

Bioterrorism



Lucy Harper discusses the use of infectious biological agents in warfare

IN 1346 TARTAR warriors camped outside the city of Kaffa in the Black Sea in an attempt to besiege it. A large percentage of the population at this time were carrying the 'black plague' — the Tartars were no exception and it wasn't long before the disease had spread through the besiegers. In an attempt to end the siege by spreading the disease, the Tartars catapulted plumed body parts over the city walls. Much later, during the battles of 1754-1767, French and British soldiers unwittingly infected American Indians by donating blankets used by settlers suffering from smallpox. The native tribes were soon killed off.

These are just two of the first recorded examples of the use of infectious biological agents in warfare. Since then, although the number of agents of biological warfare has not increased a great deal, the potential danger posed by such agents when used as weapons, remains high. Anthrax, smallpox, and botulism, have all been used as biological weapons. Despite the fundamental differences between these organisms, they exhibit some similar properties that make

them ideal candidates for biological attack. Due to the apparently inoffensive nature of their spores and / or the incubation times of these organisms, an attack is generally not easily detected until the bugs have infected substantial numbers of people. Once the organism has been identified within a population it can still take the community years to recover from such an outbreak. As such, enemies can deploy biological weapons to great effect.

Bacillus anthracis, the bacterium which causes anthrax, can be acquired through direct contact with the skin (cutaneous anthrax), through inhalation (pulmonary anthrax) and through gastrointestinal infection (gastrointestinal (GI) anthrax). The most often fatal forms are GI and pulmonary anthrax primarily because they are not generally detected until it is too late for effective treatment.

Cutaneous anthrax is the most common form of human-acquired anthrax, and is usually acquired by the handling of infected animals. A break in the skin allows the organism access and it causes a primary lesion within 2-7 days after exposure. The

primary lesion leads to the development of a characteristic ulcerated black lesion. This usually clears up without treatment but in a small amount of cases can progress to septicemia and ultimately death.

In Pulmonary anthrax, *B. anthracis* spores are inhaled and transported to the lymph nodes inside macrophages, where they germinate and the bacteria multiply. Death occurs in >80% of the infected population as a result of subsequent bacteraemia and toxemia.

GI anthrax is an extremely rare form of anthrax that results from eating insufficiently cooked meat from anthrax-infected livestock. Germination occurs within the macrophage and stimulation of a toxin known as Lethal Factor (LF) ensues. Lethal Factor then stimulates the production of TNF- α and IL-1 β . These cytokines are then released into the bloodstream through the destruction of macrophages by LF and death results due to a combination of bacterial infection of the bloodstream and cytokine-induced shock (1).

There are no known cases of human-to-human

transmission of anthrax, though death through the acquisition of inhalation anthrax results after 1-2 days after onset of symptoms. The rapid course of this disease reinforces the necessity for early therapeutic intervention (2). Information regarding the potential impact of a malicious attack of anthrax is limited as there have been few recorded incidents. In 1979 there was an accidental release of *B. anthracis* from a Russian bioweapons factory and we all remember the US anthrax 'attack' of September 2001. However, these incidents give very little information regarding an appropriate coping strategy should a widespread attack be instigated (3).

Smallpox (or *Variola*) is a member of the orthopoxvirus genus which includes monkeypox, vaccinia and cowpox. Its incubation period is normally 12-14 days and it is the only orthopox virus which is readily transmitted from person-to-person. Initially the patient experiences high fever, exhaustion, headache and backache. Severe abdominal pain and delirium may also be present at this stage. A rash then appears on the mucosa of

the mouth and pharynx, face, and forearms and spreads to the trunk and legs. Within 1-2 days the rash becomes vesicular and later pustular, with crusts developing on the 8th or 9th day of the rash. Virus titres are highest during the first week of illness and death from the toxæmia associated with circulating immune complexes and soluble variola antigens, is most likely during the second week of illness. Encephalitis may also occur (4).

In 1796 it was found that infection with cowpox protected against smallpox, but it wasn't until 1967 that the world health organisation (WHO) began a global campaign to eradicate the disease. This was declared a complete success in 1977. In 1980 the WHO recommended that all countries cease vaccination and that all labs destroy stocks of variola or transfer them to named reference laboratories. In 1999 the WHO recommended that all stocks be destroyed, however the institute of medicine (IOM) wished to maintain stocks for research purposes (4).

In response to the terrorist attacks of September 2001, the US planned to vaccinate 500,000 health workers against smallpox, but unexpected health problems meant that by the end of the programme only 39,000 had been vaccinated (5). Because the use of smallpox vaccine is contraindicated in so many cases, scientists are on the look out for alternatives. The CDC (Centers for Disease Control and Prevention) are now recommending cidofovir, a nucleotide analogue under investigation for the treatment of genital warts and CMV retinitis, be investigated as treatment for smallpox. However its use is limited as it is 'virtually not available by the oral route'. A lipid tail has been attached onto cidofovir

producing hexadecyloxypropyl-cidofovir (HDP-cidofovir), a cidofovir-derivative which is available orally and once administered penetrates cells more effectively than cidofovir (6).

At a conference in Geneva during October 2003, a number of leading experts in the field of bioterrorism put forward a number of differing opinions regarding the preparative measures and handling of potential biological threats, including smallpox. Ken Alibek is a professor at George Mason University, Fairfax, Virginia. He previously headed the former Soviet Union's secret biological weapons programme which, in 1980 embarked upon a programme to produce smallpox in large quantities and adapt it for use in bombs and intercontinental ballistic weapons (4). In 1992 he defected to the United States where he became president of Hadron Advanced Biosystems. He now leads research into developing new forms of medical protection against biological weapons (5). He '*wanted the conference to show that despite eradication in the late 1970s, smallpox was still a serious threat.*'

However, Peter Jahrling, principal scientific adviser to

the US Army Medical Research Institute of Infectious Diseases, told journalists that he '*was in favour of mass vaccination of populations in advance of any threat.*' Whereas David Heymann, a WHO infectious diseases expert said that '*WHO recommend mass vaccination in the event of an outbreak*' (5). Mr Jahrling added '*one of the problems in vaccinating health workers was the danger of infecting cancer patients, for example, through the vaccine (which is the related virus Vaccinia) for days afterwards.*' But Dr Bill Bicknell, a professor at the Boston University School of Public Health, argued that '*recent research finds negligible risk in vaccinating healthy adults, as opposed to children and sick adults, with existing vaccines.*' He rejected the WHO's four day window recommendation, saying: '*If (you are) vaccinated within four days, the severity of disease is less and you probably don't die, but you do not prevent the disease and you may transmit more disease*' (5).

Botulism is caused by infection with the anaerobic Gram-positive bacterium *Clostridium botulinum*.

Onset of botulism following inhalation of *Clostridium Botulinum* spores is dose-dependent and may occur 24hrs to several days after exposure. The first manifestations tend to be ophthalmological and include double vision, drooping of the upper eyelid, excessive dilation of the pupil, extraocular muscle palsies and photophobia. Other early signs of infection include slurring of speech, dysphonia and difficulty in swallowing. These signs are followed by a progressively descending symmetrical, flaccid paralysis which may culminate in respiratory failure. It is known that if not diagnosed and treated promptly, botulism has significant morbidity and mortality.

Clostridium Botulinum spores are widespread and are found in soil, and mud (salt-water and fresh-water). They are resilient and can live for up to 2 hours at 100°C. The neurological symptoms stem from the fact that *Clostridium Botulinum* spores trigger the production of a neurotoxin which inhibits acetylcholine release at pre-synaptic nerve-endings. Like many agents used as biological weapons, the ease of administration of minute yet lethal doses (by inhalation), together with its colourless and odourless nature, make botulinum toxin a highly effective chemical weapon. In 1995 Iraq revealed it had deployed over 11,000 litres of botulinum toxin into SCUD missiles. Also in 1995, the *Arum Shinrikyo* cult in Japan, prepared botulinum toxin before its attack on the Tokyo subway system but, despite the fact that BT is 100,000 times more toxic than Sarin, it was this chemical warfare agent they decided to employ (7). So what are the coping strategies that we should adopt to counter these threats? ▽



Should the government make guidelines available outlining the most appropriate course of action? One might think this would be sensible. For example, a scientist in Texas was arrested for misinforming the FBI regarding vials of 'plague' (*Yersinia pestis*) which he claimed had 'gone missing'. A top security alert involved the FBI who later accounted for the missing vials claiming, "there is no danger whatsoever". However, the investigation continues.

This case highlights the requirement for governmental intervention and in June 2002, President Bush signed the *Bioterrorism preparedness and Response Act 2002*

(BPRA) in the USA. This report is currently being implemented. Although this outwardly appears to be the sensible thing to do, it raises the question of whether it will inhibit research into the long-term effects and treatment of infection with these agents. For example BSE prions are among a group of 'select agents' that, under the BPRA, require security checks for those who are handling them – including any person who has access to them in the lab (8). There is speculation over whether such restrictions place lab research at risk as they have been known to delay the certification of certain laboratories. This may push workers into making the

information available freely, for example on the Internet. This may bring into question the quality of the research in the absence of peer-review, but more importantly it allows the work to be made available to all. Most scientists would see this as a benefit, but it

may also result in information getting into the wrong hands, negating the whole principle of employing the BPRA in the first place.

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References

1. Baillie & Read (2001) *Current Opinion in Microbiology* **4**: 78-81.
2. Dixon, *et al.*, (1999) *NEJM* **341**:11;815-826).
3. Inglesby *et al.*, (2002) *JAMA* **287**:1:2236-2252.
4. Henderson *et al.*, (1999) *JAMA*:**281**:2127-2137.
5. Fleck (2003) *British Medical Journal*. **327**:948.
6. Morris (2002) *The Lancet Infectious diseases* **2**:262.
7. Caya JG (2001) *Survey of Ophthalmology*:**46**:1:25-34.
8. *The Scientist*, January 2004.