanwhile, the national Public Health Laboratory Network (PHLN) established common laboratory procedures for the safe transport and handling of suspicious ‘white powders’.[5] A workshop was held on 19 October 2001 at the Queensland Health Scientific Services Laboratory at Coopers Plains, Queensland, at which representatives of all PHLN laboratories shared methods of identification and analysis of *B. anthracis*, particularly nucleic acid detection using polymerase chain reaction (PCR).

Sample collection

Samples suspected of containing bacterial agents of bioterrorism must be examined in a physical containment level 3 (PC3) laboratory within the CIDMLS. To be handled safely, they must be free of radioactive and or toxic chemical agents and be received in a clean container small enough to be opened safely in a biological safety cabinet. This excludes large items, such as mailbags. After discussion with the Police Forensic Services Group and the NSW Fire Brigade, a staging area was established at the Police Forensic Services Group headquarters at Westmead, where articles were tested for radioactive substances and toxic chemicals, and where small samples of any suspicious substance were collected. Samples were packed in ‘hazardous material’ containers and transported to the PC3 laboratory in the same way as routine specimens, in accordance with Instruction Number 602 of the *International Air Transport Association Dangerous Goods Regulations*.[6]

Microbiological testing

Protocols for the examination of ‘white powders’ or suspicious substances include phase contrast microscopy and examination of a gram stained preparation by light microscopy for bacteria and spores, which are the basis of a preliminary report. Initially, specimens were inoculated into media that support the growth of all known bacterial agents of bioterrorism. However, within the first week, intelligence reports established that the risk was confined to *B. anthracis* and laboratory protocols were modified accordingly. Brain heart infusion (BHI) broth and two blood agar plates were inoculated. BHI broth and one blood agar plate were incubated at 35°C aerobically, and one blood agar plate was incubated in CO_2. The plates were examined after 24 and 48 hours of incubation for the presence of non-haemolytic colonies of gram positive non-motile rods resembling *B. anthracis*.

Suspicious isolates were tested by *B. anthracis*-specific PCR, initially using the method modified by Queensland Health Scientific Services from a published method,[6] and later using an in-house fluorescence detection method with a faster ‘turn around’ time. Additional testing included bacterial fatty acid analysis by gas chromatography and standard biochemical tests.[7]

RESULTS

Samples submitted and results of microbiological testing

In NSW, there were two main periods of activity: 15 October to 23 November 2001, following bioterrorism-related outbreaks of anthrax in the United States; and 2 January to 6 February 2002, following an extortion threat on the McDonalds’ food chain. More than 1,000 incidents were investigated during these periods. During the first period, the Bioterrorism Response Unit at the CIDMLS examined 475 samples; during the second period, 119 samples were examined.

Spores were not identified by microscopy in any samples submitted. *B. anthracis* grows readily on blood agar plates incubated at 35°C, and is usually detectable within 18–24 hours. A wide variety of bacteria were isolated from samples, mainly environmental, including many non-haemolytic colonies of gram positive rods (nine of which were non-motile and closely resembled *B. anthracis*; however, specific PCR was negative and all nine suspicious isolates were subsequently identified as *B. megaterium* using a combination of gas chromatography and standard biochemical tests).

The total time for the PCR assay on suspicious colonies, after an average of 16–18 hours incubation (overnight), was four hours, which allowed a provisional result to be issued within 24 hours of receipt of the specimen in the laboratory.

DISCUSSION

Bacterial agents of bioterrorism are generally classified as risk category group three (RCG3) pathogens. Handling of powders or other material suspected to contain them should be performed in a PC3 facility. Weaponised agents of bioterrorism, including *B. anthracis* spores and *Coxiella burneti* (the cause of Q fever), are developed for aerosol delivery and can remain viable for many years. Safe investigation requires staff trained to handle dangerous organisms. It also requires well-documented procedures, not only for microbiological testing but also to ensure biological security and integrity of forensic evidence.

Previous bioterrorism response protocols for the Sydney Olympic Games in 2000 were useful in managing the incidents between October 2001 and February 2002. In particular, existing procedures for a coordinated and practical decision-making that could be rapidly reactivated were invaluable. However, the 'white powder' incidents were a significant challenge to all agencies involved, including the CIDMLS. Previous procedures were designed for response to incidents of bioterrorism associated with defined events and venues. The possibility of agents of bioterrorism being disseminated through the postal system greatly increased the number of possible exposures, and the demand for resources needed to manage them.

The establishment of a staging area for screening, and pre-testing of suspicious articles, greatly facilitated handling of specimens and reduced the laboratory workload. Triage of specimens allowed some specimens that did not contain suspicious substances to be discarded. Multiple copies of specimens, such as advertising mail, that were suspected of being contaminated, were not tested once the presence of *B. anthracis* spores had been excluded in one sample.

Nevertheless, during the first six-week period, staff who normally perform routine diagnostic and public health microbiology were on call almost continuously so that samples could be processed as rapidly as possible. Accrued days off were cancelled and administrative and non-urgent maintenance procedures were postponed. The PC3 laboratory at the Centre for Infectious Diseases and Microbiology is normally used for diagnosis of tuberculosis and analysis of cultures thought to contain other RCG3 pathogens such as *Brucella* spp. Careful planning was required to ensure that the bioterrorism work did not interfere with routine work and, after the first two weeks, all but the most urgent samples were batched to make optimal use of the PC3 facility. Major public health laboratories in other jurisdictions experienced similar problems; however, because of the relatively large population of Sydney, the Centre for Infectious Diseases and Microbiology received more than half of all bioterroism-related specimens submitted to laboratories in Australia during this period. This heavy workload could not have been sustained without significantly compromising routine laboratory functions.

Even when the chance of detecting *B. anthracis* is low, rapid handling of specimens to exclude its presence as quickly as possible is essential to allow people potentially exposed to be reassured and normal business to resume in premises suspected of being contaminated. Rapid methods for on-site testing of samples for *B. anthracis* are still unreliable. At present, rapid PCR on suspicious overnight cultures provides the best balance of accuracy and speed, although further confirmatory testing is needed.

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