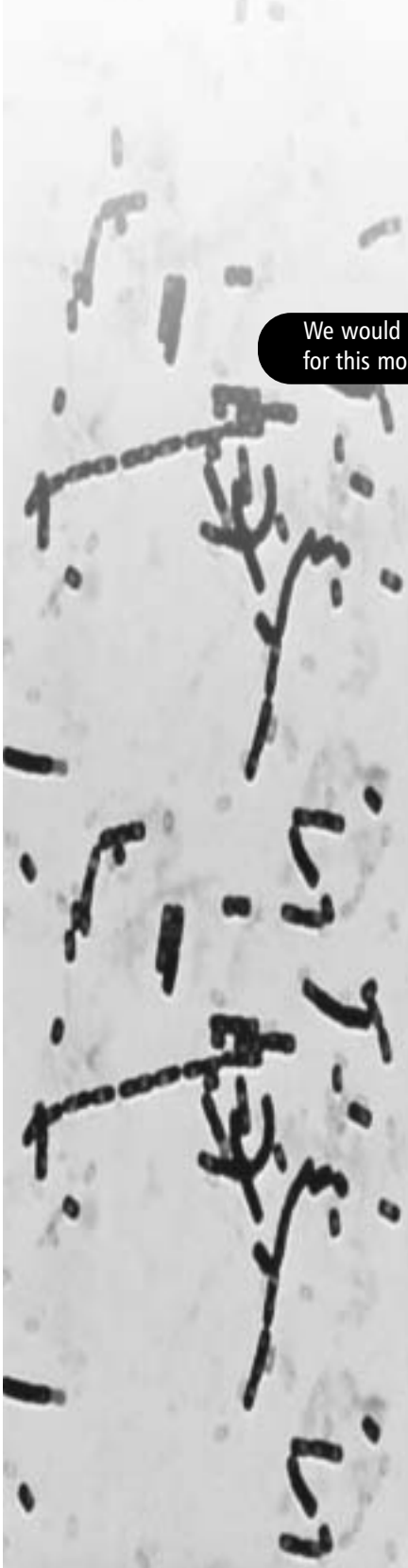


In response to recent events  
**Pamela Hunter** reviews what  
we know about this intriguing and  
historical disease

# *Bacillus anthracis*

The recent outbreak of human  
**anthrax** infections in the USA,  
apparently caused by the  
deliberate spread of spores, has  
made all microbiologists and  
infectious disease specialists rush  
to renew their knowledge

We would like to extend our grateful thanks and appreciation to **Pamela Hunter** for this most interesting and informative article



**A**nthrax has caused disease in animals and humans for many centuries. The common name is derived from the Greek word 'anthrakis', or coal, so named from the black cutaneous lesions produced. The disease anthrax is caused by the sporulating bacterium *Bacillus anthracis*, found in the soil in many parts of the world. The spores from an infected animal carcasses can contaminate the pasture for any decades and lead to further sporadic outbreaks.

Naturally acquired anthrax in humans is generally due to contact with infected animals or their carcasses. Although fatal human disease may occur, *B. anthracis* is not highly virulent, and in spite of its previous prevalence in the environment and animals, human infections were not common and are now rare.

Virulence is associated with encapsulated strains and death is caused by toxins which produce massive haemorrhaging and shock. The most common form of the disease is cutaneous, which is frequently self-limiting. To produce the far rarer and serious form of inhalation anthrax, a large number of spores, greater than 2000, and possibly 5-10,000, are necessary to establish an infection.

The genome *B. anthracis* has been sequenced recently and considerable advances have also been made in understanding the mode of action of the toxins. The plasmids coding for toxins (pX01) and the capsule-associated plasmids (pX02) have also been identified and sequenced.

**History** It is believed that the fifth and sixth plagues of Egypt, described in the Old Testament, were probably caused by anthrax, and also the descriptions of the 'black bane' which caused an estimated 60,000 deaths in cattle in Europe in the 1600s, indicate that this was anthrax.

Two giants of early microbiology, Robert Koch and Louis Pasteur both studied anthrax in the 1870s. Koch demonstrated that the disease could be produced by the transfer of infected material from one animal to another. Pasteur continued this work, confirming the 'germ theory of disease' by using membrane filtration to prove that it was the bacilli that transferred the disease.

Koch also demonstrated the ability of the bacillus to produce heat resistant spores, which had the ability to germinate and infect animals. He thus explained what had been long been a mystery; why animals continued to become infected long after infected animals had died and been removed from the pasture. Pasteur demonstrated in 1881 in a famous public experiment that cows, goats and sheep could be 'vaccinated' by use of a live attenuated strain. Soon after, vaccination of farm animals became commonplace and has remained so. The current vaccine is a live vaccine made from an unencapsulated, avirulent but toxigenic strain.



**The organism** *B. anthracis* is a large (1 x 3µm), Gram-positive, aerobic, rod-shaped, non-motile, sporulating bacillus. The spores are central and thermostable. Like many other members of the genus *Bacillus*, the spores are remarkably resistant and long-lived in the environment in the environment. The organism grows rapidly on normal laboratory media (nutrient or blood agars) not needing any special cultural techniques, but does not grow on MacConkey agar.



There are a number of morphological distinguishing characteristics; white-grey, flat or slightly convex, irregular round colonies with a ground-glass appearance. A 'Medusa head' colony is characteristic of *B. anthracis*. Cultures are not or very weakly haemolytic, but not ?-haemolytic. In smears from infections, long chains may be formed and a capsule is present. The capsule can be seen clearly using India Ink. The lack of motility distinguished *B. anthracis* from many other *Bacillus* species.

Modern molecular techniques can be used to confirm strains of *B. anthracis*. Multiplex PCR has been used in recent outbreaks and immunohistochemical staining can be valuable.

**Occurrence** The disease is naturally one of animals, particularly herbivores, who ingest spores on the grass and from the environment. Until the advent of an effective vaccine, the disease was relatively common in cattle, sheep, goats, horses and pigs, but is now far rarer. Infections in humans were most often associated with the handling of infected animal carcasses or hides. 'Wool sorter's disease' and 'Tanner's disease' was contracted by those handling infected fleeces or hides, often from inhaling sores released in the processing of the fleeces or hides. Anthrax is still endemic in some parts of the world, notably Africa, the Middle East, Asia and some parts of the US and Australia.

Anthrax is now a rare disease with only 16 cases reported in the UK between 1980 and 2000. All were cutaneous cases, mostly associated with people working with bonemeal, animal carcasses or skins.

A human case occurred in North Dakota, USA, in 2000, the first since 1992. The case was associated with an epizootic among livestock. The man had handled cows dying of anthrax, and although he had worn leather gloves, he subsequently developed a lesion on his face, characteristic of anthrax. The disease was mild and responded to ciprofloxacin therapy. For several months during 2000, there were cases of anthrax among livestock on 31 farms in the area, compared with only two during each of the preceding 40 years.

Inhalation anthrax is extremely rare under normal conditions, the last death reported in the UK was in 1974 and that was the first since 1965. In the US, even though anthrax is endemic in several states, only 18 cases have been reported in the 20th Century, the last in 1976.

**Human disease** The commonest form of the disease, occurring in up to 95% of cases, is cutaneous. It is seen most often on the face, hands, forearms and neck since infection is generally from handling infected material. The organisms, usually spores, invade from a skin abrasion or cut. After germination of the spores, the vegetative bacilli multiply locally and a small, visible papule appears. This develops into a characteristic black, painless eschar with surrounding oedema.



In most cases cutaneous anthrax is self-limiting, the lesion resolving within 10 days. In some cases systemic anthrax may develop, but unless therapy is initially very late by which time extensive toxin production has occurred, the disease responds well to chemotherapy. In untreated cases the death rate can reach 20%, but is less than 1% when treated. Inhalation anthrax is rarer, comprising only approximately 5% of cases. Once established, however, this is a far more serious disease with a higher death rate. The organisms do not multiply in the lungs but are carried by alveolar macrophages to the mediastinal lymph nodes where the spores germinate and multiply. From this focus they are able to spread rapidly throughout the body. Initial symptoms are insidious and flu-

like, generally mild and non-specific. In the second phase there is acute respiratory distress, sepsis and an acute haemorrhagic mediastinal widening. X-ray symptoms can be confused with those of tuberculosis. Blood culture may be positive at this stage, and if the disease has progressed to this stage, it is frequently fatal as toxin production has already advanced and therapy can thus be ineffective.

Gastro-intestinal anthrax is even rarer and is contracted by consuming large numbers of spores, which infect the intestinal tract or the oesophagus. Unless there is a suspicion of anthrax, the difficulties in distinguishing these forms from other possible disease means that, like in the case of inhalation anthrax, treatment may often be ineffectual. Meningitis is a rare complication of any form of anthrax, and is generally fatal since, by the time it is diagnosed, toxin production is well advanced.

**Toxin production** Three toxins are produced by *B. anthracis*, all thermolabile proteins; a protective antigen (PA, 83kDa), an oedema factor (OF, 89kDa) and a lethal factor (LF, 90kDa). The individual toxins have no adverse effects, they need to produce the characteristic toxicity seen in the disease. They target the macrophages and have little effect on other cells. The protective antigen binds to receptors on the mammalian (macrophage) cell surface and is cleaved by a cell-surface protease. This produces a cell-bound C-terminal 63kDa protein (PA63). This cleaved portion, PA63, has high affinity binding sites for the other two toxins. The bound complex enters the cell by receptor mediated endocytosis. The OF toxin has calcium and calmodulin-dependent adenylate cyclase activity. The LF toxin is a protease which interferes with the protein kinase signal transduction pathway. The assembled toxin has powerful proteolytic activity which causes the eventual death of the macrophage. The release of cytokines, including IL-1 and tumour necrosis factor, from the damaged macrophages is believed to cause the damage to the blood clotting system and to contribute to the septic shock and oedema.

### **Treatment and susceptibility to antibacterial agents**

Most strains of *B. anthracis* are susceptible to a wide range of antibacterial agents. The organism was traditionally highly susceptible to penicillins and, until recently, these were the drugs of choice. Wild-type strains often produce a constitutive cephalosporinase. Penicillin-resistance has been noted, albeit rarely, in addition, strains with resistance to penicillin are believed to have been

engineered during the 'cold war' era. For these reasons, the first choice is now generally a fluoroquinolone, ciprofloxacin, although other fluoroquinolones are probably also effective. Most authorities still recommend that therapy be switched to a penicillin (penicillin G, penicillin V, ampicillin, amoxicillin) if antibacterial-susceptibility is confirmed. Alternative drugs include doxycycline, minocycline, erythromycin, clindamycin, vancomycin and chloramphenicol. Cotrimoxazole is not active and 3<sup>rd</sup> generation cephalosporins are not recommended.

### Current dosages recommended in most countries are as follows:

**Penicillin G** (procaine) 600,000 units intramuscular every 6 or 8 hours  
**Penicillin V** 500mg orally every 6 hours  
**Amoxicillin** 500mg orally 3 times a day  
**Doxycycline** 100mg orally 3 times a day  
**Ciprofloxacin** 500mg twice a day

For localized or uncomplicated cutaneous anthrax these doses are normally given for 7 days. The therapy is recommended to continue for 60 days for suspected or known inhalation anthrax to protect against the possibility of delayed germination of spores. High intravenous doses are recommended for any cases of systemic anthrax, penicillin plus streptomycin is often suggested. In the recent American cases of inhalation anthrax ciprofloxacin and rifampicin are being used together with clindamycin, penicillin or vancomycin (See Table 1).

Fluoroquinolones and tetracyclines are not normally recommended therapy for children and should thus be used with caution. Therapy should be switched to penicillins or alternatives as soon as possible.

Antimicrobial susceptibility of 11 of the 22 isolates (as of October 24th) from the recent American cases of anthrax have been reported from the Centers for Disease Control (CDC).

The strains have all proved susceptible to ciprofloxacin, penicillins, tetracyclines, clindamycin, imipenem and clarithromycin, but erythromycin and azithromycin were less active (See Table 1).

**Vaccination for humans** An absorbed culture filtrate vaccine became available for humans some four decades ago and is generally used for those likely to be at high risk of exposure, including veterinarians and military personnel.

Protection is not long lasting and repeat vaccination is advised. Local reactions may occur. Because of the rarity of the naturally occurring forms of the disease, however, there is little information on the degree of protection afforded against inhalation anthrax. Supplies of this vaccine in the US are reputed to be scarce, with only one company producing it. This company has had problems and is awaiting clearance from the FDA to commence large scale production. A live, toxigenic unencapsulated avirulent vaccine is available for animals, but this is not regarded as sufficiently safe to give to humans, although a live vaccine is apparently in use in Russia. Several groups are working on the development of new vaccines, but most are still at the experimental stage.

**Anthrax as a weapon** As has been illustrated so clearly in the US recently, anthrax can be used as a weapon of terror.

It is one of the favoured species for 'germ warfare' or bioterrorism, but is not necessarily an ideal one. It is not highly pathogenic, requiring a large number of spores to produce infection by any route. It is not transmitted from person-to-person other than in exceptional circumstances. The spores need to be transmitted in a very fine aerosol to be highly effective. To do this, very fine milling and the use of anti-caking agents are required to prevent the spores clumping. Those known to have been exposed can be protected by immediate chemoprophylaxis.

Nevertheless, as has been illustrated so graphically in the US recently, it can be the cause of great 'nuisance' and fear. If spores have been spread over a large area, because they are so resistant and long lasting, disinfecting a contaminated area is not easy.

## Table 1

### Antimicrobial Susceptibility of 11/12 isolates of *B. anthracis* (as on 24 October 2001 - MMWR Vol 50; No 42, 909)

MICs (mg/l) are reported for the following drugs:

#### 'Susceptible'\*

Doxycycline  $\leq$  0.03, Tetracycline 0.06  
 Ciprofloxacin  
 Amoxicillin  $\leq$  0.06, Penicillin G < 0.06 - 0.12  
 Rifampicin  $\leq$  0.5  
 Clarithromycin 0.25  
 Clindamycin 0.5  
 Vancomycin 1-2  
 Chloramphenicol 4

Imipenem (fewer isolates tested) < 0.12

#### 'Intermediate'\*

Erythromycin 1  
 Azithromycin 2  
 Ceftriaxone 16

\*In the absence of susceptibility for *B. anthracis*, breakpoints were used for *S. aureus*.

Preliminary studies report the presence of a Class B cephalosporinase (constitutive) and the possibility of an inducible penicillinase.

#### Therapy of current inhalation cases:

Combination intravenous treatment with  
 Ciprofloxacin and rifampicin  
 plus clindamycin  
 or plus vancomycin  
 or plus penicillin

A number of countries have attempted to develop anthrax as a bioweapon during the 20th century. The Germans were believed to have deliberately infected large numbers of cattle, goats and sheep in the first World War and a major outbreak of anthrax in Iran, which killed 1 million sheep was widely rumoured to have been part of a weapons programme. During the 2nd World War the Germans were believed to have developed anthrax as a weapon, and to investigate how effective a weapon anthrax could be, the British Government deliberately infected a small uninhabited island, Gruinard, which lies off the Scottish coast, with anthrax spores. This illustrated the longevity of the spores, as over four decades later the soil contained fewer, but still unacceptably high concentrations of viable spores. At this stage, the soil was decontaminated successfully with formaldehyde, which is sporicidal. Such a task is not always practical and illustrates how

disruptive and effective the blanket use of a finely-dispersed aerosol of an-thrax spores could be as an offensive weapon.

Later, during the 'Cold War' era, according to Dr Alibek, a top Russian bioweapons expert who defected to the US, Russia developed highly sophisticated ways of using anthrax as a weapon. The accidental release of finely aerosolised spores from an extractor unit in one of the bioweapons sites, Sverdlovsk, in 1987 resulted in the death of over 60 civilians in the vicinity.

**What of the future?** It is clear from the recent outbreaks in the US that even if the delivery of anthrax spores has not been optimized, such attempts can pose a serious threat. In the early stages of an attack, if anthrax is not suspected, and if sufficient spores have been inhaled, fatal inhalation anthrax can occur. If the suspicion of anthrax

is high and cases or those potentially exposed are identified rapidly, the disease is normally highly susceptible to a wide range of antibiotics. It is encouraging to hear that out of ten people in the US with confirmed inhalation anthrax, six have survived, two of whom have been discharged from hospital. Rapid identification of the disease proved to be a key factor in their survival. As inhalation anthrax has many symptoms in common with influenza-like illnesses, CDC have issued guidelines to aid in distinguishing between the conditions (See Table 2). Resistance can be developed experimentally to most of the current agents of choice, and it remains to be seen whether widespread use of ciprofloxacin and doxycycline will create a selective pressure on the organism. Vaccination is not a viable option currently for large numbers of people and supplies are not plentiful. In addition, the strain employed in the bioterrorist attacks in the US is thought to be the Ames strain, used originally in the US and the UK for their 'Germ warfare' programmes. Vaccination is not thought to give full protection against this strain. Better methods of protection from the lethal effects of the toxins are required and various recent approaches may hold out some promise. A DNA-based vaccine has proved itself to be of value in experimental work in mice. Plasmids coding for the PA and LF toxins were used by Galloway et al. to immunise mice who were able to survive over five times the usual lethal dose of anthrax. (*Infect Immun* 2001; 69:4509). Work published recently by Mourez et al. describes the identification of a peptide that binds to PA63. Multiple copies of this peptide were produced which functioned in vitro to prevent the binding of OF and LF to PA63. This peptide has also protected rats from a high dose of anthrax toxin. (*Nature Biotechnology* 2001; 19:958). An alternative approach has been described by Waters et al. who have isolated a gene, Kif1C, which encodes a protein responsible for organizing the movement of proteins within the microphage. By modifying this gene they have been able to protect mice from lethal effects of anthrax toxin. (*Current Biology* 2001; 11:503). New methods of identifying strains can help in tracing the source of infection. A new technique (multilocus variable number tandem repeat analysis) can produce a genetic fingerprint of strains. Other approaches include the development of a mass spectrometer which can distinguish between anthrax and related species rapidly.

*Pamela Hunter*  
Medical Writer

**Table 2**

**Summary of the Current Situation in the US From Morbidity and Mortality Weekly Reports (MMWR) up to 9 November**

<b>Total numbers of anthrax cases</b>	(as of 9th Nov)	22
Inhalation anthrax	Confirmed	10
Cutaneous anthrax	Confirmed	10
Cutaneous anthrax	Suspected	5
Number of deaths: 4	(all inhalation anthrax)	

Potentially exposed persons who received initial prophylaxis: 32,000  
Numbers who continued with prophylaxis (60 days): 5000

**Important clinical characteristics of inhalation anthrax:**

- total WBC normal or slightly elevated ( $7.5-13.3 \times 10^3 / \text{cumm}$ )
- increased % of neutrophils or band forms
- abnormal radiographs in 10/10 - but 2/10 needed very careful examination to detect the mediastinal widening
- pleural effusions present in 7/10 including the 2 patients with only marginal mediastinal widening
- blood cultures positive in 7/10
- if already receiving antibacterial therapy, cultures may be negative
- in those with negative blood cultures, PCR, immunohistochemical staining or a four-fold rise in IgG can confirm anthrax

**Important features in distinguishing inhalation anthrax from influenza-like illnesses (ILI)**

	<b>Anthrax</b>	<b>ILI</b>
Abnormal chest radiograph	10/10	<5%*
Shortness of breath	8/10	6%
Nausea and vomiting	8/10	12%
Sore throat	2/10	>60%
Nasal congestion & rhinorrhea	1/10	70-80%

\*pneumonia more common in the elderly