

Bioterrorism

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Introduction

Although bioterrorist attacks have taken place over the last two decades, it is still an area of ignorance amongst UK medical staff, including pathologists. This is paradoxical since it is pathologists and anatomical pathology technologists (APTs) in the mortuary who are more likely to be involved closely than many other medical and paramedical staff. The purpose of this chapter is to outline some of the history of bioterrorism (BT), the responses of government, what the likely agents are and their clinical pathologies, medico-legal aspects and what everyone in mortuary work should be prepared for. By definition, much is uncertainty, although the expert consensus is that all industrialised countries could experience a BT attack sooner or later. Following the initiative of the USA, many countries (including the UK) have drawn up plans and produced guidance documents. These have been freely adopted here, accompanied by personal observations where there is no agreed evidence base.

Biowarfare and, to a lesser extent, bioterrorism have been with us for hundreds of years, but the modern industrialised world has only woken up to the actuality in the last decade (1)—see Box 1 and Box 2. Although the potential was beginning to be addressed in the 1990s (and really only in the USA) at government and institutional levels (2), it was the 2001 episode of anthrax-contaminated letters in the mail (in the USA) that truly focused minds. The civil and criminal investigation responses to that event are well documented (3), but the critical role of pathology in that investigation needs to be emphasised (4–6).

Bioterrorism is defined as “the use or threatened use of biologic agents against a person, group, or larger population to create fear or illnesses for purposes of intimidation, gaining an advantage, interruption of normal activities, or ideologic activities. The resultant reaction is dependent upon the actual

Box 1. Historical examples of the utilisation of this mode of attack include (9, 10):

- Fourteenth century, Black Sea city of Kaffa: catapulting plague-infected human cadavers into the city under siege (biowarfare)
- Fifteenth century, South America: the conquistador Pizarro introduced smallpox naturally into a naïve population and furthered the infection rate by presenting the local population with infected clothes (inadvertent biowarfare and bioterrorism)
- 1930s, China: experiments with anthrax (fatal) on humans and subsequent autopsy analysis (biowarfare research)

These examples also indicate some of the agents most prominent in BT considerations: anthrax, plague, smallpox. Their virulence and mortality rates are well attested.

Box 2. More recently, the significant BT events have been:

- USA 1984: contamination of salad bars with *Salmonella typhimurium*, apparently for local political purposes (3, 10); 751 persons affected; no deaths.
- Iraq 1990s: following the first Gulf war in 1991, it became evident that the country had prepared and stockpiled vast quantities of botulinum toxin, aflatoxin and anthrax spores (8, 21, 31). These were never found—presumed to have been destroyed on site—by the subsequent USA and Europe science teams that went in search of weapons of mass destruction.
- Japan 1995: when the attack on the Tokyo metro with the neurotoxic sarin gas by the Aum Shinrikyo cult was investigated, it emerged that members had visited Zaire in order to obtain samples of Ebola virus for BT purposes (10). They were not successful. They had also attempted to disseminate *Clostridium botulinum* toxin, unsuccessfully (31).
- USA 1996: contamination of food in a laboratory staff rest room with *Shigella dysenteriae*; motive unknown (3). Twelve persons became ill; no deaths.
- USA 2001: about 10 gm of anthrax spores was sent in multiple envelopes by post, by person(s) unknown, which resulted in 22 cases. The presumed intended targets were news media and government personnel (36). The index case had anthrax meningitis (4). Twenty of the victims handled mail, and one person was probably accidentally infected by indirect letter-to-letter contamination. Eleven cases were cutaneous anthrax (all survived); 11 cases were inhalational or meningeal anthrax of whom five died (3, 5, 23); 32,000 people received antibiotic prophylaxis.

event and the population involved and can vary from a minimal effect to disruption of ongoing activities and emotional reaction, illness, or death” (7).

Bioterrorism should be distinguished from state-initiated biowarfare research projects and applications. The governments of the UK, USA, Germany, USSR, Japan and Iraq, to list only the most publicised examples, have all supported research into the potential use of biological agents in order to harm enemy military and/or civilian personnel in the twentieth century. They have officially ceased such activities now, but much important information has been gleaned from accidents and other events during this phase of activity.

It was the 1990s events in Japan and Iraq that forced everyone to think of BT as a serious possibility that could affect any country, no matter how well prepared militarily for conventional or nuclear attack. In 1997, the *Journal of the American Medical Association* devoted a whole issue to BT, which reached a wide audience (8, 9).

The Bioterrorism Potential: The Anthrax Scenario

Partly because of the relatively simple process of disseminating the agent in a BT attack, and partly because there has already been a BT attack using anthrax spores, this is the infection that most planning scenarios have considered the most likely in the future. In a relatively densely populated country such as the UK, with conurbations of up to 8 million people, deliberate release could leave up to 5 million people exposed, particularly if it was multiple simultaneous attacks in urban areas.

The actual lethal dose is not known although experiments indicate a LD₅₀ (50% of those infected develop disease and die) of 10,000 spores; the elderly may require a smaller dose, as suggested by one patient in the USA during the anthrax-by-post attack in 2001 who had no connection with primarily infected mail.

Perhaps 10% (= 500,000 people) would be at significant risk of infection (quantified as >0.1% risk), and 2% (= 100,000 people) would be at high risk of infection (>1%). Thus of those exposed about 7500 could become infected without prophylactic antibiotic therapy. As determined from the 1979 Sverdlovsk episode (see Box 4), the risk of infection would extend to 50 km downwind.

As a result, a large number of people would present to hospitals with respiratory disease and fever over the following week or so. How they would be managed will depend on:

- Whether the attack was overt or covert
- How many present and to how many health centres
- How rapidly the diagnosis is made in those living
- How rapidly the diagnosis is made from those dying, if not made before death
- The facilities available at the health care centre (hospital), e.g. ICU
- The polices drawn up in preparation for such events

Once it became public that an anthrax BT event had occurred, which would probably be within the usual incubation period time of a week, a larger number of people within the target zone (including the downwind area) would present to health care centres or contact health phone lines for advice. They can be grouped as:

- Well but worried
- Chronically ill with another disease, but perceive themselves at high risk from anthrax
- Symptomatic although the cause is more likely be something else than anthrax
- Symptomatic due to anthrax

There will also probably be a greater number of people who could not have been exposed directly (for geographical reasons) but who are concerned for their health and those who have been in contact with people within the target zone since the attack. The exact numbers involved here are unknowable, but would almost certainly swamp the health centres and resources, and divert resources from the normal disease management processes.

There would be demands for antibiotic prophylaxis and vaccination (although in this case there would be no available vaccines for the public). Without stockpiles, there would not be enough antibiotics to go round, let alone the health care staff to distribute them. Public anger at perceived ill preparation on the part of the government and the health systems could result in public disorder, with extra demands on the forces of law and order.

Because anthrax spores (which are viable for years) would be distributed over the ground, through air conditioning systems (probably), and get into immediate water supplies, infra-structures such as public transport and schools would be closed for decontamination. This would disrupt the social economy as well as the business and financial economies, and reduce the active workforce (child-minding, workplace closed) and so on.

From this brief scenario, it will be seen that the objectives of terrorists—to inflict harm upon a population and disrupt the social systems—will have been achieved. The message that emerges from consideration of the possible effects of a BT attack is the need to focus on several key issues, so as to minimise the effects on individuals, the wider public, the health systems and the economy. These include:

1. Preparation of evidence-based, or at least expert opinion, plans for all reasonable eventualities
2. Awareness among front line staff of the clinical features that should raise suspicion of a BT incident
3. Robust and rapid means of establishing or refuting the diagnosis of a BT-related infection in life
4. Similar means for autopsy diagnosis
5. Plans for reference centre involvement and epidemiological surveillance

6. Plans for the in-hospital management for such diagnoses or suspected patients, both for the first and then subsequent likely cases
7. Plans for advice, prophylaxis and/or vaccination as appropriate for health staff and for the wider public
8. Plans for the disposal of the dead resulting from a BT attack
9. Plans for the medico-legal systems' involvement in dealing with the dead

The Means and Cost of Terrorism

The four physical means of terrorism (conventional weaponry, biological, chemical and nuclear) have very different consequences for societies and their preparations for such events. Of the four means, BT is the most insidious. Assuming a BT attack is covert (not announced—see below), it would be days to weeks before anything was noticed and by then the effects would be widespread (regionally, nationally or even internationally), and then it would be up to individual doctors and health centres to notice the effect and notify the authorities. Hence the attention is now being paid to new methodologies of potential disease surveillance and diagnosis (see below). Chemical attacks have effects within minutes to hours and will be restricted in their radius.

These four main means for terrorists to cause harm and disrupt society are listed in [Table 1](#), along with the estimated costs. The cost is the calculation of the expense to kill 50% of the population in a given area per square kilometre (10).

The Motives for Bioterrorism

The purposes of terrorism in general are varied and mainly beyond the scope of this chapter; hereon the focus is only on BT. From the events so far documented, the purposes include local personal grudges, a desire to influence voting patterns, a doomsday cult's activity and the simply unknown (the 2001 anthrax attack). Of more concern in the twenty-first century (and since the attack on the World Trade Center in New York in 11 September 2001) have to be the effect of

Table 1 Relative costs of the different modes of terrorism to kill civilians

The weapon	Cost in US dollars to kill 50% of a population
Conventional arms and weapons	2000
Nuclear weapons	800
Chemical weapons	600
Biological agents	1

international conflict, particularly in the Middle East and augmented by the 2003 Iraq invasion, and the perceived anti-Islam stand of (particularly) the USA and Europe. Broadly, the purposes of BT include:

- To cause morbidity and mortality
- To disrupt health services
- To induce fear in the population

The consequences of this are, presumably in the minds of bioterrorists, to

- Disrupt society
- Force change of government and/or government policies

The Potential Agents for Bioterrorism

There is no ideal bioweapon for terrorist purposes. One can draw up a list of criteria that would characterise the ideal agent, including:

- Easily available from other laboratories
- Easily prepared from local materials
- Safe to generate and weaponise
- Easily disseminated as an aerosol of 1-5 μm size particles
- Safe to disseminate
- Long lasting and stable in the environment to prolong infectivity
- Readily transmitted from person to person (contagious secondary spread)
- High infectivity, virulence and mortality rate
- No effective treatment of those with clinical disease
- No effective prophylaxis for infected, asymptomatic people (chemotherapy and/or vaccine)
- Major public health impact
- Cause public panic and social disruption
- Require special action for public health agencies

In the late 1990s, the Centers for Disease Control and Prevention (CDC), the federal public health institute in the USA, consulted experts and drew up a consensus list of the most likely and dangerous agents that bioterrorists might use. They comprise the Category A list (2, 7).

Category A

- Smallpox (*variola*)
- Anthrax (*Bacillus anthracis*)
- Plague (*Yersinia pestis*)
- Tularaemia (*Francisella tularensis*)
- Botulism toxin (*Clostridium botulinum*)
- Viral haemorrhagic fevers (Ebola, Lassa, Congo-Crimea haemorrhagic fever, Marburg viruses)

Following the 2001 anthrax attack, these were consolidated. Two further categories of infective agents were then considered that might be used in BT attack, but carried a lower mortality than the Category A list agents. One stimulus to including these was the perception at the CDC that the diagnostic capabilities for these agents needed to be improved and expanded nationally.

Category B

- Q fever (*Coxiella burnettii*) (11)
- Brucellosis (*Brucella* spp)
- Glanders (*Burkholderia mallei*)
- Arthropod-borne encephalitis (Venezuelan, eastern and western)
- Water- and food-borne gut pathogens (*Salmonella* and *Shigella* spp, *E. coli*, cholera (*Vibrio cholerae*), *Cryptosporidium parvum*)

A further Category C list has been compiled by the CDC, of emerging or re-emerging pathogens that might be engineered for mass dissemination, are easily available and have potentially high mortality. They include:

Category C

- Nipah virus
- Hantaviruses
- Tick-borne haemorrhagic fevers
- Tick-borne encephalitis
- Yellow fever virus
- Multi-drug resistant tuberculosis (*M. tuberculosis*)

Obviously none of these lists is exclusive and exhaustive, but represent the consensus of the most likely possibilities for BT. Table 2 summarises many of the Category A infection characteristics.

Availability, Weaponisation and Dissemination of the Proposed Agents

With the exception of smallpox (see below), all the agents in the three categories cause disease naturally and are present in nature, globally or locally. In addition, there will be freeze-dried preparation in many laboratories. Apart from smallpox, the most difficult to obtain by potential terrorists would be Ebola, as it is still not clear in which animal reservoir the virus resides in the wild in central Africa (12). But there are many isolates in several laboratories. To obtain smallpox would involve a source within one of the two laboratories (in USA and Russia) known to house isolates under secure conditions—unless, secretly, a scientist previously working there has already taken samples with him or her.

Table 2 Properties of biological agents in Category A (data from multiple USA and UK public health sources)

Agent/ disease	ACDP hazard group	Survival in nature	Infectious dose	Transmission	Incubation period	Person to person transmission	Case fatality (%)	Specific therapy
Smallpox	4	2 days	10–100	Contact; inhalation inoculation; ingestion	10–16 days	Yes	15–95	?
VHF	4	2 days	Unknown	Inhalation; inoculation; ingestion	1–21 days	Yes	20–90	+/-
Anthrax	3	40 years	LD ₅₀ ~10,000, but may be 10–100	Inhalation; inoculation; ingestion	1–10 days, but may be up to 6 weeks	Not in life, but possible during autopsy	20–90	Yes
Plague	3	4 h	100–500	Inhalation; inoculation by infected flea	1–4 days via inhalation	Yes, from pneumonic plague	33–95	Yes
Tularaemia	3	Long	10–50	Inhalation; inoculation; ingestion	2–5 days	Not in life but possible at autopsy	30–50	Yes
Botulinum toxin	3	2 days	~1 ng/kg	Ingestion	3–4 days	No, although it is just possible from an externally contaminated cadaver	?	No

To establish a laboratory capable of manufacturing BT agents on a large scale is not difficult, and the estimated cost of setting up the facility is about \$100,000 only (1, 10).

The various means of dissemination of BT agents include:

- Aerosol dispersion
- Contamination of food
- Contamination of water supplies
- Contamination of milk tankers
- Direct inoculation into people

The consensus is that aerosol dissemination is the most likely for mass BT attacks. The two previous gut pathogen BT attacks contaminated food and were targeted locally and specifically; widespread harm did not result. Similarly milk tanker contamination would have limited, brief and local effect only. Most of the Category A list pathogens are not disseminated by water, and those that could be would require vast amounts to be placed in reservoirs to overcome the dilution effect. Direct inoculation is simply inefficient, and is detectable.

Aerosol Dispersion

All the Category A list agents can potentially be disseminated in a fine particle aerosol of sizes 1-5 μm . This is invisible and small enough to be inhaled and passed to the alveoli without filtering and capture. The means of spreading the agents are various, and include:

- Paint-sprayers
- Fogging machines that are used to disseminate insecticides
- Hand-held perfume atomisers
- Hand-held drug delivery devices (like asthma inhalers)
- Airplanes, as for crop-dusting

Basic Definitions in BT Terminology and the Public Health Organisations' Responses

The UK Health Protection Agency (HPA) has issued, from 2005 with updates, interim guidelines for action in the event of deliberate release of a range of chemical, nuclear (radioactive) and biological agents (see their website www.hpa.org.uk/infections/topics_az/deliberate_release/menu.htm for updates). For the biological agents, they include comprehensive sections on Biology of the agent, Epidemiology, Transmission, Communicability, Clinical features, Mortality, Antimicrobial sensitivity, Clinical procedures, Treatment, Infection

control, Immunisation, Decontamination, Protection of health care workers, Post-exposure prophylaxis, Laboratory diagnosis, Public health procedures and contact names and addresses. Autopsy is considered briefly, in terms of what samples to take for diagnosis but also stressing that they should not be performed if the infection is suspected.

However, there is more detail in an earlier HPA document “Initial investigation and management of outbreaks and incidents of *unusual illnesses* – a guide for histopathologists” (2004) (13). As well as specific advice on what samples to take and where to refer them for confirmation, the role of the *coroner*¹ is acknowledged in the autopsy process; and there is the guidance not to perform an autopsy “on any patient recognised as having an unusual illness until expert advice has been sought”. An update on microbiological sampling has been issued in 2006 (14). This encompasses the possibilities of genuinely new infections (see below) and enables the full panoply of molecular diagnostics to be applied to material for rapid identification of known and unknown infections.

Unusual illnesses are described as being in/of:

1. Patients presenting with signs and symptoms which do not fit any recognisable clinical picture
2. Known aetiology but not usually expected to occur in the UK or setting where it has been observed
3. Known aetiology that does not behave as expected, e.g. failure to respond to standard therapy
4. Unknown aetiology

An *outbreak* is said to occur where:

- The number of cases is greater than the number expected over a given time period.
- One or more cases are linked by epidemiological, toxicological, microbiological or radiological features.

Obviously, one case of a serious unusual illness such as inhalational anthrax is of public health concern, but would be termed an *incident*. Whilst an outbreak or incident of unusual illness may be the result of natural or accidental processes, they could be due to *deliberate release*; this may or may not have had an underlying malign intent. Deliberate release may be *overt*, where it is immediately evident that release has occurred (e.g. a phone call from perpetrators to the police), although the nature of the release may or may not be clear. A deliberate release may also be *covert*, with the first indication of a release being the presentation of people with unusual illnesses—alive and/or dead.

¹ Coroners are part of an independent national medico-legal system for investigating unexpected deaths, potentially unnatural deaths and deaths of unknown cause. They are not integrated into the British health service.

Thus health professionals have a crucial role to play in the identification of covert releases. Previously unrecognised syndromes may also be due to *new* or *emerging* or *re-emerging* conditions. New infections would be those previously unknown or known in animals but not known to affect man (probably all significant human infections arose in this way, from animal to man transmission). Emerging infections are those previously described but of low natural prevalence and incidence, but now becoming more widespread. Re-emerging infections are previously described, but considered eradicated or of such low natural prevalence as not to pose a risk to man, but are now found to be occurring more frequently.

Acute incidents are those where recognition of an event occurs rapidly, within minutes to hours. *Delayed incidents* are those where presentation of affected persons is delayed by hours, days or weeks: this is the situation in all biological agent incidents.

These descriptions and definitions are presented to demonstrate how public health authorities are preparing themselves and the wider network of medical and paramedical personnel. All the points could be criticised for vagueness or over-inclusion (e.g. this version of unusual illness would cover HIV disease with its complex presentations), but that is less important than the fact of consciousness-raising and placing the concepts before a broader audience.

Mortuary Plans and Provisions

BT cases can arrive at any mortuary, whether hospital or free-standing local authority (public mortuary), with prior known diagnosis or of unknown cause of death before autopsy. What happens once it is established that a BT outbreak, with mortality, is definite or likely will depend on scale. If there are only a few identified or suspected cases, then they will probably be handled at the place of arrival or in a specialist referral unit for autopsy.

However, if the outbreak is larger and is designated as a “disaster”, then many local authorities will institute established plans and concentrate all cases in one designated public mortuary in an area, or go further and create a temporary “resilience mortuary”. For example, in London, the coroners and local authorities have drawn up plans for designating disaster mortuaries as that most geographically and facility appropriate. Each such mortuary will have a maximum agreed capacity, and if this is exceeded, a resilience mortuary will be created to concentrate all the cases on one site.

This could be an existing public (or even hospital) mortuary, but is more likely to be a free-standing entity for logistic reasons. Because such BT outbreaks are by definition homicide and by the nature of the infections, there is the need for a multi-agency investigative team, forensic science access, identification teams, high inter-agency communications traffic, all-hours vehicular access, relatives’ communication and viewing areas, media communication centre, etc. The

investigations could go on for weeks depending on the scale and cadence of the BT attack. All these factors, in the event of a major attack, would overwhelm most existing hospital and public mortuaries, and—critically—prevent them from undertaking their normal day to day activities (normal mortality and funereal requirements do not stop because of terrorism). The utility of this approach was demonstrated in the aftermath of the July 2005 London terrorist bombing, where a temporary mortuary facility and communications centre was erected in a territorial army campus in central London, and all the above activities could proceed smoothly; it was then taken down when all the bodies had been studied and prepared for disposal (London resilience plan, May 2005).

The Disease That May Present as Bioterrorism

Smallpox and anthrax are described in some detail, and the other four infections in Category A are more briefly depicted. Colour images of the clinical and gross and histopathological features of all the infections are available for viewing and downloading on the USA CDC and the UK HPA websites. Table 2 depicts the essential data on infectivity and contagiousness.

Smallpox

Smallpox is one of the six Category A infections considered most likely to be used in a BT attack. The following fairly full account of the disease—its presentation, investigation and management and its potential impact and autopsy issues—is presented as an exemplar of the aspects of which an involved pathologist needs to be aware (15–17).

Smallpox was declared extinct in 1980 by the WHO. The USA and Russia have maintained reference stocks of the virus (in one institution each) (16), and there are no known illicitly held smallpox virus stocks. Thus the possibility of deliberate release is low; however, the likely consequences are so severe that contingency plans are prepared against the event. The number of people who have active immunity from routine vaccination programmes prior to 1980 is low since the complete efficacy of the vaccine is believed to be not more than 5 years' duration.

Variola is a DNA virus. There is no natural animal reservoir. Transmission is usually from droplet aerosols of infected persons, inhaled into the respiratory tract of another. Direct skin-lesion-to-skin contact can transmit, as can contact with infected body fluids. The most infectious period in a patient is after the incubation period, during the first week of the rash, when the virus is released from the respiratory tract. Several of the critical facts of smallpox are in the Table 1.

Clinical Features

During the asymptomatic first week, there is viraemia and dissemination to the lymphoreticular system. A second viraemia commences on about day 8 from infection and is associated with the characteristic illness:

- Sudden high fever
- Macular rash 1–3 days later in the oropharynx, then face, forearms and trunk
- The rash becomes popular 2 days later, then vesicular after another 1–2 days. Typically it is more severe on the face and extremities—centrifugal pattern
- The vesicles become pustular after another 2–3 days, forming scabs 5–8 days after the onset of the rash
- The scabs separate leaving characteristic pitted scarring, most prominent on the face.

That is the typical pattern. Smallpox can have less common atypical patterns that have caused late diagnosis through confusion. The two forms are

- Haemorrhagic smallpox—with haemorrhage into the mucosal and skin lesions.
- Malignant smallpox—the lesions do not develop to the pustular stage but remain soft and flat.

Differential Diagnosis of Smallpox

With no natural cases of smallpox for >30 years, few doctors will recall the clinical features from personal experience. The classic differential has been chickenpox (varicella-zoster virus, VZV). Other skin rashes that could be confused with smallpox, and almost inevitably will do so in the event of an outbreak, when the threshold for suspecting smallpox will be lowered, include (15):

- Monkeypox
- Generalised vaccinia
- Herpes simplex virus
- Molluscum contagiosum
- Measles
- Parvovirus (B19)
- Rubella
- Enteroviral infections
- Hand-foot-and-mouth disease
- Syphilis
- Impetigo
- Drug eruptions and Stevens–Johnson syndrome
- Atypical forms of skin lesions in immunosuppressed persons (e.g. anergic cutaneous cryptococcosis in advanced HIV disease can resemble herpetic and smallpox rashes in the pustular stage)

For haemorrhagic smallpox, the differential diagnosis is:

- Meningococcal sepsis
- Acute leukaemia

For malignant smallpox:

- Haemorrhagic chickenpox

The World Health Organisation has produced training materials to help health care workers recognise smallpox and its differential diagnosis; see the WHO website www.who.int/emc/diseases/smallpox/slideset/index/htm.

Treatment

There are no proven antiviral drugs effective against smallpox (there has been no need to develop any against an eradicated infection, until now). However, cidofovir is active against other orthopox viruses, is active against variola in vitro and would be tried if cases arose. It has to be administered IV and is potentially nephrotoxic.

Vaccination

Stocks of smallpox vaccine have inevitably been depleted since 1980, but are now being regenerated precisely because of the threat of BT. It is most effective before exposure to smallpox, but vaccination does reduce the clinical attack rate if given after exposure. Previously vaccinated people may have an accelerated response in the development of the vaccination pustule. Vaccine “effectiveness” means reducing the attack rate to <10% and mortality to <1%.

In the UK, stocks of vaccine are limited and only a few health care professionals, mostly laboratory staff, and emergency service personnel have been vaccinated; the supply is controlled by the Department of Health. No pathologists or APTs have been vaccinated at the time of writing. A further problem is that of vaccine complications. Apart from generalised malaise, specific complications include (18)

- Generalised vaccinia
- Progressive vaccinia
- Post-vaccination encephalitis
- Fetal vaccinia
- Myopericarditis

None of these is common, but are sufficiently notable to advise a cost–benefit approach as to who needs vaccination in the event of a definite or suspected smallpox BT attack. A further issue, not seen in the days before smallpox

eradication, is the susceptibility of HIV+ persons to a greater complication rate than non-infected persons. Whether potential vaccinees should have an HIV test or be screened by questioning for HIV risk factors and their own knowledge of their HIV status awaits clarification (19).

Diagnostic Procedures and the Autopsy

In life, there are protocols produced by the HPA on clinically suspecting smallpox and how to confirm or exclude the disease. In the laboratory, the diagnosis will be made by real-time PCR; electron microscopy identifies the virus but the morphology is similar to that of other orthopoxviruses (15).

For those who die, the HPA guidance is fairly specific (17):

- If the diagnosis is already known there is no requirement for autopsy.
- If a case is suspected but examination procedure can be kept to a minimum of invasiveness, to reduce the likelihood of infection to pathologists and APTs, there is no need to open the cadaver to prove smallpox.
- All staff involved in an autopsy must be immunised.
- Full respiratory protection must be used, in addition to the standard universal precautions in dress (see below).
- Skin samples, both uninvolved and obviously abnormal can show the virus through standard virological techniques
- Postmortem blood through cardiac puncture is a useful source of virus
- The samples must be transported to the reference centre laboratory in leak-proof secondary containers, complying with the UN602 standard packaging, and labelled BIOHAZARD.
- Bodies should be cremated, and not buried or expatriated, and thus disposed of rapidly. They must be placed in double body bags (not cotton shrouds).
- Bodies should not be embalmed
- Funeral director staff must be fully informed and involved with disposal, and should also be vaccinated.

However, this does not immediately answer the question of what to do when the likelihood of smallpox is actually low, and a fuller, invasive, autopsy will or might be needed to identify the proper cause of death. Following the previously published Royal College of Pathologists' guidelines on autopsy practice for Hazard Group 4 serious communicable diseases (20), it is recommended that the results of rapid virological studies, transported promptly to the reference centre, on the skin and blood material are awaited. If smallpox is excluded, and any similarly hazardous infection likewise, the autopsy can proceed under standard conditions. If smallpox is proven, but for forensic reasons there is a need for further examination, then the autopsy can take place in a mortuary designated suitable for such diseases, if the body is not there already.

The Pathology of Smallpox (6)

Skin. The rash has been described above. Histopathologically, there is intra-epidermal oedema, ballooned epidermal cells and necrosis. The characteristic Guaneri bodies are intra-cytoplasmic granular, basophilic viral inclusion bodies in epidermal cells. There is marked dermal inflammation. Similar inclusions are seen in the mucosal epithelium.

Heart. In fatal cases of smallpox, there is a myocarditis.

Lung. In fatal cases there is often a direct smallpox pneumonitis and secondary bacterial infections.

The Impact of Smallpox

In the absence, so far, of a smallpox BT event, any estimation of its impact on society is imprecise. It will depend on the number of people directly affected and how rapidly the health authorities diagnose the infection and act to limit its spread. However, it is worth reiterating what happened in Yugoslavia in 1972 when a person returned from the haj in Mecca having acquired smallpox infection there (1). The country had continued an active vaccination programme although there had not been a case since 1927. See Box 3.

What would happen during a smallpox outbreak in an industrialised country now is unpredictable, particularly where government does not have a similar built-in control over its population as pertained during the communist era in Yugoslavia. In a covert BT attack with smallpox, there would be no warning that such a febrile rash could be smallpox, and 7–10 days would lapse after the attack before any presentations. Because of delays in diagnosis, it might be another few days before the diagnosis was suspected and made (though now the diagnosis would be made virologically within 24 h once a sample was presented to a reference centre). Suppose 100 patients in a large city developed the disease, presenting to different hospitals. Coping with them using isolation precautions, with the exposed health care workers and with the friends and relatives of the cases would probably bring the hospitals to a halt in the discharge of their normal function.

The Impact in the Mortuary

What would happen in the mortuary should a case be autopsied, without anyone realising at the outset that it was smallpox, is also unpredictable. If the pathologist or APT suspects the diagnosis, then the diagnostic and logistic procedures outlined above should be followed. But the staff are unlikely to have been already vaccinated (as required by the guidance) and there will probably be a degree of panic. Also there may not be established and available protocols between the mortuary, the microbiology department and the reference centre as to how to proceed in the evaluation of suspected smallpox. Occupational health

Box 3. The 1972 Yugoslavia smallpox outbreak

The index patient developed an undiagnosed fever. Friends and relatives came to visit him. Two weeks later, 11 of them had developed fever and a rash; no clinicians consulted diagnosed smallpox.

One of the 11 secondary cases developed atypical haemorrhagic smallpox; he was taken from a local clinic to a larger hospital, to an ICU in a third institution, and died without a diagnosis. Two days later, the first case of smallpox was diagnosed. Now, a month after the index patient's return, 150 people were ill with the disease, and before the first diagnosis had been made. The patient with haemorrhagic smallpox had transmitted the infection and the disease, in transit through hospitals, to 38 other people.

The cases of smallpox were distributed over the country. Neighbouring countries closed their borders with Yugoslavia. The government launched a nation-wide vaccination campaign, and 20 million persons were vaccinated. Hotels and residential blocks were taken over as quarantine centres, and all people exposed to cases of smallpox interned, totalling 10,000 people who spent two weeks or more therein.

The Yugoslavia outbreak stopped 9 weeks after the index patient became ill; 175 persons had developed the disease and 35 died. And this was in a generally well-vaccinated population. One commentator noted that "it was, in fact, a small outbreak" (1).

units (in a hospital environment) will be involved and vaccines would have to be obtained from the Department of Health rapidly. In a public mortuary environment, there are usually no such on-site health advice facilities, and tissue sampling is also usually more problematic.

If the diagnosis was not suspected by the end of the gross autopsy examination, there are two possibilities:

1. The diagnosis is not made at all, no tissue having been retained and another cause of death provided
2. The diagnosis becomes evident later on histopathological and/or microbiological analyses

If only histopathology is used, it is likely to be days to weeks before the diagnosis is suspected and confirmed (the viral inclusion bodies in the skin can be confirmed by immunocytochemistry). Thus the opportunity to protect the staff by vaccination will either be lost or made perhaps too late, unless the diagnosis becomes retrospectively evident for other, clinical, reasons and/or from other similar patients.

Once the first case has been identified at autopsy, then the information will pass rapidly to all other mortuaries in the country. It is likely that only the few

designated mortuaries will then undertake even the skin sampling procedure for diagnosis in subsequent suspected cases, following a reasonable fear among the unvaccinated APT and pathology staff nationally. Unfortunately, at the time of writing, there are no such designated mortuaries in the UK; only a few anecdotally known to be capable of undertaking the relevant procedures safely.

The conclusion is that, despite preliminary planning and published guidance, the first time that such a BT attack takes place, the autopsy pathological process will be messy, probably chaotic and possibly late. The role of the medico-legal authorities has not been considered in this scenario, but as coronial jurisdictions are independent, there may be no consistent approach, at least in the initial phases of a BT attack. It will be a learning experience, and it is to be hoped that no staff become infected accidentally as a result.

Deliberate Release?

Any confirmed case of smallpox signifies deliberate release.

Anthrax

This is the only one of the Category A list pathogens yet to have been used in a BT attack (21). It is an aerobic gram-positive bacillus that is readily grown on artificial media. Once the growth is saturated, it forms spores, which also happens when bacilli in man are exposed to air. Infection is by the spores, which measure 2 μm . In nature, many animals are normally infected, form spores in the soil, where they can remain viable for decades (22).

There are three routes of infection for *Bacillus anthracis*: inhalational, cutaneous and gastrointestinal. However, the clinical presentation expands to include meningitis (half the autopsied victims of the Sverdlovsk inhalational anthrax outbreak (Box 4) also developed meningitis; the first case in the USA 2001 anthrax attack presented with meningitis). From the forensic epidemiological viewpoint (see below), an index case of anthrax meningitis should suggest deliberate release.

Clinical Presentation

For inhalational anthrax, the incubation period is usually between 2 and 10 days. The initial symptoms are non-specific fever, non-productive cough and malaise. Then sudden shortness of breath, hypotensive shock, stridor and cyanosis develop, and within a day or so death occurs, despite intensive care. Radiologically the critical finding is widening of the mediastinum and pleural effusions.

Box 4. The Sverdlovsk episode, 1979 (1, 21).

There was an accidental release of a small quantity (estimates vary from 1 to 100 gm) of anthrax spores into the atmosphere from a military bioweapons facility at Sverdlovsk (ex-Ekaterinberg) in the then USSR. Within a radius downwind of 4 km, 77 persons became ill and 66 died, from official announcements. It is suspected that more were infected, perhaps 300, with 100 deaths.

The modal incubation period (IP) was 9–10 days, but the longest IP was 43 days. Irrespective of the IP, death from inhalational anthrax followed 1–4 days from the beginning of symptoms.

Animals were affected up to a radius of 50 km from the source, causing death in sheep and cows. Anthrax spores reside viable on earth for decades.

The clinical suspicion of inhalation anthrax is raised in a previously healthy person if there is:

- Rapid onset of severe unexplained febrile illness or febrile death
- Rapid onset of severe sepsis not due to a predisposing illness, or respiratory failure with a widened mediastinum
- Severe sepsis with gram-positive rods or *B. anthracis* identified in the blood, chest effusions or cerebro-spinal fluid, and assessed not to be a contaminant.

Pathologically, this is not a pneumonia or pneumonitis, but a haemorrhagic effusion involving the mediastinal lymph nodes (hence the mediastinal widening on chest X-ray) and pleura. There is necrosis of the lymph nodes with immunoblast proliferation, abundant gram-positive bacilli, but little acute inflammation. The lung parenchyma shows oedema and haemorrhage and acute lung injury (acute respiratory distress syndrome, ARDS) in some cases. If the patient has been treated with chemotherapy for 72 h or more, the gram-positive rods may not be visible, but immunohistochemically are still evident (23).

The meningitis is also haemorrhagic. On CSF examination it is like other acute bacterial meningitides except that the gram-positive bacilli are plentiful.

Cutaneous lesions developed in half of the 2001 USA anthrax patients. These are ulcers that become eschars. The clinical differential diagnosis has included skin haematoma and non-specific erosions (23). Histologically, there is epidermal and dermal oedema and necrosis, acute inflammation and characteristic gram-positive rods; treatment will reduce their number, but immunocytochemistry will identify the antigens.

Autopsy in cases of known or suspected anthrax in the UK is discouraged (22). But if they take place, then full protective clothing and equipment are to be utilised (see below).

Deliberate Release?

This should be considered with a single confirmed case of inhalational anthrax or a single confirmed case of cutaneous anthrax in someone who does not routinely have contact with animals or animal hides, two or more cases of suspected anthrax that are linked in time and place.

Plague

Plague is caused by *Yersinia pestis*, a gram-negative cocco-bacillus. It is a zoonotic infection widespread globally (24, 25).

In the UK, the last outbreak was in 1919. Infection is usually transmitted by the bites of infected fleas, but human-to-human transmission can occur through infectious respiratory droplets. The common natural plague infection is bubonic, i.e. from fleas, with lymphadenopathy, sepsis and secondary pneumonia. Pneumonic plague is the likely outcome from BT attack, as the bacilli can be stored and aerosolised readily.

The incubation is 1–4 days, and once pneumonia has developed, transmission to others is possible from sputum; this phase could last up to 3 days after commencing antibiotic therapy.

The clinical suspicion of plague is raised by the following clinico-pathological presentations, in previously healthy persons, especially of two or more cases that are linked in time and place (24):

- Sudden onset of severe unexplained febrile respiratory illness
- Unexplained death following a short febrile illness
- Sepsis with gram-negative cocco-bacilli identified in clinical specimens

From the autopsy pathology perspective, this and tularaemia are the potential BT diseases that most mimic other septic conditions commonly seen in the mortuary (see Table 3). Other clinical patterns that could present are pharyngeal infection, meningitis and septic shock syndrome.

The gross pathology of primary pulmonary plague is acute pneumonia. Histologically there is alveolar oedema, acute inflammation and abundant bacilli. Septicaemic plague results in skin vasculitis with numerous bacilli, vascular obstruction, skin infarction and gangrene (4). Lymphadenopathic plague shows large haemorrhagic and necrotic nodes, with abundant bacilli (6).

Autopsy in cases of known or suspected plague are discouraged in the UK (24), but if the standard precautions are used (see below) the risks to staff are minimal.

Tularaemia

Francisella tularensis type B is endemic across northern Europe, but the more virulent type A strain, which would be used in BT, is restricted to North

Table 3 The pathological syndromic approach to surveillance for BT agents (expanded from Guarner and Zaki (4) and Nolte et al. (7))

Clinico-pathological syndrome (biopsy or autopsy)	Common causes, including infections of public health interest	Potential bioterrorism illness
Vesicular skin rash	Varicella, immunological blistering disorders	Smallpox
Diffuse haemorrhagic skin rash	Measles, rickettsioses, meningococcaemia, dengue, toxic shock syndrome, enterovirus, other thrombo-cytopaenias, leukaemia	Viral haemorrhagic fever, smallpox
Community-acquired pneumonia	<i>Strep. pneumoniae</i> , <i>Legionella</i> , influenza, hantavirus pulmonary syndrome, tuberculosis, other bacterial and viral pneumonias	Plague, tularaemia, Q fever
Haemorrhagic mediastinitis and pleural effusion	Carcinoma and mesothelioma, pulmonary leptospirosis	Anthrax (if gram+ve rods present, highly likely)
Sepsis syndromes, including disseminated intra-vascular coagulation (35)	Streptococcal and staphylococcal infections, meningococcaemia, malaria, leptospirosis, yellow fever, rickettsioses, tuberculosis, haemophagocytic syndrome, lymphoma, HIV	Plague, tularaemia, viral haemorrhagic fever, anthrax
Haemorrhagic meningitis	Herpes simplex encephalitis	Anthrax (if gram+ve rods present, highly likely)
Encephalitis, meningitis	Viral, bacterial, fungal and parasitic meningitis and encephalitis	Venezuelan equine encephalitis, Nipah virus
Swallowing, muscle movement, eye movement and breathing difficulties	Myasthenia gravis, Eaton–Lambert syndrome, Guillain–Barre syndrome, rabies	Botulinum toxin
Hepatitis, fulminant hepatic necrosis	HBV, HCV, septic shock	Brucellosis, viral haemorrhagic fevers
Haemorrhagic colitis	Bacillary dysentery, infarction	<i>E. coli</i> , <i>Shigella</i> , gastrointestinal anthrax
Pharyngitis, epiglottitis	Common viral and streptococcal sore throat	Viral haemorrhagic fever (Lassa)

America where it is zoonotic. It is a gram-negative bacillus, transmitted by arthropod (tick) bites, contact with infectious animal material and inhalation of infected aerosols, e.g. damp hay. The minimum infective dose may be as low as 10 organisms (26, 27).

Clinically, BT tularaemia will be pneumonic or septicaemic, and the mortality is 30–50%. Gentamicin is the treatment for cases, but for prophylaxis ciprofloxacin is indicated.

Clinical suspicion of tularaemia arises with:

- A severe unexplained febrile illness or febrile death in a previously healthy person
- Severe unexplained respiratory illness in an otherwise healthy person
- Severe unexplained sepsis or respiratory failure not due to a predisposing illness
- Severe sepsis with unknown gram-negative cocco-bacillary species that fails to grow on standard blood agar, identified in blood or cerebro-spinal fluid (26).

Person-to-person transmission does not occur in life, but transmission during an autopsy is possible. The UK HPA discourages autopsy of suspected cases, but if one is required for diagnostic or forensic reasons, then universal precautions and FFP3 mask respiratory protection as minimum is required.

Pathologically there is a pneumonia and multi-organ failure from septic shock. Histology shows necrotising haemorrhagic pneumonia and reactive necrotic lymph nodes, with abundant bacilli which can be proven to be *F. tularensis* by immunocytochemistry in fixed material if fresh microbiological studies have not already identified the infection (4). The relevant samples to take include lung tissue, blood and lymph node.

Viral Haemorrhagic Fevers

Deliberate release of these viral agents may not actually occur as they are difficult to weaponise by aerosolisation; only in experimental animal situations has aerosol transmission been proven. However, because clinical person-to-person transmission is frequent, some of them have high mortality, and when known to the public, they are included among Category A pathogens. The four agents considered are (28, 29):

- Lassa fever
- Crime/Congo haemorrhagic fever (CCHF)
- Ebola virus
- Marburg viruses

Lassa fever presents insidiously as malaise, headache and a sore throat. Ebola infection starts as an acute fever and diarrhoea (which may be bloody) and vomiting. Marburg is similar to Ebola. CCHF starts abruptly with fever and malaise. All the VHF's develop into multi-organ failure (but the lung is not usually affected) with haemorrhages from gut, pharynx and skin. The mortality rates vary with that for Ebola (50–90%) the highest.

The differential diagnosis includes falciparum malaria, yellow fever, dengue and gram-positive and gram-negative bacterial septic shock syndromes.

Treatment is with intensive care as appropriate. Ribovirin is effective in Lassa fever and might be useful for CCHF, but is ineffective in Ebola and Marburg infections.

Pathologically, the organ injury is more necrosis than inflammation, notably in the liver and kidney (30). Gut haemorrhage is common. Intra-cytoplasmic viral inclusions are seen in the liver in Ebola and Marburg (6). Immunohistochemical and electron microscopical analysis shows vast amount of virus in most organs. Skin punch biopsies also show virus with this technique, which can be used to screen potential cases without a full autopsy (4).

The UK guidance on autopsy is that these are not allowed for known or suspected VHF disease (28). This follows from guidelines issued by the Advisory Committee on Dangerous Pathogens (mainly concerned with management of living patients) which are enforceable by the Health and Safety Executive. Nonetheless cases of undiagnosed suspected imported VHF have been examined at autopsy in the UK, using proper respiratory protection; they have excluded VHF and provided other diagnoses (personal observations). The only two definite cases of VHF in the UK over the last decade were not autopsied (28).

Botulinum Toxin

The clostridial neurotoxins are among the most potent lethal substances known (31). The human LD₅₀ is about 1 ng/kg. Botulinum toxin, in nature, is usually ingested in contaminated food. It causes muscle paralysis and respiratory failure. Apart from failed attempts in Japan to use it for BT purposes, it is known that Iraq had prepared some 19,000 L of toxin, and weaponised much of it, in the 1980s.

The toxin could be disseminated either in food or liquid (e.g. milk) or aerosolised for airborne transmission. Natural toxicity results in neurological symptoms from 12 to 36 h (range 8 days to 8 days) after ingestion (32). These comprise progressive motor paralysis and difficulties in breathing. The management of cases is ventilation; vaccination is ineffective as it takes about 12 weeks to undergo. Vaccination for those predicted to be exposed is possible. The supply of antitoxin is limited everywhere. Diagnosis is currently by an in vivo mouse assay, tested against serum, faeces or respiratory secretions. Clinically the diagnosis is made by electromyography.

Differential diagnosis of botulism: this is limited to myasthenia gravis, Eaton–Lambert syndrome, Guillain–Barre syndrome and some cases of rabies.

The role of the autopsy pathologist in suspected botulinum toxin attack is limited. Gathering samples—if not already collected in life—is important, although it is unlikely that there will be detectable toxin in cases of inhalational botulism. There is also the differential diagnoses and evaluating co-morbidities that may have contributed to death.

Surveillance for Bioterrorism

There has been much discussion on the optimum means of identifying BT attacks, either before they have affected anyone or as early as possible after people become ill. The four basic modes are (1):

1. Regional syndromic surveillance for unusual diseases
 - Monitoring reported cases of fever, diarrhoea, rash, respiratory tract infections
2. Data mining
 - Analysis of school and work absentees
3. Regular air sampling from sentinel sites
 - For example, detecting anthrax and tularaemia agents in the air
4. Local reporting of unusual cases
 - For example, PUO + pustular rash = ?smallpox
 - Pneumonia + haemoptysis = ?plague
 - Fever, SOB, mediastinal widening on chest X-ray, rapid demise = ?anthrax

The present consensus is that local reporting systems are the most likely to be useful and efficient in real time for identifying BT. The pathologist has a role both as surgical biopsy and autopsy diagnostician, and the most important aspects are (i) being aware of the differential diagnoses that include BT agents and (ii) being able to pursue the possibilities so as to confirm and exclude them. Table 3 presents the commoner clinico-pathological scenarios that may present to the mortuary that include the possibility of a BT-related disease, alongside the commoner infectious and non-infectious causes of those presentations.

Needs and Standards of Autopsy Practice

In the circumstance of a pandemic bird influenza epidemic (33), it is agreed that once the initial cases have been identified from a combination of clinical pre-mortem investigation and autopsy investigation, the presenting syndrome will have been described and clarified. Subsequent cases will then be identified syndromically and will not require positive identification of the infecting agent by laboratory analysis in life or from autopsy. It is agreed that the syndromic diagnosis will suffice for medico-legal purposes and death certification.

However, deaths from proven bioterrorist attacks are homicide (7, 6), so consideration will need to be given to the degree of autopsy examination if there

are many cases; compromise may affect the stringency of evidence in individual cases required to bring a successful prosecution against alleged perpetrators at a criminal trial. Here, forensic epidemiology comes into play.

Forensic Epidemiology

Epidemiologists have been investigating the patterns and causes of outbreaks of infectious disease (and other diseases) with increasing sophistication for more than a century. But the recent concept of “forensic epidemiology” has emerged when public health investigations overlap with criminal investigations; and BT attacks are an evident cause for this. Because the training and experience of epidemiologists is quite different from that of criminal investigators, there are different priorities and thus conflicts when the two groups interact, and the new cadre of forensic epidemiologists is intended to bridge the gap. Forensic epidemiology is defined as:

the use of epidemiological methods as part of an ongoing investigation of a health problem for which there is suspicion or evidence regarding possible intentional acts of criminal behaviour contributing to the health problem (3).

In the USA, there is now a standard training programme in forensic epidemiology being rolled out (see CDC website: www.bt.cdc.gov/). In brief, the important issues when investigating an outbreak of infectious disease focus on:

1. Understanding how public health investigations proceed
 - i. Defining exposed populations
 - ii. Providing prophylaxis to exposed persons
2. Identifying the source, i.e. perpetrators or reservoir
3. Recognising that certain unusual or unnatural findings in a disease investigation may suggest intentional (deliberate) or covert action
4. Identifying procedures and mechanisms to communicate suspicions of intentionality to law enforcement officials
5. Understanding how a public health investigation differs from and is similar to a criminal investigation
6. Assessment and credibility of a threat
7. The laws surrounding entry into and obtaining samples within homes and workplaces
8. Establishing chain of custody of evidence
9. How to conduct concurrent public health and criminal investigations

A little thought about what happens during an autopsy in cases when there is no initial consideration of criminality or homicide, particularly regarding points 3, 4, 5 and 8 in the above list, exemplifies the lack of relevant training and the problems likely to ensue for pathologists in the event of a covert BT

attack that results in fatalities with no diagnosis. Further, in the UK, the general lack of training in, and experience of, infectious diseases for pathologists (as opposed to cancer, the bedrock of histopathology training) is a concern. The situation already exists whereby pathologists may refuse to undertake Hazard Group 3 infectious autopsy cases (e.g. HIV, TB, HCV) for fear of infection of themselves and their APTs. There is ethical debate as to the degree to which medical practitioners are expected to potentially put their lives at risk in managing patients. It seems likely that the continuing greater awareness of possible BT will affect the patterns of mortuary activity across the country, with such cases being more concentrated in a restricted number of centres with the resources and protocols to handle them.

UK Medico-legal Systems and Bioterrorism

Several organisations in the USA (e.g. New York City, Nov 2004) have made formal agreements for conducting joint public health and law enforcement investigations following a BT attack. The impression is that planning consideration for BT deaths is more advanced in the USA than in the UK, with a greater degree of central control and network organisation of the relevant medico-legal authorities (medical examiners and/or coroners) and laboratories. In the UK, the initial investigations of deaths will almost certainly take place under the coronial system (or procurator fiscal in Scotland), which is currently fragmented, under-resourced and non-standardised. There is statutory reform planned and it is hoped that considerations of terrorism (all forms) will feature more prominently in the contingencies and planning.

Fundamentally, if the likelihood of a BT-related infection is not highlighted at the commencement of an autopsy, there is currently no standardised approach to considering the possibility of BT and how to resolve the differential diagnosis among the pathologist community at large. Table 3 shows how similar BT infections are to many non-BT infections. It is not impossible that early cases may be missed through inadequate investigation.

Personal Protection for Pathologists and APTs During Autopsy in BT Cases

Historically, pathologists have acquired a wide range of diseases from working infected cadavers, including streptococcal sepsis, tuberculosis, tularaemia, erysipeloid fever, diphtheria, glanders, scrub typhus, systemic mycoses (blastomycosis, coccidioidomycosis), toxoplasmosis, HIV-1, hepatitis B and C, rabies, smallpox and viral haemorrhagic fever (34). Deaths among prosecutors from these infections—which include agents in the BT Category A–C lists—have occurred.

It is likely that the threat of BT will reinforce the historical trend amongst mortuary workers to utilise greater levels of personal protection against

accidental infection. Both in the USA and UK, the standard recommendation dress for all exposed staff for *all* autopsies is (20, 34):

- Surgical scrub suit
- Hat
- Water-impermeable gown covering arms, trunk and upper legs
- Plastic apron over the gown
- Respiratory protection—see below
- Eye protection
- Reinforced rubber boots
- Multiple glove layers, ideally latex gloves either side of cut-resistant neoprene glove

The respiratory protection prevents inhalation of aerosols and contamination of the mucosa by droplets. Standard surgical masks provide protection against droplet splashes, but finer aerosols readily get round the leaky sides. When there is a risk from inhaling a pathogenic aerosol, respirators that prevent nearly all particles $\geq 1 \mu\text{m}$ getting into the lungs are necessary, following guidelines (34). There are two basic types:

- The modified disposable mask (N-95 respirator in USA; EN149 FFP3 respirator in UK)
- The powered air-purifying respirator (PAPR) with high-efficiency particulate air (HEPA) cartridge filters. These are ventilated hoods that cover the head; they may be part of a whole body suit or applied on top of a separate suit. In the UK, the standard PAPR is the EN12941 with PP3 filters.

The latter provides better protection for those with beards, and also protects against chemical toxic agents. It is intuitive that PAPR would be preferred when dealing with suspected BT agents such as smallpox and VHF_s (34); but it should be emphasised that there is (as yet) no evidence base for such a statement, and there is no official public health service guidance on the issue, in either USA or UK. At present, it is a matter of preference and equipment resource. One downside to currently available PAPR is the somewhat reduced visibility of the dissection area compared with usual goggles or visor.

Generic Protocol for the Autopsy and Specimen Collection in Suspected Bioterrorism Cases (14)

1. Autopsies should be performed within 24 h of the patient's death, to increase the validity of culture and PCR results
2. Aseptic techniques must be applied as rigorously as possible to minimise contamination, from within and without the body
3. All samples should be collected in duplicate for

- Histopathology—fixed in formalin
 - Microbiology—unfixed in sterile containers, fresh and frozen
4. Normally site-sterile samples (with no commensal flora to complicate interpretation) are preferred whenever possible, as long as they are relevant to signs and symptoms displayed
 5. Tissues to be sampled
 - Local inflammatory lesions or abscesses
 - Liver
 - Spleen
 - Lung
 - Heart
 - Kidney
 - Lymph nodes
 - Bone marrow
 - Other organs with gross pathological abnormality
 6. The tissue fragments for microbiology should be 1 cc in size
 7. Samples for microbiology should be both fresh and frozen at -70°C
 8. Heart blood to be collected
 - Whole in one tube
 - Spun for postmortem serum in a separate tube
 9. Cerebro-spinal fluid to be collected—fresh and frozen
 10. Urine to be collected—frozen
 11. Faeces and gut contents to be collected, for microbe and toxin detection
 12. Cytological preparations from tissue smears done at the time of autopsy
 13. All samples to be clearly labelled with patient's name, date of autopsy and site of sample
 14. Chain of custody of sample evidence ensured

Once samples have been gathered, then microbiological analysis can be conducted through the local microbiology departments or directly with the HPA laboratory network.

This sample list is necessarily exhaustive and is hardly likely—or needed—to be followed in every instance. The nature of the case and the material actually available will determine what is taken. However, the important fact is that mortuaries undertaking autopsies of this nature must be equipped with necessary facilities and material. This includes:

- Good ventilation, lighting and water provision
- Sterile specimen containers and labels
- Formalin fixative
- Plentiful sterile instruments
- Centrifuge
- Freezer

- Storage facilities for specimens
- Anatomical pathology technologists (APTs) skilled for the task
- Appropriate personal protective equipment
- Protocols for safe practice in the mortuary
- Communication facilities for pathologist–microbiologist–other professional conversations

Disposal of the Cadaver

For all the likely used BT infections, the guidance on disposal of the cadaver is uniform (Table 4). Embalming is not to be done, and the body should be cremated rather than buried. Funeral directors must be informed about the case and associated hazards. There is no reason why relatives may not view the body after death so long as skin contact is not made if there is a possibility of skin contamination.

The degree of reconstruction of cadavers autopsied following BT infections is not defined in guidelines. But common sense indicates that for blood-borne infections the reduction of potential glove penetration by not using needle and thread is evident. As the cadavers are to be cremated, then binding them up with sticky tape is recommended.

Pacemakers, for all BT infections, should be removed (as for all cremation cases), treated with hypochlorite, bagged and disposed of (but not by incineration) (26).

Pathological Identification of BT Agents

The clinico-pathological syndrome is the starting point for consideration (4). Table 3 depicts some of the syndromes that could present as BT-related illness, with the other common causes of such disease. If a BT infection is suspected, confirming or excluding it depends on the scenario and what material is available. Fresh tissue for microbiological analysis is obviously optimal. But formalin-fixed, paraffin-embedded material can be precisely categorised in many cases, using H&E, empirical special stains, immunocytochemistry and (potentially) molecular diagnostics.

H&E stains identify viral inclusion bodies, but do not necessarily specify them. The histological special stains most useful in evaluation of BT infections and their differential diagnosis are: Gram, Grocott silver, Ziehl-Neelsen and Warthin-Starry (6). Immunocytochemical antibodies are not generally available for specifying the relevant infectious agents, at least in the UK. The USA Centers for Disease Control, however, does maintain a large panel of antibodies that can reliably identify most of the BT agents (and all those in Category A) (4). Molecular technology, including PCR, is in its infancy as regards identification

Table 4 Summary checklist of relevant issues if an autopsy is performed on a Category A infection case

	Anthrax	Smallpox	Plague	VHF	Tularaemia	Botulinum toxin
HEPA respiratory protection	Yes	Yes	Yes	Yes	Yes	No
Samples for microbiology	Lung, pleural fluid, spleen, lymph node	Skin, blood	Lung, spleen, lymph node	Blood, liver, spleen	Lung, spleen, lymph node	Blood, faeces
Samples for histopathology	Standard set	Standard set including skin	Standard set	Standard set	Standard set	Standard set
Vaccinate staff	No	Yes	No	No	No	No
Antibiotic prophylaxis for staff, assuming universal precautions used during autopsy	Yes	No	Yes	No. Only if direct skin contact with infected material	Yes	No
Antitoxin for staff	n/a	n/a	n/a	n/a	n/a	No
Decontaminate body and surfaces with hypochlorite	Yes	Yes	Yes	Yes	Yes	No
Embalm body	No	No	No	No	No	No
Cremate body	Yes	Yes	Yes	Yes	Yes	Yes
Autoclave instruments	Yes	Yes	Yes	Yes	Yes	No

(with high sensitivity and specificity) of BT-related infections in fixed material; but it is increasingly the gold standard for microbiological identification in fresh material (5).

Summary

Bioterrorism attacks have not yet occurred in the UK, but are possible and considered likely in some quarters. Patients will present live and dead, and pathologists (and APTs) are therefore in the front line of exposure. This chapter has depicted some of the infections considered more likely to be used in BT, what they look like clinico-pathologically and how pathologists can approach the differential diagnoses. Health and safety issues are also highlighted. It is possible that the first episodes of fatal BT in the UK will be managed erratically and much will be learned from the experience. More robust and all-embracing guidelines can then be produced.

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