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**Guidelines for the Surveillance and Control of Anthrax in
Human and Animals. 3rd edition.**

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Guidelines for the Surveillance and Control of Anthrax in Humans and Animals

THIRD EDITION

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Figures and Diagrams

Figure 1

Cycle of infection in anthrax. The spore is central to the cycle, although infection can also be acquired through uptake of the vegetative forms when, for example, humans or carnivores eat meat from an animal that died of anthrax or when biting flies transmit the disease.

Figure 2

Importation from endemic to other countries and cycles within the importing country.

Figure 3 see Figure 1.

Figure 4

Pictures featured in a book and on a poster conveying the story of the problems the Liseli family suffered following the death of their cows from Anthrax.

A.1.1.4.1 Flow diagram of suggested procedure for isolation and identification of *B.anthraxis* and confirmation of diagnosis.

A.1.1.4.2 Simplified version of A.1.1.4.1 (following Route B for old animal specimens or environmental samples).

Figures: front cover

Terminal haemorrhage from the nose, mouth and/or anus following sudden death is characteristic of anthrax and plays an essential role in the cycle of infection of *Bacillus anthracis* (see Figure 1, p.2).

Top left figure (cow) kindly supplied by Mr Nigel Durnford, Trading Standards Department, Gloucestershire County Council, Gloucester, UK.

Figures top right (zebra), bottom left (springbok) and bottom right (elephant) reproduced from Culture 1997; 18 (2): 5-8, by permission of Euromed Communications Ltd, Haslemere, UK.

Figures A-F

Confirmation (see Appendix I [A.I.1 and A.1.2]).

- A** Spores and vegetative cells of *B. anthracis*. This species belongs to *Bacillus* species morphological group 1; the spores are centrally placed in the sporangium and do not cause it to swell.
- B** Capsulated *B. anthracis* in the blood of an animal that has died of anthrax stained with polychrome methylene blue (M'Fadyean stain). *In vivo*, the bacilli are in short chains (*in vitro*, they form endless strings). The clearly demarcated capsule surrounding dark blue "box-car" bacilli in blood from a dead animal is a definitive diagnostic confirmation of anthrax (unstained "ghosts" around bacterial cells are not acceptable for diagnosis and may be putrefactive organisms).
- C** Colonies of *B. anthracis* on selective PLET agar after 42 hours at 37 °C. The colonies are "bee's-eye" in appearance: dome-shaped with a matt texture but not dry.
- D** On blood agar, the colony is non-haemolytic and characteristically tacky;

it can be teased up in the manner shown with an inoculating loop. Note also the curly tails sometimes seen with *B. anthracis* colonies.

E *B. anthracis* (east and west) and two non-anthrax *Bacillus* species (north and south) on blood agar. *B. anthracis* is non-haemolytic and sensitive to the diagnostic "gamma" phage and to penicillin. Note the haemolysis around the growth of the top culture and that neither top or bottom cultures show sensitivity to phage or penicillin.

F PCR is useful for confirming the virulence of an isolate. Lanes 2 and 6 show the presence of both capsule and toxin genes, representing fully virulent *B. anthracis*. Lane 3 shows the presence of the toxin gene only; the *B. anthracis* would be similar to the Sterne vaccine strain 34F2. Lane 4 shows the presence of the capsule gene only; the *B. anthracis* would be similar to the Pasteur vaccine strain. The DNA in lane 5 lacks both genes. Lane 7 is a water control. (Lanes 1 and 8 are molecular weight markers). (See Appendix I [A.I.6]).

Figures A and F kindly supplied by Jane Bowen, CAMR, Porton Down, UK.

Figure D kindly supplied by Mr R. Charlton, Bulawayo, Zimbabwe; reproduced from Topley & Wilson's *Principles of Bacteriology, Virology and Immunity*, 8th ed, Vol 2, Figure 9.7, by permission of Edward Arnold/Hodder & Stoughton.

Figures G–L (see Section 8.1.3 and Appendix III [A.III.3]):

G to J show incineration of an anthrax carcass using a commercial incinerator. Note in G the bag to prevent further spillage of blood from the nose and mouth and that, in H, incineration is from underneath the carcass. Note also in J that the ground underneath the carcass has been well-scorched.

K and L show an alternative approach to on-site incineration using down-directed blow-torches.

Figures G–J kindly supplied by Mr Nigel Durnford, Trading Standards Department, Gloucestershire County Council, Gloucester, UK;

Figures K–L kindly supplied by the Notifiable Diseases Section, Ministry of Agriculture, Fisheries and Food, Tolworth, UK.

Figures M–R

Stages in the development and resolution of cutaneous anthrax lesions (see Section 4.4.1).

M As first seen (day 1)

N On day 2 or 3

O Day 4

P Day 6

Q Day 11

R Day 150. Resolution of all but the most severe lesions is usually complete without surgical intervention, leaving only light scarring. Sensitive areas, such as the eyelid, may need surgical attention.

Not visible in these pictures is the extensive accompanying oedema which is an important diagnostic sign, and can be life threatening from the risk of asphyxiation when the lesions are on the face or neck.

Figures M, N, P, R kindly supplied by Dr W.E. Kobuch, 07-Gynecologie Obstetrique, Toulouse, France. Figure P reproduced from Figure 15-1 of *Medical Microbiology*, 4th ed, University of Texas Medical Branch, Galveston, USA, by permission of the editor.

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1 The disease and its importance

Anthrax is primarily a disease of herbivores although few, if any, warm blooded species are entirely immune to it. From earliest historical records until the development of an effective veterinary vaccine mid-way through the present century (Sterne, 1937; Sterne *et al.*, 1939), together with the subsequent advent of antibiotics, the disease was one of the foremost causes of uncontrolled mortality in cattle, sheep, goats, horses and pigs worldwide. Humans almost invariably contract anthrax directly or indirectly from animals. Today the Office international des épizooties, Paris, France (OIE), reports (OIE, 1997a) show that the disease is still enzootic in most countries of Africa and Asia, a number of European countries and countries/areas of the American continent and certain areas of Australia; it still occurs sporadically in many other countries.

2 Etiology and ecology

Anthrax is a bacterial disease caused by the spore-forming *Bacillus anthracis*, a Gram positive, rod-shaped bacterium (see section 6).

2.1 Spores and vegetative forms

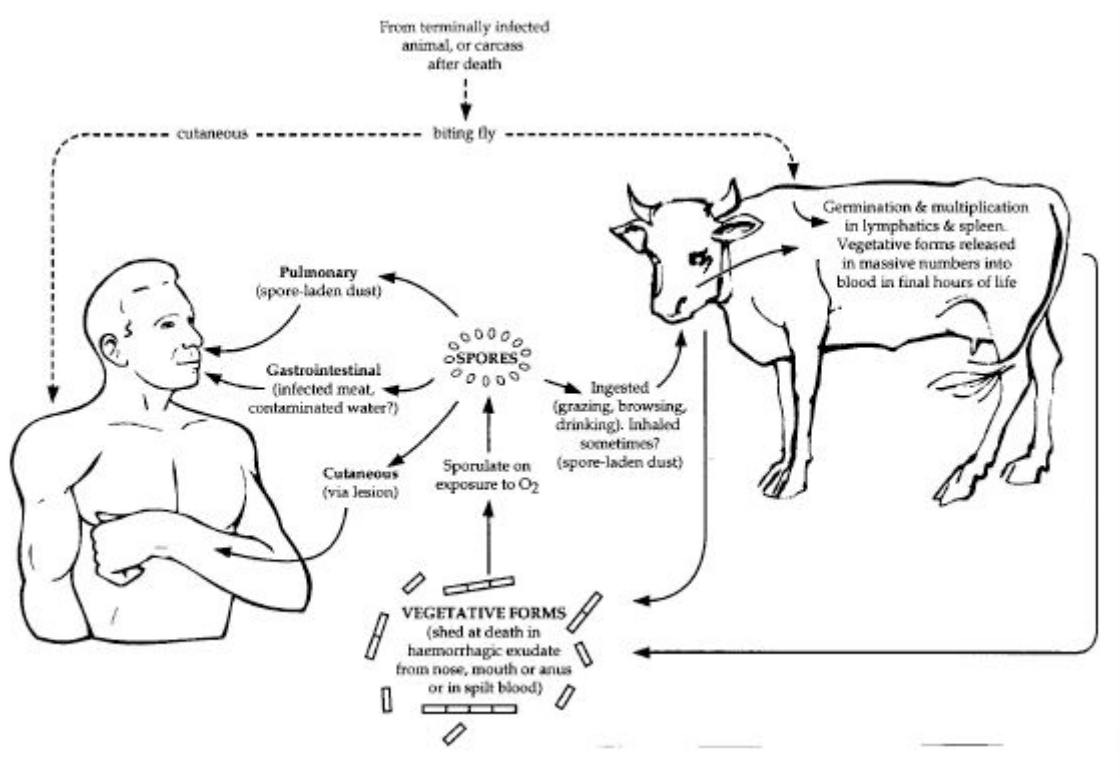
When conditions are not conducive to growth and multiplication of the bacilli, they tend to form spores. Sporulation requires the presence of free oxygen; within the anaerobic environment of the infected host the organism is in the vegetative form.

Although the vegetative forms of *B. anthracis* grow and multiply readily on or in normal laboratory nutrient agars or broths, the evidence is that they are more “fragile” than the vegetative forms of other *Bacillus* species, dying more spontaneously in simple environments such as water or milk, and more dependent on sporulation for species survival (Turnbull *et al.*, 1991; Bowen *et al.* 1992; Lindeque 1994). *B. anthracis* is, to all intents and purposes, an obligate pathogen.

The spore forms are markedly resistant to biological extremes of heat, cold, pH, desiccation, chemicals (and thus to disinfection), irradiation and other such adverse conditions. Therefore, the spore forms are the predominant phase in the environment and it is very largely through the uptake of spores that anthrax is contracted.

Within the infected host the spores germinate to produce the vegetative forms which multiply, eventually killing the host. A proportion of the bacilli released by the dying or dead animal (see Section 5) into the environment (usually soil under the carcass) sporulate, ready to be taken up by another animal. This cycle of infection is illustrated in Figure 1.

Figure 1. Cycle of infection in anthrax. The spore is central to the cycle, although infection can also be acquired through uptake of the vegetative forms when, for example, humans or carnivores eat meat from an animal that died of anthrax or when biting flies transmit the disease.



The rate and extent of sporulation by vegetative cells shed from infected animals is affected in a complex manner by the environmental conditions into which they fall. Temperature, pH, oxygen availability and the presence of certain cations such as Mn^{++} are the principal influencing factors.

Spores will germinate outside an animal if they fall into appropriate conditions, i.e. a temperature between about 8° and 45°C, a pH between about 5 and 9, a relative humidity greater than 95% and the presence of adequate nutrients. The extent to which subsidiary cycles of germination, multiplication and resporulation occur in the environment remains a topic of debate, but research (Bowen *et al.*, 1992; Turnbull *et al.*, 1991; Lindeque, 1994) suggests that the level of nutrient required for this to become possible is unlikely to occur very frequently under natural conditions. In fact, it seems that the fragile vegetative forms die rapidly in most environmental conditions, depending on sporulation in a proportion of the population for passing the infection to the next host (Bowen *et al.*, 1992; Lindeque, 1994).

2.2 Climate and other influencing factors

There is little dispute that anthrax is a seasonal disease; its incidence in any one place is related to temperature, rains or drought; however, examination of the literature shows that the conditions which predispose to outbreaks differ widely from location to location. Climate probably acts directly or indirectly by influencing the way in which an animal comes into contact with the spores (for example, grazing closer to the soil in dry periods when grass is short or sparse, or movement of herds to restricted sites when water becomes scarce), or by affecting the general state of health of the hosts and thereby affecting their level of resistance to infection.

Much has been written and hypothesized about the effects of season, rainfall, temperature, soil, vegetation, host condition and population density on the epidemiology of anthrax, but little agreement exists on the roles played by these factors in the incidence of the disease. Most of the theories are based on concepts of conditions under which *B. anthracis* may germinate and multiply in the environment, but hard scientific supportive data are not readily available and, as said in 2.1, the conditions under which multiplication in the environment could take place probably only occur in exceptional circumstances.

No one model satisfactorily explains the varying observations on the relationship between the factors listed above and the incidence and persistence of anthrax in a locality. This is an area worthy of further structured research.

2.3 Strains

B. anthracis appears to be one of the most monomorphic species known. That is to say, isolates from whatever type of source or geographical location are almost identical phenotypically and genotypically. Phenotypically, strain differences are only apparent in non-quantifiable or semi-quantifiable characteristics, such as colonial morphology, flocculation in broth culture, cell size, LD₅₀ in animal tests, and so on. The biochemical, serological or phage typing methods available in the case of other pathogens have proved of no value for identifying different strains of *B. anthracis*. At the molecular level, genomic differences have also proved hard to detect, although, some progress is being made in attempts to devise chromosomally-based strain differentiation systems Henderson 1996; Anderson *et al.* 1996; Keim *et al.* 1997; Jackson *et al.* 1997.

It seems reasonable to attribute this exceptional degree of species monomorphism to the fact that *B. anthracis* encounters opportunities to multiply less often than most other bacterial and pathogenic species. Given the truth of the statements in 2.1 and 2.2 that opportunities to multiply in the environment are rare, multiplication cycles depend almost exclusively on infections in animal hosts – which, in turn, may only occur following considerable time intervals between sequential hosts. Furthermore, since multiplication occurs almost exclusively in the animal host, the vegetative form of the organism is rarely exposed to the mutagens, phages or other environmental factors responsible for strain variation in other bacterial species.

3 Anthrax in animals

3.1 Host range and susceptibility

As said at the outset (Section 1), anthrax is primarily a disease of herbivores. However, reports of its occurrence in dogs scavenging anthrax carcasses and in carnivorous animals in zoological gardens and wildlife sanctuaries or parks are not entirely uncommon, though outbreaks affecting large numbers of carnivorous animals are very rare. Published LD₅₀s for anthrax by the parenteral route range from <10 for a guinea pig through 3×10^3 for the rhesus monkey, 10^6 for the rat, 10^9 for the pig and 5×10^{10} for the dog (Watson and Keir, 1994). Minimum infectious dose (MID) estimates are only rarely available, but an aerosol MID for sheep of 35 000 spores has been recorded (Fildes, 1943). De Vos and Scheepers (1996) record that 100 spores of a strain isolated from a kudu in the Kruger National Park consistently resulted in death from anthrax when administered parenterally in impala. In another recent study on 50 pigs given doses of 10^7 to 10^{10} spores in feed containing grit (Redmond *et al.*, 1997), the majority showed clinical illness with recovery, and just two died with confirmed anthrax 6 and 8 days respectively after ingestion of the spores; these were estimated to have received 1.6×10^7 and 7.8×10^7 spores respectively.

Since it is thought that animals generally acquire anthrax by ingestion of spores, and that some sort of lesion is necessary for the establishment of infection (see 3.3), LD₅₀s, particularly parenterally determined, only provide a rudimentary guide to natural susceptibility. Extrapolating experimental findings to the natural situation must also be done cautiously, taking into consideration the many factors influencing infectivity, such as the strain of *B. anthracis*, the route of infection, the specie, breed or strain and state of health of the animal tested, the times and sites at which tests are done and so on.

3.2 Incidence of anthrax in animals

The following information on the occurrence of anthrax in animals has been derived from official reports (OIE, 1997) as well as from data collected from other sources (Hugh-Jones; personal communication).

During the past three decades, there has been a progressive global reduction in livestock cases in response to national programmes. As a result, the disease in animals is now truly absent or only sporadic in the middle and higher latitudes of Europe; it is still common in the European countries adjoining the Mediterranean (Greece, Italy, Spain, Turkey and Yugoslavia). In Canada, apart from its continued incidence in bison in the MacKenzie Bison Range, North West Territory, and in the Wood Buffalo National Park, northern Alberta, it is sporadic in southern Alberta and Saskatchewan, with a recent singular outbreak in Ontario. In the USA it is confined to a few persistent pockets with sporadic cases in South Dakota, Nebraska, and Oklahoma but probably a hyperendemic situation persists throughout the south western quadrant of Texas.

The true situation in Latin America awaits definition; under-reporting and failure to diagnose unexpected livestock deaths certainly occurs, especially in small ruminants; it is enzootic in Central America, Mexico and Guatemala, with decreasing occurrence as one goes farther south—it is absent in Belize, as it is throughout the Caribbean with the exception of Haiti. Anthrax is enzootic in Peru, Bolivia and Venezuela.

In South Africa, the annual number of outbreaks is less than 5 and occasionally zero, despite the continued occurrence of the disease in wildlife in the various parks. Good control programmes have been established in Botswana, Zimbabwe and Zambia, but the disease remains well-known in the latter two countries at least. In equatorial African countries, anthrax is enzootic or hyperenzootic.

The Middle Eastern and adjoining countries of former USSR republics continue to experience hyperendemic anthrax. Anthrax is enzootic in southern India but is less frequent to absent in the northern Indian states where the soil is more acid, while in Nepal it is endemic.

Some south-east Asian countries are severely affected—Myanmar, Vietnam, Cambodia, western China—while Thailand used to be free, now it is endemic and continually threatened and afflicted by its neighbour. Malaysia and Taiwan are free, and the disease is limited to single regions of the Philippines and Indonesia. All these countries differ from the rest of the world in that pigs and water buffalo, as opposed to ruminants, are the animals commonly affected.

As countries become free of anthrax or the annual incidence of outbreaks approaches unity, the numbers of animals affected in an outbreak increase. This seems to be due to the decreasing veterinary experience in recognizing cases and in dealing appropriately with outbreaks. The mere absence of reported livestock anthrax does not mean that a country is free of the disease. Reporting deficiencies and insufficient examination of unexpected livestock deaths are common throughout the world. Also, while civil unrest in northern latitudes can be expected to result in a fall in anthrax cases, in warmer countries, especially in Africa and Asia, civil wars invariably result in anthrax epidemics in livestock, which then readily spill over into human populations. The reason for this is that the northern countries import meat and bone meal and other commonly contaminated materials but imports are cut off in times of war. For example, Norway, which was occupied and pillaged in World War II, quickly ceased to experience outbreaks of anthrax; meanwhile the disease continued unabated in neutral Sweden. In contrast, during the civil war in Southern Rhodesia/Zimbabwe at the end of the 1970s, livestock vaccination ceased and by the end of the war there had been some 10 000 human cases, as compared with the previous normal annual rate of a dozen cases or less (Turner, 1980; Davies, 1982).

3.3 Transmission in animals

In economic and public health terms, the importance of the disease lies in its ability to affect large numbers of livestock at one time. Carcasses then pose a hazard to humans and other animals both in the vicinity and at a distance through their meat, hides, hair, wool or bones. Hides, hair, wool and bones may be transported large distances for use in industries, feedstuffs or handicrafts. Livestock may acquire the disease through contaminated feedstuffs or from spores that have reached fields in sewage sludge.

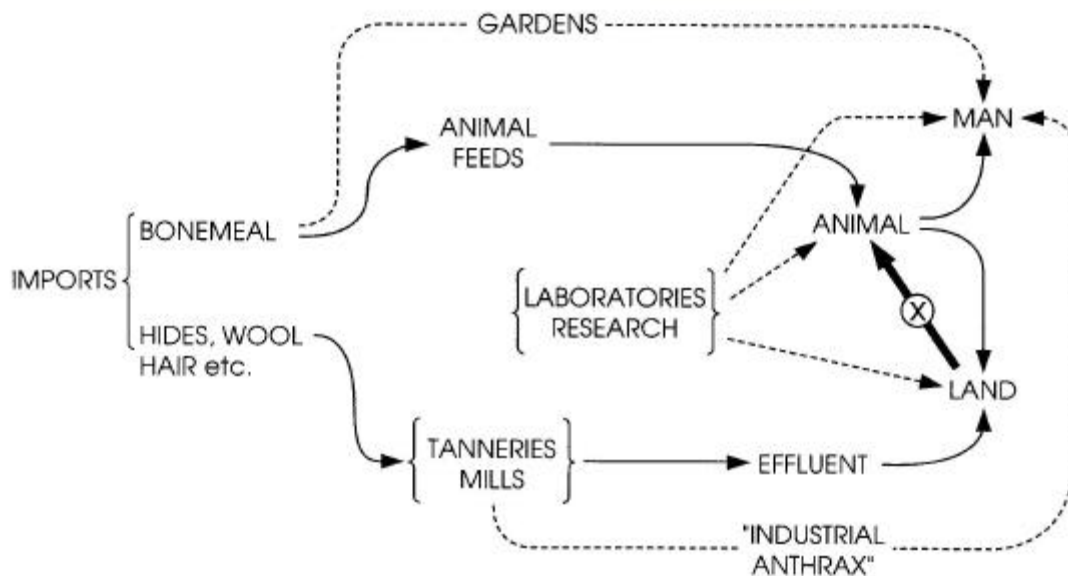
Although anthrax has been recognized for centuries, much remains unknown about the disease, including the precise manner by which grazing animals acquire it. The sporulated forms shed by an animal dying or dead from anthrax generally provide the source of infection of other animals (Figure 1). It is generally assumed that ingestion of the spores while grazing is a frequent mode of uptake; since *B. anthracis* is apparently non-invasive, it is believed a lesion is necessary for the initiation of infection. In view of associations between times of higher incidence and dry, hot conditions, theories have arisen that, at such times, the animal is forced to graze dry, spiky grass close to the soil. The spiky grass and grit produce the gastrointestinal lesions and if the soil is contaminated with anthrax spores, there is a high chance of infection occurring.

In countries with advanced agriculture, contaminated feedstuffs are or have been a common and significant source of infection, especially for dairy cows. The source can either be improperly treated locally produced meat and bone meals salvaged from moribund stock or imported infected bones/contaminated meat and bone meals. Cross-contamination can occur during shipping if the hulls of cargo ships and other containers are not cleaned out between shipments or if infected dry hides are placed on top of feed; this practice has in the past produced contaminated vegetable high-protein feeds which normally would not be expected to contain *B. anthracis* spores. Similar cross-contamination following the re-use of feed sacks has resulted in outbreaks of anthrax.

In England, Germany, Italy and Canada, pastures flooded by tannery waste water historically have posed a long-recognized hazard to grazing stock (Figure 2).

The examination of associations between climatic conditions and peak anthrax periods around the world have resulted in a number of other theories. Some contribute to the hypothesis that an animal can harbour the spores for long periods only manifesting the disease when stressed or compromised immunologically; seasonal stress may play a role in this regard. Others believe that acquisition of the disease by inhalation of spore-laden dust is not infrequent. The extent to which this might occur would vary with season. It is widely accepted that, in some regions, certain types of flies transmit anthrax; again this would be associated with season.

Figure 2. Importation from endemic to other countries and cycles within the importing country.



3.4 Pathogenesis and symptoms

Except when taken up by the pulmonary route, *B. anthracis* needs a lesion through which to enter the body. Following entry, the spores, which may have commenced germination, are carried to the lymphatics where they multiply and, in terms used by workers in the 1950s, continuously feed the blood stream with the vegetative bacilli in a manner analogous to continuous culture. Initially, during the incubation period, the bacteria are filtered out by the spleen and other parts of the reticuloendothelial system. However, the system finally breaks down due to toxin action and, during the last few hours of life (fulminant systemic phase), the bacteria build up rapidly in the blood (doubling time approximately 0.75 to 2 hours depending on host species) to levels of $>10^8$ /ml together with massive toxæmia at the time of death. The action of the toxin on the endothelial cell lining of the blood vessels results in their breakdown, internal bleeding and the characteristic terminal haemorrhage to the exterior which is an essential part of the organism's cycle of infection (Figure 1 and front cover).

The incubation period in the susceptible herbivore ranges from about 36 to 72 hours and leads into the hyperacute systemic phase, usually without easily discernible prior symptoms. The first signs of an anthrax outbreak are one or more sudden deaths in the affected livestock, although farmers may reflect retrospectively that the animals had shown signs such as having been off their food or having produced less milk than usual. During the systemic phase, the animals become distressed, appear to have difficulty breathing and cease eating and drinking. Swellings in the submandibular fossa may be apparent; temperatures may remain normal for most of the period or may rise. The animal can remain responsive to treatment well into this

period but if treatment fails it lapses into coma followed by death from shock. In highly susceptible species, the period between onset of visible symptoms and death may be just a few hours; the course of these events is more protracted in more resistant species.

3.5 Diagnosis in animals

The history is of major importance in the diagnosis of anthrax. Clinical manifestations to look for are:

Ruminants: Sudden death, bleeding from orifices, subcutaneous haemorrhage, without prior symptoms or following a brief period of fever and disorientation should lead to suspicion of anthrax.

Equines and some wild herbivores: Some transient symptoms — fever, restlessness, dyspnoea, agitation — may be apparent.

Pigs, carnivores, primates: Local oedemas and swelling of face and neck or of lymph nodes, particularly mandibular and pharyngeal and/or mesenteric.

At death in most susceptible species (the pig being a notable exception), the blood contains 10^7 to 10^8 bacilli per ml provided the animal has not been treated (numbers may also be lower in immunized animals which succumb to the disease). For reasons unknown, numbers of *B. anthracis* at death are very low in pigs (hundreds per millilitre or less).

A blood smear should be obtained with a swab from a small incision in the ear or from an ear clipping (the ear is usually recommended as being accessible, supplied with an extensive capillary network), or by means of a syringe from an appropriately accessible vein (the blood characteristically clots poorly or not at all upon death in anthrax victims and is dark and haemolysed). The smear is dried, fixed and stained with polychrome methylene blue (M'Fadyean stain) as described in Appendix I (A.I.2.1.2). Large numbers of the capsulated bacilli will be seen in smears from relatively fresh carcasses of most species (see preceding paragraph). A Gram stain will not reveal the capsule and may result in mistaken diagnosis, particularly if the carcass is not very fresh. *B. anthracis* does not compete well with putrefactive bacteria and, with increasing age of the carcass, the capsulated bacilli become harder to visualize (see also four paragraphs below).

The bacterium can also be cultured from the blood or from a swab of a ear clipping or other appropriate specimen on blood agar or other nutrient agar (see Appendix I). The haemorrhagic nasal, buccal or anal exudate will also carry large numbers of *B. anthracis* which can be cultured from swabs or from samples contaminated with the exudate.

If anthrax is suspected the carcass should not be opened; contamination of the environment by spilled body fluids with subsequent spore formation, is thereby avoided. If, mistakenly, the carcass has been opened, the dark unclotted blood and markedly enlarged haemorrhagic spleen are immediately apparent. The mesentery may be thickened and oedematous and peritoneal

fluid may be excessive. Petechial haemorrhages may be visible on many of the organs and the intestinal mucosa may be dark red and oedematous with areas of necrosis. Where anthrax has been diagnosed after a carcass has been opened, special attention should be paid to decontamination of the site at which the postmortem was carried out and of the tools and materials that were used (see section 8).

In horses, the intestine and parenchymatous organs may be less affected than in sheep and cattle and the subcutaneous and intramuscular tissues may be oedematous. In pigs, as indicated above, blood smears may not reveal the capsulated bacilli and, if cervical oedema is present, smears and cultures should be made from fluid from the enlarged mandibular and supratharyngeal lymph nodes. If intestinal anthrax is responsible for death in pigs, this may only become apparent at necropsy; smears and cultures should be made from the mesenteric fluid and lymph nodes. In other animals, in addition to any haemorrhagic exudates that may be observed, severe inflammation and oedematous swelling of the lips, tongue, gums, jowls and throat may be diagnostic indicators.

In the unopened carcass, the bacilli, unable to sporulate in the absence of oxygen, are destroyed by the putrefactive processes. Smears, as a diagnostic procedure, become unreliable about 24 hours after death although "shadons" (capsular material) may still be observed some time after the bacilli themselves can no longer be seen. *B. anthracis* can often be cultured from carcass skin residues for some days after death but, with increasing length of time between death and examination, this becomes progressively less easy. Diagnosis then becomes increasingly dependent on isolation of spores from soil or other environmental samples contaminated by the oral, buccal or anal exudates.

As mentioned in Appendix I (A.I.1.2), it may not be possible to find the bacilli in smears or to isolate *B. anthracis* from animals that were treated before death; treatment can sterilize the blood and tissues but, if sufficient toxin has been formed, the animal may still go on to die.

The thermostable antigen precipitin test devised by Ascoli (1911) (see Appendix I [A.I.3.1]) is still used in several countries of Europe and the Far East for detecting residual antigens in tissue in which it is no longer possible to demonstrate *B. anthracis* microscopically or by culture. However, it should be borne in mind that the antigens being detected are shared by other *Bacillus* species and that the test relies on the fact that, if *Bacillus* antigens are present in the tissues, this probably represents *B. anthracis* infection since infections with other *Bacillus* species are rare. Care has to be taken if the tissue being examined has been grossly contaminated with environmental materials (soil, sand, etc.) which frequently harbour large numbers of other *Bacillus* species.

A simple, rapid and highly sensitive and specific chromatographic device, utilising a monoclonal capture antibody detecting the anthrax-specific protective antigen, has now been designed (Burans *et al.*, 1996) and hopefully will become available in the near future as a more reliable and more sensitive alternative to the Ascoli test.

3.5.1 Differential diagnosis

For differential diagnosis, other causes of sudden death should be considered. Amongst these are botulism, blackleg (*Clostridium chauvoei*), peracute babesiosis, chemical poisoning (heavy metals, other poisons), ingestion of toxic plants, snake bite, lightning strike or metabolic disorders such as lactic acidosis, magnesium deficiency, bloat. A recent outbreak of Rift Valley Fever in Kenya was initially thought to be anthrax (WHO, 1997a).

3.5.2. Retrospective diagnosis (seroconversion)

Historically, there has been little need for serological support for the diagnosis of anthrax in animals. Either the animal had anthrax, recognized from the recent history of the herd or site, and was treated accordingly, or it died. Most of the interest in developing serological testing has been for research on humoral responses in humans, and—to a lesser extent—animals, to vaccines and for epidemiological studies involving naturally acquired seroconversion in humans, livestock and wild mammals.

Currently accepted as the best serological procedure is the ELISA in microtitre plates coated with the Protective Antigen (PA) component of the anthrax toxin at 3–5 µg/ml in high pH (9.5) carbonate coating buffer. The toxin antigens appear to be truly specific for *B. anthracis*, although there is at present no commercial source of these. This tends to mean that anthrax serology is currently confined to a few specialist laboratories. Various versions of the ELISA exist and can be found in standard laboratory manuals; any version will do for anthrax serology, although certain sera appear to be more "sticky" than others. A useful tip appears to be to use reconstituted dried milk as the blocking agent and to raise its concentration until control negative sera are giving reliable negative results. For bovine sera, this may be a 10% suspension or higher.

Examples of the successful field application of anthrax serology are given elsewhere (Turnbull *et al.*, 1992; Redmond *et al.*, 1997).

4 Anthrax in humans

4.1 Incidence

The major sources of human anthrax infection are direct or indirect contact with infected animals, or occupational exposure to infected or contaminated animal products. Other possible sources are rare and epidemiologically trivial. Human anthrax incidence is dependent on the level of exposure to affected animals and national incidence data for non-industrial cases reflect the national livestock situation. Historical analysis of epidemiological data globally reveals the following approximate ratios: (a) one human cutaneous anthrax case to ten anthrax livestock carcasses; (b) one incident of enteric human anthrax to 30–60 anthrax-infected animals eaten; (c) in humans, 100–200 cutaneous cases for each enteric case that occurs.

Industrial anthrax incidence data can be inferred from the volume and weight of potentially affected materials handled or imported, taking into account the quality of prevention, such as vaccination of personnel and forced ventilation of the workplace. These relationships are essentially all that can be used for many countries where human anthrax is infrequently, erratically or incompletely reported. In addition, certain countries suppress anthrax reporting at the local or national levels.

Human case rates for anthrax are highest in Africa, the Middle East and central and southern Asia. Where the disease is infrequent or rare in livestock, it is rarely seen in humans.

4.2 Susceptibility. Data for risk assessments

4.2.1 Historical information

Circumstantial evidence indicates that man is moderately resistant to anthrax. Before vaccines and antibiotics became available, and at a time when understanding of industrial hygiene was relatively basic, workers in at-risk industrial occupations processing animal products were exposed to significant numbers of anthrax spores on a daily basis. In Britain, 354 cases of anthrax in such industries were notified during the 13-year period 1899–1912 (Anon, 1918). Although the numbers of persons exposed is not known, it must have been many thousands, and the number of cases clearly represented only a very small proportion of the number exposed.

In 4 mills in the USA, in which unvaccinated workforces, varying in size from 148 to 655, were “chronically exposed to anthrax”, annual case rates were only 0.6 to 1.4% (Brachman *et al.*, 1962). In one mill, workers were found to be inhaling 600 to 1300 anthrax spores over an 8-hour shift without ill effect (Dahlgren *et al.*, 1960) and in two goat-hair mills, *B. anthracis* was recovered from the nose and pharynx of 14 of 101 healthy persons. Despite extensive exposure to anthrax, cases among workers in wildlife reserves are exceedingly rare (Quinn and Turnbull, 1998).

Nevertheless, outbreaks and epidemics do occur in humans; sometimes these are sizeable, such as the epidemic in Zimbabwe which began in 1979, was still smouldering in 1984–5 and had by that time affected many thousands of persons, albeit with a low case fatality rate (Turner, 1980; Davies 1982; Kobuch *et al.* 1990). Occasionally, the case fatality rates are substantial, such as in the Sverdlovsk incident in Russia in 1979 (Abramova *et al.*, 1993; Meselson *et al.*, 1994). The outbreak in a mill in New Hampshire, USA, in 1957 was not associated with any unusual change in occupational exposure but seems to have been an isolated event within a prolonged period of exposure (Brachman *et al.*, 1960).

4.2.2 Infectious dose

Infectious doses, which have not been established for man, and the severity of the resulting infection clearly depend on several factors such as route of infection, nutritional and other

states of health on the part of the infected person, and probably on the relative virulence of the infecting strain. For the purpose of risk assessments, dependency on information from animal tests is unavoidable. The published data on infectious and lethal doses in animals have been collated elsewhere (Watson and Keir, 1994).

Cutaneous infections. It probably does not take many spores to initiate a cutaneous infection, but it is generally accepted that the spores must gain access to subepidermal tissue through a cut or abrasion before this can occur and risk of infection reflects the chance of this happening. This risk is greatly reduced in at-risk occupations by appropriate clothing and gloves, dressing of wounds, and other hygienic practices.

Pulmonary (inhalation) infections. Recorded inhalation LD₅₀s in non-human primates range from 2500 to 760 000 spores (Meselson *et al.*, 1994; Watson and Keir, 1994). The US Department of Defence bases its strategies on an estimate that the LD₅₀ for humans is 8000 to 10 000 spores (Meselson *et al.*, 1994). However the only hard data on inhalation infectious doses in humans come from the studies in goat hair processing mills referred to in 4.2.1. In any event, substantial exposure is evidently necessary before the risk of inhalation anthrax becomes significant. In a recent study (Turnbull *et al.*, 1998) the highest levels found in air sampled 3 to 9 m downwind from disturbed dry, dusty anthrax carcass sites in Namibia were 20 to 40 colony-forming units of spores per cubic metre. This corresponds to the conservative estimate that it would require about 2.5 minutes for an average human undergoing moderate activity to inhale 1 spore. It is, furthermore, well established that, at sizes above 5 µm, particles face increasing difficulty in reaching the alveoli of the lungs. The likelihood of inhaled spores penetrating far enough to induce inhalation anthrax therefore depends greatly on the size of the particles to which they are attached.

The overall conclusion from the available evidence is that the risks of pulmonary anthrax outside industrial situations are very low.

Oral route infections. There is very little information on infectious doses by the oral route, but what is true for the skin is probably largely true for the oropharyngeal and gastrointestinal epithelium. The chance of infection is likely to be enhanced by, if not dependent on, the existence of a lesion in the epithelium through which spores can gain entry and establish an infection.

Treatability. The fact that anthrax is readily treated if diagnosed at a sufficiently early stage of infection also needs to be taken into account when assessing risks. Awareness of the likelihood of exposure having taken place is clearly an important part of the equation.

4.2.3 Biological warfare associations

In the developed parts of the world where it is now seen rarely, anthrax has developed something of a “doomsday bug” status in the mind of the public, and the name frequently engenders unnecessary fear, for example, in relation to contaminated burial or industrial (e.g.

tannery) sites. This anxiety results from the association of anthrax with the topic of biological warfare.

There is, in fact, no conflict between the statements and evidence given in 4.2.1 and 4.2.2 that humans are fairly resistant to fatal anthrax infection and the possible aggressive use of anthrax spores. The “worst case” natural contamination in the environment is found at the carcass sites of animals that have died of anthrax. In a study in Namibia of 106 such sites, the highest contamination level found was just over 1 million anthrax spores per gram of soil, but 79% had less than 1000 per gram and 25% less than 10 per gram (Lindeque and Turnbull, 1994). Levels in other types of inadvertently contaminated environments (soils at tannery sites, horsehair plaster, etc.) rarely exceed a few units or tens of spores per gram (Turnbull, 1996). Natural environmental exposure to infectious doses in the normal course of human life and endeavour is, therefore, a fairly unlikely event.

Aggressive scenarios, in contrast, envisage exposures to overwhelmingly massive doses (many millions of spores) which can only be created artificially. The Figure of 100 kg of dried anthrax spores, given in one article (Taylor, 1996) on the subject as technically feasible for aggressive delivery, represents dose levels in the order of 10^{13} human LD50s. It must be supposed that this could cause substantial devastation to human and animal communities within selectively targeted areas. The public health implication of deliberately induced anthrax outbreaks and its use as a biological weapon have been reviewed elsewhere (WHO, 1970).

4.3 Epidemiology and transmission: the forms of anthrax

Anthrax in humans is classically divided in two ways. The first type of classification, which reflects how the occupation of the individual led to exposure, differentiates between **non-industrial** anthrax, occurring in farmers, butchers, knackers, veterinarians and so on, and **industrial** anthrax, occurring in those employed in the processing of bones, hides, wool and other animal products. The second type of classification, reflecting the route by which the disease was acquired, distinguishes between **cutaneous anthrax** acquired through a skin lesion, **gastrointestinal tract anthrax** contracted from ingestion of contaminated food, primarily meat from an animal that died of the disease, or conceivably from ingestion of contaminated water and **pulmonary (inhalation) anthrax** from breathing in airborne anthrax spores.

Non-industrial anthrax, resulting from handling infected carcasses, usually manifests itself as the cutaneous form; it tends to be seasonal and parallels the seasonal incidence in the animals from which it is contracted. Cutaneous anthrax transmitted by insect bites and intestinal anthrax from eating infected meat are also non-industrial forms of the disease. Industrial anthrax also usually takes the cutaneous form but has a far higher probability than non-industrial anthrax of taking the pulmonary form through inhalation of spore-laden dust.

Humans almost invariably contract anthrax directly or indirectly from infected animals. Records of person-to-person spread or laboratory-acquired anthrax are rare (Heyworth *et al.*, 1975; Collins 1988; Lalitha *et al.*, 1988; Quinn and Turnbull, 1998).

It is generally believed that *B. anthracis* is non-invasive and cutaneous and gastrointestinal tract anthrax infection require entry through a small cut, abrasion or other lesion (insect bite, ulcer, etc.). Thus anthrax eschars are generally seen on exposed regions of the body, mostly on the face, neck, hands and wrists.

As inferred earlier, in some countries mechanical transmission by biting insects is believed to be at least an occasional mechanism by which anthrax is contracted by humans (Rao and Mohiyudeen, 1958; Davies, 1983); that this can happen has been demonstrated experimentally (Sen and Minett, 1944; Turell and Knudson, 1987).

4.4 The clinical disease

Cutaneous anthrax is said to account for 95% or more of human cases globally. All three forms, cutaneous, gastro-intestinal tract and pulmonary, are potentially fatal if untreated, but the cutaneous form is often self-limiting. Data from pre-antibiotic and vaccine days indicate that 10–20% of untreated cutaneous cases might be expected to result in death (Anon, 1918). With treatment, less than 1% are fatal.

Overt gastrointestinal tract and pulmonary cases are more often fatal, largely because they go unrecognized until it is too late for effective treatment. However, serological and epidemiological evidence suggests that undiagnosed low-grade gastrointestinal tract or pulmonary anthrax with recovery can also occur, and may not be infrequent, among exposed groups (Brachman *et al.*, 1960; Norman *et al.*, 1960; Sirisanthana *et al.*, 1988; Van den Bosch, 1996).

Development of meningitis is a dangerous possibility in all three forms of anthrax.

4.4.1 Cutaneous anthrax

The incubation period ranges from as little as 9 hours to 2 weeks, mostly 2 to 6 or 7 days.

The general scenario is as follows:

Day 0 Entry of the infecting *B. anthracis* (usually as spores) through a skin lesion (cut, abrasion, insect bite, etc.).

Days 2–3 A small pimple or papule appears (see Figures M–R).

Days 3–4 A ring of vesicles develops around the papule. Vesicular fluid may be exuded. Unless the patient was treated, capsulated *B. anthracis* can be identified in polychrome methylene blue-stained (M'Fadyean stain) smears of this fluid and isolated on conventional agars, preferably blood agar (see Appendix I). Marked oedema starts to develop. Unless there is

secondary infection, there is no pus and the lesion is not painful, although painful lymphadenitis may occur in the regional lymph nodes.

Days 5-7 The original papule ulcerates to form the characteristic eschar. Topical swabs will not pick up *B. anthracis*. Detection by polychrome methylene blue-stained smears or isolation requires lifting the edge of the eschar with tweezers (this gives no pain unless there is secondary infection) and obtaining fluid from underneath. The fluid will probably be sterile if the patient has been treated appropriately. Oedema extends some distance from the lesion. Clinical symptoms may be more severe if the lesion is located in the face, neck or chest. In these more severe forms, clinical findings are high fever, toxæmia, regional painful adenomegaly and extensive oedema; shock and death may ensue.

Day 10 (approximately).

The eschar begins to resolve; resolution takes almost six weeks and is not hastened by treatment. A small proportion of cases, if untreated, develop systemic anthrax with hyperacute symptoms.

4.4.1.1 Differential diagnosis

Boil (early lesion), orf, vaccinia, glanders, syphilitic chancre, erysipelas, ulcer (especially tropical). These lack the characteristic oedema of anthrax. The absence of pus, the lack of pain, and the patient's occupation may provide further diagnostic pointers. The outbreak of Rift Valley Fever, referred to in 3.5.1 and initially thought to be anthrax, also affected numerous humans.

In differential diagnosis of the severe forms, orbital cellulitis, dacrocystitis and deep tissue infection of the neck should be considered in the case of severe anthrax lesions involving the face, neck and anterior chest wall. Necrotising soft tissue infections, particularly group A streptococcal infections and gas gangrene, and severe cellulitis due to staphylococci, should also be considered in the differential diagnosis of severe forms of cutaneous anthrax.

4.4.1.2 Immunological tests

Subject to certain provisos, serology can, on occasion, be useful in supportive or retrospective diagnosis of anthrax (see A.I.5). The practical aspects have been covered in 3.5.2.

Similarly, in the Russian sphere of influence, a skin test utilising Anthraxin^T (Anti plague Research Institute, Sovetskaya St., 13/15, Stavropol, 355106 Russian Federation; Fax: +7 8652 260312), first licensed in the former USSR in 1962, has become widely used for retrospective diagnosis of human and animal anthrax and for vaccine evaluation (Shylakhov *et al.*, 1997). This is a commercially produced heat-stable protein-polysaccharide-nucleic acid complex, derived from oedematous fluid of animals injected with the vaccine STI or the Zenkowsky strains of *B. anthracis*. The test involves intradermal injection of 0.1 ml of Anthraxin and inspection after

24 h for erythema and induration at the site lasting for 48 hours after the injection. This delayed type hypersensitivity is seen as reflecting anthrax cell mediated immunity and was reportedly able to diagnose anthrax retrospectively some 31 years after primary infection in up to 72 % of cases (Shlyakhov *et al.*, 1997). It was used with success in a retrospective investigation of a series of cases occurring in a spinning mill in Switzerland where synthetic fibres were combined with goat hair from Pakistan (Pfisterer, 1990). The diagnostic reliability of Anthraxin, like Ascoli test antigen (A.I.3.1), depends on the nature of anthrax rather than on the specificity of the antigens involved.

4.4.1.3 Precautions

Surgical tools should be sterilized without delay after use, and dressings should be incinerated. The wearing of surgical gloves by medical staff and orderlies is recommended but risks to these staff are NOT high. Direct human-to-human transmission is exceedingly rare (see 4.3). Vaccination of medical staff and orderlies is not necessary.

4.4.2 Gastrointestinal anthrax

There are two clinical forms of gastrointestinal anthrax which may present following ingestion of *B. anthracis* in contaminated food or drink.

- i) *Intestinal anthrax*: Symptoms include nausea, vomiting, fever, abdominal pain, haematemesis, bloody diarrhoea and massive ascites. Unless treatment commences early enough, toxæmia and shock develop, followed by death. There is evidence that mild, undiagnosed cases with recovery occur.
- ii) *Oropharyngeal anthrax*: The main clinical features are sore throat, dysphagia, fever, regional lymphadenopathy in the neck and toxæmia. Even with treatment, the mortality is about 50% (Dogonay *et al.*, 1986).

The suspicion of anthrax depends largely on awareness and alertness on the part of the physician as to the patient's history and to the likelihood that he/she had consumed contaminated food or drink.

4.4.2.1 Confirmation of diagnosis. **See 4.4.3.1**

4.4.2.2 Differential diagnosis (gastrointestinal anthrax)

The differential diagnosis in **intestinal anthrax** includes food poisoning (in the early stages of intestinal anthrax), acute abdomen due to other reasons, and haemorrhagic gastroenteritis due to other microorganisms, particularly necrotising enteritis due to *Clostridium perfringens*.

In the differential diagnosis of **oropharyngeal anthrax**, streptococcal pharyngitis, Vincent's angina, Ludwig's angina, parapharyngeal abscess, and deep tissue infection of the neck should be considered.

4.4.3 Pulmonary (inhalation) anthrax

Symptoms prior to the onset of the final hyperacute phase are non-specific and suspicion of anthrax depends on the knowledge of the patient's history. In probably the best-documented set of five case reports of inhalation anthrax (Plotkin *et al.*, 1960), the illnesses began insidiously with mild fever, fatigue and malaise lasting one to several days. Headache, muscle aches, chills and fever were recorded in all four patients with development of a cough in four and mild pain in the chest in one. This mild initial phase was followed by the sudden development of dyspnoea, cyanosis, disorientation with coma and death in four of the patients, in whom treatment was unsuccessful. Death occurred within 24 hours of onset of the hyperacute phase.

4.4.3.1 Confirmation of diagnosis (pulmonary and intestinal anthrax)

As indicated, clinical diagnosis is dependent on a knowledge of the patient's history; early symptoms are non-specific and "flu-like" with mild upper respiratory tract signs in pulmonary anthrax or resembling mild food poisoning in intestinal anthrax. In fact, in pulmonary anthrax, the X-ray picture of the lung is very characteristic, with extremely enlarged mediastinal lymph nodes. Frequently, however, confirmatory diagnosis of pulmonary or gastrointestinal anthrax will usually take place after the patient has died or, if correct treatment is initiated early enough, when he or she is well recovered.

The definitive diagnosis is made by the isolation of *B. anthracis* from sputum in pulmonary anthrax and from vomitus, faeces and ascites in intestinal anthrax. Blood cultures may be positive in either form of the disease.

Depending on the treatment administered and the stage the disease has reached at the time of collection of specimens, smears stained for demonstration of the capsule (Appendix I) may be positive, or the specimens may be positive by culture. *B. anthracis* may be visualized in or isolated from sputum (pulmonary anthrax) or faeces (intestinal anthrax) but this cannot be relied upon. Specialized laboratories may be able to demonstrate anthrax toxin in fluid specimens (serum or oedematous fluid) or, in the case of patients who survive, anti-toxin and anti-capsular antibodies may be demonstrable in convalescent sera (Appendix I [A.I.5]). The Anthraxin hypersensitivity test referred to in 4.4.1.2 may also be applicable.

Death being due to the toxin, belated treatment can sterilize the blood and tissue fluids while still not preventing death. If this sterilising effect has not occurred, the capsulated *B. anthracis* may be visible in capsule-stained smears of these fluids and should be easily isolated from them by bacteriological culture.

Where anthrax has not been suspected prior to postmortem, characteristic signs are dark haemolysed unclotting blood, enlarged haemorrhagic spleen, petechial haemorrhages throughout the organs, and a dark oedematous intestinal tract, ulcerated or with areas of necrosis. In pneumonic anthrax, the mediastinal lymph nodes are always affected with haemorrhagic necrotizing lymphadenitis. Nevertheless, it may be hard to differentiate between

pulmonary and intestinal anthrax at autopsy and the decision as to how the disease was contracted may have to be based on the patient's history.

4.4.4 Anthrax meningitis

Meningitis due to anthrax is a serious clinical development which may follow any of the other three forms of anthrax. The case fatality rate is almost 100%; the clinical signs of meningitis with intense inflammation of the meninges, markedly elevated CSF pressure and the appearance of blood in the CSF (the meningitis of anthrax is a haemorrhagic meningitis) are followed rapidly by loss of consciousness and death (Levy *et al.*, 1981; Koshi *et al.*, 1981; Lalitha *et al.*, 1990; George *et al.*, 1994; Lalitha *et al.*, 1996). Only a few instances of survival as a result of early recognition of the problem and prompt treatment are on record (Khanne *et al.*, 1989; Lalitha *et al.*, 1996).

Differential diagnosis should take into account acute meningitis of other bacterial aetiology. The definitive diagnosis is obtained by visualisation of the capsulated bacilli in the CSF and/or by culture.

4.4.5 Anthrax sepsis

Sepsis develops after the lymphohematogenous spread of *B. anthracis* from a primary lesion (cutaneous, gastrointestinal or pulmonary). Clinical features are high fever, toxæmia and shock, with death following in a short time.

In the differential diagnosis, sepsis due to other bacteria should be considered. Definitive diagnosis is made by the isolation of *B. anthracis* from the primary lesion and from blood cultures.

5 Pathogenesis and pathology

5.1 Toxin as the cause of death

The events occurring between entry of infecting *B. anthracis* into a lesion or uptake from the lungs and death were covered in 3.4. At one time it was held that death from anthrax was due to capillary blockage, hypoxia and depletion of nutrients by the exceedingly large numbers of bacilli. Subsequently it was shown that death is attributable to a toxin (Keppie *et al.*, 1955).

5.2 The virulence factors of *B. anthracis*

The capsule and the toxin complex are the two known virulence factors of *B. anthracis*.

The poly-D-glutamic acid capsule is presumed to act by protecting the bacterium from phagocytosis.

The toxin complex, which consists of three synergistically acting proteins, Protective Antigen (PA, 83kDa), Lethal Factor (LF, 87 kDa) and Oedema Factor (EF, 89 kDa), is produced during the log phase of growth of *B. anthracis*. LF in combination with PA (lethal toxin) and EF in

combination with PA (oedema toxin) are now regarded as responsible for the characteristic signs and symptoms of anthrax.

According to the currently accepted model, PA binds to receptors on the host's cells and is activated by a host protease which cleaves off a 20 kDa piece, thereby exposing a secondary receptor site for which LF and EF compete to bind. The PA+LF or PA+EF are then internalized and the LF and EF are released into the host cell cytosol.

EF is an adenylate cyclase which, by catalysing the abnormal production of cyclic-AMP (cAMP), produces the altered water and ion movements that lead to the characteristic oedema of anthrax. High intracellular cAMP concentrations are cytostatic but not lethal to host cells. EF is known to impair neutrophil function and its role in anthrax infection may be to prevent activation of the inflammatory process.

LF appears to be a calcium- and zinc-dependent metalloenzyme endopeptidase (Hammond and Hanna, 1998). It has recently been shown (Duesbery *et al.*, 1998) that it cleaves the amino terminus of two mitogen-activated protein kinase kinases and thereby disrupts a pathway in the eukaryotic cell concerned with regulating the activity of other molecules by attaching phosphate groups to them. This signalling pathway is known to be involved in cell growth and maturation; the manner in which its disruption leads to the known effects of LF has yet to be elucidated. On the basis of mouse and tissue culture models, macrophages are a major target of lethal toxin which is cytolytic in these. The initial response of sensitive macrophages to lethal toxin is the synthesis of high levels of tumour necrosis factor and interleukin-1 cytokines and it seems probable that death in anthrax results from a septic shock type mechanism resulting from the release of these cytokines.

The endothelial cell linings of the capillary network may also be susceptible to lethal toxin and the resulting histologically visible necrosis of lymphatic elements and blood vessel walls is presumably responsible for systemic release of the bacilli and for the characteristic terminal haemorrhage from the nose, mouth and anus of the victim (see Figures on the front cover).

The detailed nature and mode of action of the toxin has been more thoroughly reviewed in various texts (Leppla, 1992; Quinn and Turnbull, 1998). Most of the recorded histopathological studies on anthrax were done between 1945 and 1970; these are reviewed elsewhere (Quinn and Turnbull, 1998).

6 Bacteriology

B. anthracis, the causative agent of anthrax, is a Gram positive, aerobic or facultatively anaerobic, endospore-forming, rod-shaped bacterium approximately 4 µm by 1 µm, although under the microscope, it frequently appears in chains of cells. In blood smears, smears of tissues or lesion fluid from diagnostic specimens, these chains are two to a few cells in length (see Figure B); in suspensions made from agar plate cultures, they can appear as endless strings of cells - responsible for the characteristic tackiness of the colonies (see Figure D). Also

characteristic is the square-ended (“box-car” shaped) appearance traditionally associated with *B. anthracis* vegetative cells, although this is not always very clear. In the presence of oxygen, and towards the end of the exponential phase of growth, one ellipsoidal spore is formed in each cell; this does not swell the sporangium and is generally situated centrally, sometimes sub-terminally (see Figure A).

Under anaerobic conditions and in the presence of bicarbonate (HCO_3), the vegetative cell secretes a polypeptide (poly- γ - D-glutamic acid) capsule. As covered in section 5, the capsule is formed *in vivo* and is one of the two virulence factors of *B. anthracis*. It is also a primary diagnostic aid (see 3.5 and 4.4.1, Appendix I [A.I.2] and Figure B).

6.1 Detection and isolation

In appropriate blood or tissue specimens collected within a few hours of death from animals (see 3.5) or humans with anthrax, *B. anthracis* is readily detected in capsule-stained (M'Fadyean-stained) smears and readily isolated in pure culture on blood or nutrient agar plates. The same applies to smears of fluid from cutaneous lesions of humans prior to treatment (see 4.4.1).

In old or decomposed animal specimens, or processed products from animals that have died of anthrax, or in environmental samples, detection is likely to involve a search for relatively few *B. anthracis* within a background flora of other bacteria, many of which will probably be other *Bacillus* species, in particular, the closely-related *B. cereus*. In this case, selective techniques are necessary. A procedure for the isolation of *B. anthracis* from such specimens is given in Appendix I (A.I.1).

Such is the nature of the properties of *B. anthracis* that few agents which differentially select between *B. anthracis* and other *Bacillus* species do so in favour of *B. anthracis* and those that do only do so unconvincingly. Of the selective media that have been proposed, the most successful is polymyxin-lysozyme-EDTA-thallos acetate (PLET) agar (Knisely, 1966), although care has to be taken to prepare this correctly. As yet no selective enrichment broth system has been devised for *B. anthracis* and, pending development of such a system, the sensitivity of *in vitro* detection of *B. anthracis* by conventional means in environmental samples or specimens from old or decomposed animals or from processed animal products is limited to approximately 5 cfu/g or /ml (Manchee *et al.*, 1981).

As covered in Appendix I (A.I.1.4 and A.I.4), the most sensitive method for isolating the organism is inoculation into a guinea pig or mouse. Although, in line with increasing aversion to the use of animals for scientific tests, this should strictly be a last resort, there are times when this may still be the necessary approach, for example, when confirming anthrax in individuals or animals that have been treated with antibiotics, or in essential tests on environmental samples that contain sporostatic chemicals.

Polymerase chain reaction (PCR) detection systems have been developed for *B. anthracis* (Beyer *et al.* 1996; Patra *et al.* 1996; Sjöstedt *et al.* 1996; 1997), but it will probably be a few years before they become fully reliable, adequately sensitive and robust, and generally available for use in the non-specialist laboratory.

6.2 Identification and confirmation

With occasional exceptions, it is generally easy to identify *B. anthracis* and to distinguish it from other *Bacillus* species, including *B. cereus*. For all practical purposes, an isolate with the characteristic colonial morphology (Parry *et al.*, 1983) on nutrient or blood agar (matt appearance, fairly flat, similar to *B. cereus* but generally rather smaller, more tacky, white or grey-white on blood agar, and often having curly tailing at the edges), and which is non-haemolytic or only weakly haemolytic, non-motile, sensitive to the gamma-phage and penicillin, and able to produce the capsule in blood or on anaerobic culture on bicarbonate media is *B. anthracis* (see Figures D and E, and Appendix I [A.I.2]).

The PCR is becoming more widely available as a means of confirming the presence of the virulence factor (capsule and toxin) genes, and hence that an isolate is, or is not, virulent *B. anthracis*. For routine purposes, primers to one of the toxin genes (usually the Protective Antigen gene) and to one of the enzymes mediating capsule formation are adequate (Appendix I [A.I.6] and Figure F). In laboratories not equipped for PCR tests, if doubt remains at the end of the procedures given in Appendix I (A.I.1 and 2) as to the definitive identity of a suspect *B. anthracis* isolate, inoculation into a mouse or guinea pig may be necessary (Appendix I [A.I.4]). However, as stated in 6.1, this should be a last resort procedure and confined to situations where a definitive identity is essential.

Movement of infectious or contaminated materials from the site of origin to a diagnostic or reference laboratory obviously presents a risk of spread of diseases if the materials inadvertently escape into the environment during transit. Attention is drawn to the recommendation of the United Nations Committee of Experts on Transport of Dangerous Goods for packaging, labelling and documentation in relation to transport of infectious specimens, detailed in other publications (WHO, 1997b), excerpts of which one is reported in Appendix VIII.

7 Treatment

Prompt and timely antibiotic therapy usually results in dramatic recovery of the individual or animal infected with anthrax. Almost all isolates of *B. anthracis* can be expected to be highly sensitive to penicillin and, being cheap and readily available in most parts of the world, this remains the basis of treatment schedules, particularly in animals and in humans in developing countries. The organism is also sensitive to numerous other broad spectrum antibiotics; should the use of penicillin be contraindicated, a wide range of alternative choices exist from among

the aminoglycosides, macrolides, quinolones and tetracyclines. Chloramphenicol is also a satisfactory alternative.

We are only aware of four reports of the isolation of penicillin-resistant strains (Anon, 1996). The molecular basis of susceptibility and resistance is complex (Lightfoot *et al.*, 1990). It looks as if it may prove to be the case that all *B. anthracis* strains carry the β -lactamase gene(s) on their chromosomes but, apart from the exceptions mentioned, these are not expressed.

In pulmonary or gastrointestinal anthrax in humans, symptomatic treatment in an intensive care unit in addition to antibiotic therapy may save the patient's life; as referred to in 7.2.8, if available, plasmaphoresed hyperimmune serum or gamma-globulin from vaccinated persons may be considered in life-threatening situations.

7.1 Treatment of animals

7.1.1 General principles and approaches

Following the first incident of anthrax in a herd, the remaining animals should be moved immediately from the field or area where the index case died and regularly checked at least three times a day for two weeks for signs of illness (rapid breathing, elevated body temperature) or of submandibular or other oedema. Any animal showing these signs should be separated from the herd and given immediate treatment. Clinical experience has frequently demonstrated that animals, especially cattle, will respond favourably to treatment even though apparently in the terminal stages of anthrax. Even if they go on to die (death in anthrax results from the effect of the toxin), the infecting load of *B. anthracis* will have been greatly reduced, if not entirely eliminated, thereby significantly reducing the chance of subsequent transmission from the carcass to other animals.

In endemic areas, or if there is concern that the outbreak may spread, the herd should be vaccinated (see Appendix V). Further anthrax deaths usually cease within 8 to 14 days of vaccination. Herd quarantine can be lifted 21 days after the last death. Decontamination of the site(s) where the index case or other case(s) died should be carried out (see Section 8 and Appendix III). [It should be remembered that vaccination and treatment should not be done simultaneously; the treatment will prevent the live vaccine taking effect].

In certain countries, treatment is not permitted and slaughter policies are in place (7.1.2.4).

7.1.2 Specific procedures

7.1.2.1 Antimicrobial therapy in animals

The recommended procedure for treating animals showing clinical illness in which anthrax is thought to be the likely or possible cause is immediate intravenous administration of sodium benzylpenicillin as directed by the manufacturer's instructions (usually in the range 12 000–22 000 units per kg of body weight) followed 6–8 hours later by intramuscular injection of long acting benethamine penicillin (manufacturers' instructions usually recommend dose within

range 6000–12 000 units per kg of body weight) or other appropriate long-acting preparation such as Clamoxyl[®] (15 mg/kg), a long-acting preparation of amoxycillin. If long-acting preparations are unavailable, procaine penicillin, (dose recommended by manufacturers is usually 6000–12 000 units/kg) can be used for intramuscular injection but should be administered again after 24 and 48 hours.

Streptomycin acts synergically with penicillin and penicillin/streptomycin mixtures are available commercially. Recommended doses of streptomycin to be administered together with penicillin intramuscularly are 5–10 mg per kg body weight in large animals and 25–100 mg per kg body weight in small animals.

Veterinary experience in Britain is that, in contrast to advice frequently found in textbooks, treatment with tetracyclines may not be fully effective.

Attention should be paid to manufacturers' recommendations regarding precautions and limitations of use and aspects relating to withdrawal periods after use in food animals.

Cost and availability are likely to be a major consideration in choice of treatment. For example, combined penicillin and streptomycin treatment can be expected to cost twice as much as penicillin alone.

7.1.2.2 Supportive therapy for animals

Symptomatic treatment may also be useful and a range of possible agents is available. Supportive therapy with an agent such as flunixin (an analgesic with anti-inflammatory, anti-pyretic and anti-endotoxic properties) may be advantageous although it will add significantly to the cost of the therapy.

7.1.2.3 Hyperimmune serum therapy for animals

Hyperimmune serum has been used in the past for treating anthrax cases (Sterne, 1959) and it was generally considered that homologous sera (e.g. serum prepared in cattle for bovine use, etc.) were more effective than heterologous sera. Serum treatment of livestock is apparently still practised in Russia.

The protective effect of immune serum administered therapeutically was demonstrated in monkeys (Henderson *et al.*, 1956). However, these were challenged by the inhalation route and, once the effects of the immune serum wore off at about 20 days, the animals began to die of anthrax from continued uptake into the lymphatics of spores that still remained in the lungs. The possibility of relapse after the effect of this therapy has worn off should be borne in mind.

7.1.2.4 Countries prohibiting treatment in livestock

It should be noted that treatment of animals is forbidden in several countries. Veterinary requirements in these countries are that, in a herd that has experienced a case of anthrax, other animals showing signs of illness must be killed without spilling of blood or exsanguination and the unopened carcass must be heat-treated in a rendering plant. Certain countries require the

slaughter of the entire herd following a case of anthrax; this draconian approach is unnecessary and wasteful.

7.1.2.5 Therapy in wildlife

While the use of antibiotics for controlling anthrax in wildlife is, generally speaking, unlikely to be feasible, it has reportedly been done with success in an outbreak in Tanzania where a single treatment of roan and sable antelope and kudu, 50 animals in total, by direct darting, appeared to arrest an outbreak (Dr SFH Jiwa, Faculty of Veterinary Medicine, Sokoine University of Agriculture, P.O. Box 3110, Morogoro, Tanzania, personal communication).

7.2 Treatment of humans

7.2.1 Mild uncomplicated cases

In mild uncomplicated cases of cutaneous anthrax, penicillin V, 500 mg, taken orally every 6 hours for 5–7 days is adequate, but the treatment usually recommended is 3 to 7 days of intramuscular procaine penicillin, 600 mg (1 million units), every 12–24 hours or intramuscular benzylpenicillin (penicillin G), 250 000 units at 6-hourly intervals. Cutaneous lesions usually become sterile within the first 24 hours of such regimens but, although early treatment will limit the size of the lesion, it will not alter the evolutionary stages it must go through (Kobuch *et al.*, 1990).

7.2.2 Severe or potentially life-threatening cases

In severely affected patients or when pulmonary or gastrointestinal anthrax is suspected, the initial treatment is penicillin G, 1200 mg (2 million units) per day by infusion or by slow intravenous injection (<300 mg/min) every 4–6 hours until the patient's temperature returns to normal; at this point treatment should continue in the form of intramuscular procaine penicillin administered as described above. Streptomycin, 1–2 g per day intramuscularly, may act synergically with penicillin.

General measures for treatment of shock may be life saving since death is due, at least in part, to toxin-induced shock. Intubation, tracheotomy or ventilatory support may be required in the event of respiratory problems, and vasomotor support with dopamine may be necessary when there is haemodynamic instability. Primary haematological, renal or liver function disorders are not generally seen.

7.2.3 Supportive treatment for cutaneous anthrax

As covered in 5.2, the swelling seen in an anthrax infection is due to the action of oedema toxin and there is very little associated inflammation. In theory, therefore, steroids should be of little value. In practice, some reports indicate that these have been administered with evidence of benefit but others (Kobuch *et al.*, 1990) have concluded that they were ineffective, discontinuing their use.

Early tracheotomy is advised if there is danger of tracheal obstruction; once oedema is extensive it can be difficult to find the trachea at operation.

7.2.4 Alternatives to penicillin

In the event of allergy to penicillin, several antibiotics are effective alternatives, including tetracyclines, chloramphenicol, gentamicin and erythromycin. Of the tetracycline family, tests in animals have indicated doxyxyclyne is very effective and that the quinolone, ciprofloxacin may also be suitable (Friedlander *et al.*, 1991). Trimethoprim is not effective.

7.2.5 Adequate doses

In vitro tests (Lightfoot *et al.*, 1990) have indicated that some strains of *B. anthracis* grown in the presence of sub-inhibitory concentrations of flucloxacillin become resistant to penicillin and amoxycillin either by induction of beta-lactamase or through some other, unidentified mechanism. It is therefore important to use adequate doses of penicillin when this is being used for treatment.

7.2.6 Duration of treatment

The appropriate duration of treatment is a subject for debate. *B. anthracis* cannot be isolated from cutaneous lesions 24 to 48 hours after commencement of antibiotic therapy and Kobuch *et al* (1990) could see no advantage to continuing treatment beyond 4 days. A report from Ethiopia (Martin, 1975) records that 100 patients with cutaneous anthrax were treated with a single intramuscular dose of procaine penicillin, 600 000 units, and 99 of these were sent home with the invitation to return if complications occurred. Only 5 returned on account of further developments. Physicians tend to continue treatment for 7 to 14 days, but it needs to be kept in mind that the lesion will continue to progress through its cycle of development and resolution regardless of the elimination of the infecting *B. anthracis* (7.2.1). Excessive treatment may be wasteful and counterproductive.

In cases of inhalation anthrax, anthrax spores have been shown to persist for many weeks in the lungs of monkeys infected experimentally by the aerosol route and kept on antibiotics (Henderson *et al.*, 1956; Friedlander *et al.*, 1991); the animals succumbed to the disease once the antibiotic treatment was stopped. Although this is unlikely to occur in the natural situation, in cases where known or suspected inhalation of anthrax spores has taken place, especially if this was likely to have been substantial, it may be prudent to consider the administration of a non-living vaccine simultaneously with treatment. The treatment should be continued for about 6 weeks to allow for development of adequate vaccine-induced immunity. (The issue of simultaneous treatment and vaccination in countries that administer live vaccines to humans is discussed elsewhere (Pomerantsev *et al.*, 1996; Stepanov *et al.*, 1996).

7.2.7 Treatment of anthrax meningoencephalitis

Lessons from the few recorded instances of survival in cases of anthrax meningoencephalitis suggest that penicillin (2 million units of crystalline penicillin intravenously every 2 hours initially) remains the antibiotic of choice because it diffuses into the CSF through highly inflamed meninges. Chloramphenicol (1 g intravenously every 4 hours) is a suitable alternative for hypersensitive patients. The effectiveness of newer antibiotics, known to achieve a satisfactory degree of penetration into the CSF, has not been determined.

Essential supportive therapy includes the early institution of anticerebral oedema measures, such as 100 ml of 20% mannitol intravenously every 8 hours and hydrocortisone, 100 mg every 6 hours. Useful references to anthrax meningoencephalitis are given in 4.4.4.

7.2.8 Hyperimmune serum therapy

The use of anti-anthrax serum was abandoned years ago in most western countries as of negligible value, but it is apparently still done in China (Dong, 1990) and the Russian Federation (Anon, 1996). With the availability of modern plasmaphoresis techniques, specific human gamma-globulin from vaccinated individuals could well be of life-saving value in an emergency. The disease is readily responsive to early antibiotic treatment but, as has been pointed out before, death is due to the toxin and delayed treatment may sterilize the blood and tissues while not preventing death. Thus, if initiated after 24–36 hours following clinical onset, antibiotic treatment may fail to save life in pulmonary, intestinal or complicated cutaneous cases. In these cases, specific gamma-globulin containing anti-toxin antibodies may be life-saving.

7.2.9 Recurrence after treatment

Recurrence of disease on termination of treatment is very rare but convalescent cases should remain under observation for at least a week after treatment has ceased.

8 Control

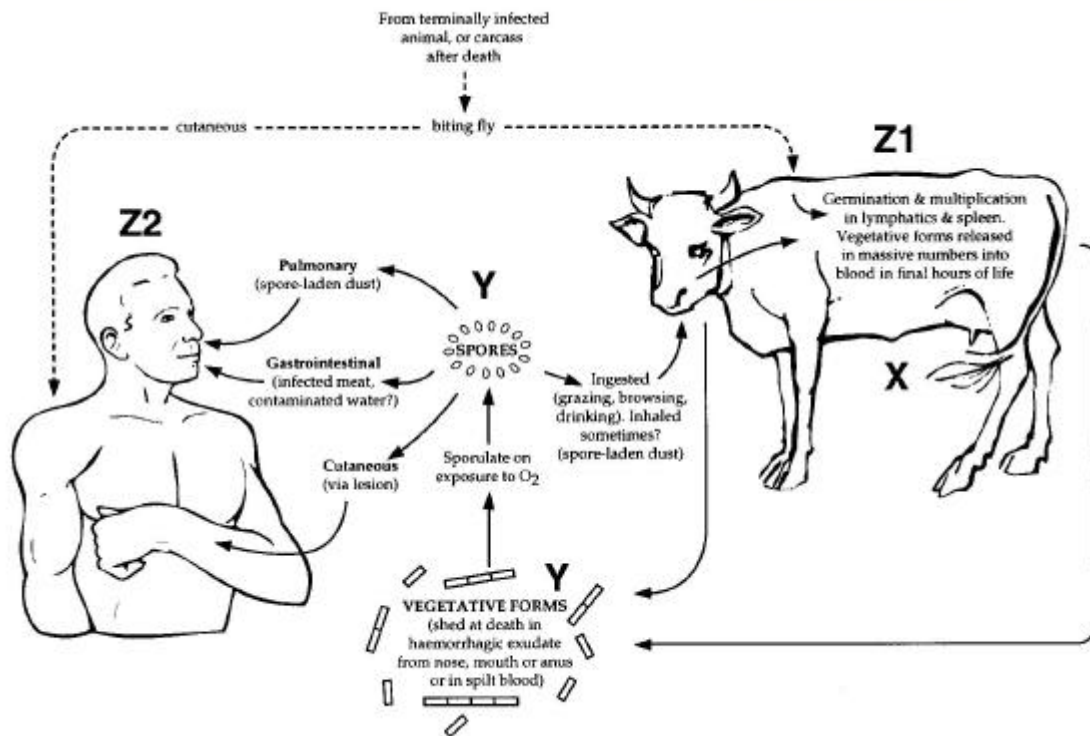
Control measures are aimed at breaking the cycle of infection depicted in Figure 3. Each of the following actions must be rigorously implemented.

- correct disposal of anthrax carcasses (point X)
- correct disinfection, decontamination and disposal of contaminated materials (point Y)
- vaccination of exposed susceptible animals (point Z1) and humans in at-risk occupations (point Z2).

8.1 Disposal of anthrax carcasses (point X in Figure 3)

Because sporulation of *B. anthracis* requires oxygen and therefore does not occur inside a closed carcass, regulations in most countries forbid postmortem examination of animals when anthrax is suspected. Most, if not all, the vegetative *B. anthracis* cells in the carcass are killed in a few days by putrefactive processes. Nevertheless, with the characteristic terminal serosanguinous exudates from the nose, mouth and anus, some organisms may escape and sporulate. The precise length of time after which no viable *B. anthracis* remain within the carcass is unpredictable but depends greatly on climatic conditions, particularly temperature.

Figure 3. (see Figure 1 [section 2.1] for further notes).



8.1.1 Alternatives

In most countries, the preferred method of disposal of an anthrax carcass is incineration, although, in some countries, it is considered that the best approach to the disposal of anthrax carcasses is an effective controlled heat-treatment or "rendering". Where neither of these approaches is possible, for example due to lack of fuel, burial is the remaining best alternative. In some developing country situations, however burial, incineration or rendering may not be feasible. The last resort in such situations is to leave the carcass unmoved and adequately closed off from other animals, particularly scavengers, or people. The carcass should be fenced

off and covered using branches of trees, corrugated iron or any other available materials, and hazard signs should be posted around the site. *B. anthracis* within the animal carcass does not sporulate and is inactivated by the putrefactive process in a few days. However, environmental contamination due to bloody exudate escaping from the mouth, nose and anus of the death animals may still occur. The resulting environmental contamination could be minimised by scorching the site with fire, after the carcass has effectively putrefies, though this may be many months later. Clearly, this is far less satisfactory than incineration, rendering or burial which should be carried out as the preferred option unless absolutely impossible.

8.1.2 Burial

Periodic reports of viable anthrax spores at burial sites of animals which died many years previously have testified to the unreliability of burial procedures for long term control of the disease. Disturbance of such sites, for example by ploughing, or laying drainage, brings the spores to the surface; even without site disturbance, spores can work their way up to the soil surface. In either case, this may result in new livestock cases.

Further disadvantages to burial sites are that scavengers may dig down to reach the carcass, and, in dry, dusty areas, the digging process can spread the contaminated soil extensively. In Wood Buffalo National Park, Canada, where burials of bison that had died of anthrax were carried out in the 1960s, the raised burial mounds became excavation sites for foxes and wolves building their dens and nesting sites for ants (D.C. Dragon, Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, Canada, personal communication).

In summary, burial should be discouraged in favour of incineration or rendering where possible.

8.1.3 Incineration

Guidelines on incineration procedures are given in Appendix III (A.III.3). Ideally, the soil surrounding and under the carcass, particularly around the nasal and anal regions, should be decontaminated (see 8.3.8) and then incinerated with the carcass.

Incineration must be done with appropriate care to ensure complete burning from beneath. Usually this involves raising the carcass off the ground before the process is started (see Appendix III [A.III.3]). Commercial incinerators designed to ensure this are available (see Figures G–J). It must be appreciated that spores that have soaked into the soil may survive the incineration process although isolation of *B. anthracis* from incineration sites is rare. The down-directed blow-torch shown in Figures K and L (see also Appendix III [A.III.3.3]), illustrates an alternative incineration procedure which ensures severe scorching of the soil to several centimetres of depth.

Comments are occasionally encountered opposing incineration on the basis that anthrax spores may survive the fire and become aerosolized in the updraft. Circumstantial evidence does not support the contention that incineration of anthrax carcasses results in the transmission of anthrax in this manner.

Blenkharn and Oakland (1989) were able to isolate Gram positive bacteria, predominantly *Bacillus* species, from the base of the exhaust stack of a hospital waste incinerator with design-specified operating temperatures of 800 °C and 1000 °C in the primary and secondary chambers respectively, thereby demonstrating that there is no room for complacency. However, numbers were very low, averaging 56 cfu per cubic metre (range 0–400 cfu per cubic metre) and would be subject to rapid further dilution on leaving the chimney. A badly constructed pyre producing smoke with little or no flame might result in a higher survival rate of organisms collected by the updraft. However, as discussed in relation to windborne spores from anthrax carcass sites (Turnbull *et al.*, 1998), the dilution effect on anthrax spores dispersed in airborne form from a carcass is such that, for even the most susceptible animal species, the risks of infectious doses arising are absolutely minimal.

A further consideration is that the anthrax organisms in an unopened carcass are in the vegetative form (see 8.1) and are highly susceptible to heat and other adverse conditions. The spores will be confined to where the blood has been shed through the mouth, nose or anus, and will mostly be in the soil beneath these points. Consequently, relatively few spore forms will enter the fire and updraft; vegetative forms are very unlikely to survive.

8.1.4 Rendering

Rendering is essentially a cooking process which results in sterilisation of raw materials of animal origin such that parts of carcasses may be utilized safely for subsequent commercial purposes.

There are a number of variations of the rendering process, broadly divided into batch processes and continuous processes. In general, the raw materials are finely chopped and then passed into a steam-heated chamber and subjected to temperatures ranging from 100 °C to 150 °C for 10–60 minutes (this does not include the time taken to bring the material to the peak temperature or the subsequent cooling period time).

The rendering procedure involves correct performance at each of three stages: collection, transport and treatment of the carcass (Riedinger *et al.*, 1975; Riedinger, 1980; Strauch, 1991). These should be supervised by veterinary authorities. The rendering plant must be properly divided into "dirty" and "clean" areas. The dirty side must be suitably equipped for disinfection of the transport vehicles and other equipment involved. Wastewater from the dirty side must be collected and autoclaved. Before the heat treatment, carcasses have to be broken down in a closed system into pieces not larger than 10 cm³. Controlled heat treatment is then carried out with temperature, pressure and time of sterilisation recorded.

The level of hygiene being maintained in the clean side of the rendering plant should be monitored at least twice yearly by the veterinary authorities.

8.2 Infection control in the management of human anthrax cases

As said in 4.3, the risk of human-to-human transmission is not a serious one given that sensible precautions are taken. For example, cutaneous anthrax lesions should be dressed for the first 24–48 hours after treatment; disposable gloves should be worn, or gloves that can be sterilized, while applying the dressing and during subsequent disposal of specimens or sterilisation of materials and equipment (see 8.3.9).

Prophylactic antibiotics and vaccination are not necessary for health workers or family contacts, though the latter should be instructed to consult their physicians in the event of suspicious signs or lesions.

In fatal cases, postmortem should be discouraged; cremation is preferable to burial where local custom permits. It is advisable for the body to be placed in an impervious body bag for transport from the place of death and preferably the body should not be extracted from the bag. Where only burial is permitted, the bagged body should be placed in a hermetically sealed coffin and buried without re-opening. Useful guidelines are available elsewhere (Healing *et al.*, 1995; Young and Healing, 1995).

Bedding and contaminated materials should be bagged and incinerated, autoclaved or fumigated as appropriate. Whether room fumigation is necessary will depend on the perceived level of contamination in the room where the patient died (see 8.2.3).

8.3 Disinfection, decontamination and disposal of infected/contaminated material (site Y in Figure 3)

In addition to helping break the local cycle of anthrax infection, disinfection, decontamination and correct disposal of infected/contaminated material are of considerable importance in preventing international transmission of anthrax. In non-endemic countries, risks arise largely from animal products – wool, hair, hides, bone, etc. – imported from endemic regions. Regulations regarding importation of untreated animal products vary from country to country but several importing countries take the view that the financial costs that would be incurred from legislating for the sterilisation of such imports would be disproportionate to the benefits.

In many countries, some or all of the following requirements are in place to limit the risks of importation or dissemination of products contaminated with anthrax spores:

- products must be accompanied by a certificate signed by a veterinary official in the country of export certifying that they derive from anthrax-free sources (see Appendix IV)
- products regarded as having a high chance of containing anthrax spores are subject to some form of monitoring or control

- finished or raw materials from certain countries may be subject to import restrictions
- finished or raw materials from certain countries may have to undergo a general sporicidal treatment before processing or distribution.

In the case of hides and skins (where no sterilisation procedure that does not damage the materials has been devised), the exporting countries nowadays often require the initial processing stages to be carried out before export for financial benefit. This has meant that far fewer anthrax-contaminated hides and skins now reach non-endemic countries than was the case a few years ago.

Sections 8.3.1–12 aim to supply information by which each country can formulate its own control programme according to its particular circumstances. However, it is clear that long-term global control depends almost entirely on the application of appropriate measures to prevent the disease among livestock in enzootic exporting countries. Ideally, national policies should ensure that materials known to be contaminated with anthrax spores are appropriately disposed of and are not included in any industrial process. The procedures in sections 8.3.10–12 are essentially designed to protect workers and the environment in situations where inadvertently contaminated materials may enter the processing line.

8.3.1 Decontamination of manure, dung, bedding, unused feed, etc.

Where possible, anthrax-contaminated materials to be disposed of, such as bedding, feedstuffs, manure, etc, should be incinerated or autoclaved (121±1 °C core temperature for 30 min). Immersion in 4% formaldehyde (10% formalin) for ≥12 hours is an alternative but full penetration of the fluids must be ensured. (*Caution: avoid skin contact with formaldehyde solutions or inhalation of formaldehyde vapour*).

8.3.2 Disinfection of surfaces in rooms, animal houses, vehicles, etc.

For general principles on disinfection and sporicidal disinfecting agents, see Appendix III.

Disinfection of contaminated surfaces involves a three-step approach aimed at (i) preliminary disinfection, (ii) cleaning, and (iii) final disinfection. Effective disinfection of spores can be extremely difficult and, under some circumstances, it may not be possible to achieve this completely. It is important, therefore, to act promptly following cases of anthrax in order to prevent, as far as possible, the release and sporulation of vegetative cells from the dying or dead animal.

Stage 1: Preliminary disinfection

One of the following disinfectants may be used in amounts of 1–1.5 litres per square metre for an exposure time of 2 hours (*Caution: avoid skin contact with the disinfectants listed below or inhalation of their vapours*):

- 10% formaldehyde (approximately 30% formalin), or

- 4% glutaraldehyde (pH 8.0–8.5)

A high pressure cleaner may be used but, to avoid spreading the contamination, the pressure should not exceed 10 bar.

Stage 2: Cleaning

Where practical, cleaning of all surfaces should be done by straightforward washing and scrubbing using ample hot water. The operator should wear protective clothing, face and hands included. Cleaning should be continued till the original colours and surfaces are restored and the waste water is free of dirt particles. At the end of the process, residual water should be removed and the surfaces dried. High pressure cleaners are again discouraged because of the greater potential to spread the contamination through aerosols. If used, however, the water jet should be applied at a pressure of 80–100 bar delivering 13–15 litres/ minute.

Stage 3: Final disinfection

For final disinfection, one of the following disinfectants should be applied at a rate of 0.4 litres per square metre for an exposure time of at least 2 hours:

- 10% formaldehyde (approximately 30% formalin)
- 4% glutaraldehyde (pH 8.0–8.5)
- 3% hydrogen peroxide
- 1% peracetic acid.

Hydrogen peroxide and peracetic acid are **not** appropriate if blood is present. When using glutaraldehyde, hydrogen peroxide or peracetic acid, the surface should be treated twice with an interval of at least one hour between applications. Formaldehyde and glutaraldehyde should not be used at temperatures below 10 °C. After the final disinfection, closed spaces such as rooms or animal houses should be well ventilated before re-commissioning.

The effectiveness of the disinfection procedure cannot be assumed and attempts should be made to confirm it has been adequate by means of swabs and culture.

In the case of surfaces within a room, it may be considered appropriate to finish the disinfection process by fumigating the room itself as described in 8.3.3.

8.3.3 Fumigation of closed spaces – cabinets, rooms, etc.

Safety cabinets should be treated as fumigation chambers and fumigated according to the procedure given in paragraph 2 of 8.3.9.

Rooms where surfaces cannot be cleared before decontamination and disinfection, such as laboratories, can be fumigated by boiling off (for rooms up to 25–30 m³) 4 litres of water containing 400 ml of concentrated formalin (37% w/v formaldehyde) in an electric kettle (fitted with a timing or other device to cut off the electricity when the fluid level has reached the

element) and leaving overnight (or no less than 4 hours from the point in time when the boiling process has been completed) before venting. Room temperature should be $\geq 15^{\circ}\text{C}$.

Before fumigation commences, all windows, doors and other vents to the outside should be sealed with heavy adhesive tape. Hazard warning notices should be posted on the door(s) and, if appropriate, windows. Proper chemical respirators should be on hand and at least one nitrocellulose disk or filter paper which has, beforehand, been dipped in a spore suspension (preferably an accepted standard, such as *B. subtilis* var *globigii* [NCTC 10073] or *B. cereus* [ATCC 12826], but failing the availability of these, the spores of the Sterne vaccine strain (34F2) of *B. anthracis* would do) and dried should be placed at some point in the room distant from the kettle. (*Caution: avoid skin contact with formaldehyde solution or inhalation of formaldehyde vapour.*)

At the end of the fumigation, the spore disk(s) should be retrieved into a sterile petri dish and the windows or vents to the outside air should be opened up. *A chemical respirator should be used for this.* A fan, or fans, assist the extraction. Doors into the room should be kept closed and other personnel prevented from passing near or through them until venting is complete. If a formaldehyde meter is available, venting should not be considered complete until levels of less than 2 ppm have been reached. In the absence of a meter, the odour of formaldehyde should have become almost undetectable before entry into the room without a respirator is allowed.

The effectiveness of the fumigation procedure is checked by placing the spore disk(s) on plates of nutrient agar containing 0.1% histidine (final concentration and added as a filter-sterilized solution after the agar has been autoclaved and cooled to 50°C). After overnight incubation at 37°C , if fumigation was properly effective, the disks should show no bacterial growth.

For health and environmental protection reasons, there are moves to replace formalin with hydrogen peroxide vapour as the recommended general purpose microbiological fumigant. Indeed, the use of formalin is being increasingly forbidden in some countries. However, the equipment needed for hydrogen peroxide fumigation is, at present, cumbersome, elaborate and expensive and is not universally available. It would not lend itself to routine fumigation of safety cabinets after every use. This section will be updated when the appropriate progress has been made.

8.3.4 In the laboratory – spills, splashes, accidents

Chlorine solutions. Commercially-prepared hypochlorite frequently takes the form of stock solutions having approximately 10% available chlorine (100 000 ppm). Thus, what is familiarly referred to in laboratories as "10% hypochlorite solutions" is a 1:10 dilution of the stock solution containing 10 000 ppm available chlorine. If solid precursors of hypochlorous acid is available, stock solutions containing 100 000 ppm available chlorine should be prepared and the required dilutions made from this.

Chlorine solutions are not highly stable and stock solutions should be titrated periodically to ensure that the correct level of available chlorine is present (see Appendix III (A.III.2.1.4)). Since

stability is affected by concentration (and also by temperature and pH), subsequent dilutions should be made only as needed and these solutions should be changed frequently (at least weekly). It should be remembered that chlorine solutions corrode metals and perish rubber and that chlorine is rapidly neutralized by organic materials, including wood (as in wooden benches), soil, or specimens of blood or tissues.

Simple chlorine solutions are slow to kill spores (Jones and Turnbull, 1996). The sporicidal rate can be increased by using 50% methanol or ethanol to make the dilutions of the stock solution.

Rapid turnover items such as pipettes, disposable loops, microscope slides, sampling spoons, etc., should be immersed overnight in hypochlorite solutions with 10 000 ppm available chlorine and then transferred to an autoclave bin or bag for autoclaving, or to a bag for incineration.

Benches should be wiped down after use with hypochlorite solutions containing 10 000 ppm available chlorine. Because of their neutralising effect on chlorine, wooden benches should be replaced by more suitable materials or covered with plastic or laminated sheeting, or with a proprietary covering designed for the purpose, such as Benchcote^T (Whatman International Ltd, Maidstone, UK).

Spills and splashes on surfaces. Some thought must be given to the nature of the material spilled. For example, freshly growing *B. anthracis* cultures will have few, if any, spores and these will be incompletely dormant and more susceptible to disinfection procedures than, at the opposite extreme, purposely prepared spore suspensions.

In general, spills and splashes on floor, bench or apparatus should be flooded with hypochlorite solution containing 10 000 ppm available chlorine and vertical surfaces should be washed or wiped down thoroughly with cloths soaked in this solution (*the operator should wear gloves and safety spectacles while doing this*). Spills and splashes from fresh cultures can be mopped up with towelling after 5 minutes; the towelling should be placed in an autoclave bin or bag and autoclaved or in a bag for incineration. Spills or splashes of spore suspensions should be left for 30–60 minutes before mopping up unless the area can be sealed off and fumigated, in which case mopping up can be done after a few minutes and fumigation carried out immediately as in 8.3.3.

An alternative approach is to cover the contaminated area with absorbent material and wet this with an excess of disinfectant. Solutions of 10% formalin, 4% glutaraldehyde or 1% peracetic acid may be more appropriate than hypochlorite, but the choice must be weighed against the greater personal protection needed when using these. HAZ-TAB disinfectant granules (Guest Medical, Edenbridge, Kent TN8 6EW, UK) are commercially prepared for this purpose.

Spills and splashes on clothing. Laboratory gowns (surgical type) are the best type of overclothes to wear in laboratories working with *B. anthracis* and disposable versions are available. In their absence, a plastic apron should be worn over the laboratory coat.

Contaminated gowns/aprons/coats should be removed immediately and placed in autoclave bins or bags and autoclaved (disposable gowns can be incinerated). Personal clothing that may still be contaminated – shoes, socks/stockings/upper garments if sleeves or collars are contaminated – should be removed as soon as possible and fumigated as in 8.3.3.

Spills and splashes on skin, in eyes. For skin contamination the area should be bathed in hypochlorite solution containing 5000 ppm available chlorine for one minute and then washed thoroughly with soap and water. Where the skin is broken (including needle-stick punctures), bleeding should be encouraged and the injury washed with copious amounts of water. The current Occupational Health wisdom is that immersing punctured skin in hypochlorite solutions does more harm than good. The appropriate medical officer should be informed and the affected person kept under observation for at least a week.

For splashes in the eye, the eye must be flushed out with copious quantities of water immediately. Avoid rubbing the eye. The appropriate medical officer should be informed and the affected person kept under observation for at least a week.

Contamination in the mouth. At the outset laboratory workers should be reminded that mouth pipetting in a microbiology laboratory is **totally unacceptable**. For contamination of the mouth with known or possible anthrax organisms, the mouth contents should be immediately spat out followed by a thorough mouth wash with hypochlorite solution containing 2000 ppm available chlorine and several subsequent mouth washes with water. The appropriate physician should again be informed and the affected person be kept under observation for a week.

8.3.5 Decontamination of liquid manure

Slurry from pigs and cattle suffering outbreaks of anthrax should be disinfected with formaldehyde added with thorough stirring to a final concentration of 2–4% depending on the content of dry matter. This is approximately equivalent to 50–100 kg of formalin (37% formaldehyde solution) per m³ of slurry. The mixture should be left a minimum of 4 days with stirring for at least one hour each day before being removed elsewhere. (*Caution: avoid skin contact with formaldehyde solution or inhalation of formaldehyde vapour*).

The formalin degrades naturally and the treated slurry can be spread on uncultivated land and ploughed in or otherwise buried.

8.3.6 Decontamination of sewage sludge

Sewage sludge containing effluents from tanneries may contain anthrax spores. Dewatered sewage sludge up to a dry matter content of 8% should be disinfected with 5% formaldehyde for 10 hours or 3% peracetic acid for 30 minutes. The disinfection process is not affected by the use of lime or polyelectrolytes for dewatering the sludge (Lindner *et al.*, 1987). (*Caution: avoid skin contact with formaldehyde or peracetic acid solutions or inhalation of their vapours*).

8.3.7 Treatment of water

It is difficult to give general advice on treatment of water. The approach chosen depends on what type of body of water is to be treated, the likely extent of the anthrax spore contamination, what volumes are involved and where the water is to go and what it may be used for after treatment. However, the choices are much the same as with other materials covered within this section 8.3.

Autoclaving is the surest way of killing spores but is only applicable to fairly small volumes of water.

Treatment with formaldehyde (5–10% final concentration) for at least 10 hours is feasible for volumes up to about 100 000 litres, as may result from industrial wastes, but holding tanks must be available and methods of neutralisation and discharge without danger to the environment must be established. Cost is a major factor in this approach also.

The merits of chlorination are debatable; the levels of chlorine necessary to ensure effective killing of spores may be hard to attain in large volumes and, if the body of water is on open ground, it is likely to contain organic matter which rapidly neutralizes the chlorine.

Filtration, as for water treatment, is probably effective as far as the emerging water is concerned but leaves unsolved the problem of contaminated filter beds.

In general, each situation must be considered on an individual basis and the best solution worked out for the particular circumstances that exist.

8.3.8 Treatment of soil

If possible, soil at the site of an anthrax carcass should be removed up to a depth of 20 cm and incinerated or heat treated (121°C throughout for 20 min). If this is not possible, it should be disinfected with 5% formaldehyde solution at 50 litre per m². Where it is necessary to decontaminate soil to greater depths, such as burial sites of anthrax carcasses, 5% formaldehyde solution should be injected below the soil surface at a rate of 30 ml for every 10 cm of depth at 0.5 m horizontal intervals across the contaminated area. (*Caution: avoid skin contact with formaldehyde solution or inhalation of the vapour*).

It is sometimes not possible to achieve sufficient penetration of even small clods of soil by formaldehyde or other sporicide solutions to result in complete kill of anthrax spores (Turnbull *et al.*, 1996), especially in the case of water-saturated or heavy soils. Decontamination failure can result when attempting chemical disinfection and the effectiveness of any such attempt should be checked by subsequent culture.

The decision on the best approach to making a contaminated site safe depends substantially on what the site is to be used for in the future. Where it is not feasible to incinerate or chemically decontaminate the soil or to remove it to an incinerator, the alternative is to close or seal off the site. Covering with concrete or tarmac for, say, a car park, is an alternative used in

industrialized countries; planting with thorny bushes surrounded by a secure fence can be an aesthetic approach.

Further guidelines are supplied elsewhere (Turnbull, 1996).

8.3.9 Other materials – clothing, tools, etc.

Where possible, contaminated materials should be incinerated or autoclaved at 121 °C for 30 minutes. In the case of non-disposable items, such as clothing, boots, tools, etc., excess dirt should be scraped off into incineration or autoclave bags and the items themselves should be soaked overnight (at least 8 hours) in 4% formaldehyde solution or 2% glutaraldehyde (pH 8.0–8.5). (*Caution: avoid skin contact with formaldehyde or glutaraldehyde solutions or inhalation of their vapours*).

Materials and equipment that cannot be autoclaved, boiled or immersed in formalin or other solutions may be fumigated in fumigation chambers of 1 to 3 m³ using 15–50 ml of 37% formaldehyde solution diluted 2–3-fold with water and boiled off with an electric element. The temperature should be ambient ($\geq 18^{\circ}\text{C}$) and exposure time overnight (at least 4 hours, but preferably more than 12 hours, especially if contamination is likely to be heavy or penetration by the fumigant into the material being fumigated is likely to be slow). The chamber should be properly constructed, airtight with a system of venting to the outside away from places of human or animal movement at the end of the fumigation procedure. The relative humidity within the chamber should be $\geq 90\%$ during the fumigation procedure.

Ethylene oxide may be used as an alternative to formaldehyde if the facilities are available. The gas is acutely toxic at concentrations of >50 ppm and can cause skin burns and blistering; it is also explosive under alkaline conditions or if exposed to certain other chemicals. Although highly effective, it is really only to be recommended where the correct facilities and expertise in its use are available.

8.3.10 Decontamination of wool and hair

Disinfection stations exist in a number of countries which import wool, hair or bristles from endemic regions and the names and addresses of these can be obtained from the relevant veterinary authorities. One established disinfecting protocol is the Duckering process. This involves five stages, each of 10 min duration at 40.5 °C: (i) immersion in 0.25–0.3% soda liquor; (ii) immersion in soap liquor; (iii), (iv) two immersions in 2% formaldehyde solution; and (v) rinsing in water. The wool or hair is finally dried in hot air and baled.

In countries where irradiation facilities are available, the preferred approach is to test samples of wool and hair from a consignment and, if positive for *B. anthracis*, to sterilize the consignment by irradiation. The dose needed to guarantee freedom from viable spores in a contaminated lot is very high; the D100 in spore suspensions of 10^8 to 10^{10} per ml have been

found to exceed 40 kGy (Bowen *et al.*, 1996). Calculations of exposure times need to take into account the size and density of the bales being irradiated.

8.3.11 Hides and skins

No hazard need be expected in situations where hides come from properly supervised slaughtering. Dry hides of uncertain origin within enzootic countries must, on the other hand, be regarded as being of high risk in terms of anthrax. Where possible these should be decontaminated by formaldehyde or ethylene oxide fumigation or by irradiation prior to processing. It is considered in some quarters, however, that no pre-processing disinfection protocol has been devised for hides and skins that does not damage them (Anon, 1959). However, the dehairing stage, which involves sodium sulphide liming with a mixture of sodium sulphide and calcium hydroxide, exposes the skins to a significant level of sodium hydroxide at high pH which probably kills any *B. anthracis* spores present (Robertson, 1948; Lindner and Böhm 1985).

Control processes in tanneries must, therefore, be primarily targeted at stages before dehairing, particularly dust control and treatment of effluent from initial washing and rehydrating stages. In tanneries processing raw hides from anthrax endemic areas, these effluents should be treated with 5% formaldehyde (starting level) for 10 hours, with adequate time being allowed for natural degrading of the formaldehyde, before discharging to sewerage. Peracetic acid (3%) for ≥ 30 minutes is an alternative but more expensive treatment.

Precautions should be taken to avoid cross-contamination of hides and skins pre- and post-treatment through appropriate controls on movement of personnel, equipment and the hides themselves (Appendix IV).

8.3.12 Bone, hoof and horn

Feed ingredients of bone, hoof and horn origin imported from endemic countries are still the cause of incidents among livestock in non-endemic importing countries. Similarly, bonemeal in fertilizers is periodically suspected of being the source of anthrax infection in humans and animals. However, in many developed countries, anthrax is becoming very infrequent. It is considered in these countries that mandatory requirements for sterilisation of these types of products for international commerce would raise the costs of these products disproportionately to the human and animal health risks involved. Consequently few countries have statutory requirements of this nature. However, some manufacturers consider it standard good practice to sterilize bonemeal before placing it on the market and this is certainly to be encouraged. Similarly it should be a reasonable policy aim in any country to collect and process separately those raw bone, hoof and horn products obtained from regular and supervised slaughtering and those obtained from sources of uncertain origin which present a higher risk in terms of anthrax.

Long-term control will be dependent on improved and effective control measures in the exporting countries. In the interim, control must depend on close adherence to the International Zoo-Sanitary Code (Appendix IV).

8.4 Prophylaxis

8.4.1 The nature of protection in anthrax

Four decades of research have shown that protection against anthrax in the susceptible host is dependent almost entirely on that host's immune response to a single antigen – the protective antigen (PA) component of the anthrax toxin, a well-defined protein of molecular weight 83 kDa (see Section 5). Immune responses to the other two toxin components, the lethal and oedema factors, may contribute to protection, but only in a relatively minor way (Ivins and Welkos, 1988; Turnbull *et al.*, 1988). No other antigens have been identified as contributory.

The effectiveness of both animal and human vaccines is dependent on the induction of anti-PA antibodies. However, the immune response is complex and involves cellular immunity in some, as yet, undefined manner; measurable anti-PA antibodies in the blood of an individual are not, in themselves, a guarantee of protected status but, on the other hand, they must be there for the individual to be protected (Ivins and Welkos, 1988; Turnbull *et al.*, 1988; Ivins *et al.*, 1990; Turnbull *et al.*, 1990). The enhanced efficacy of future vaccines over existing ones will be depend on improved stimulation of the cellular immune response in parallel with the humoral response to PA (see 8.4.4) (Quinn and Turnbull, 1998).

The mechanism by which an immune process based on anti-toxin immunity protects against a disease characterized by rapid *in vivo* growth of the bacilli is not known yet. Neutralisation of the lethal action of the lethal toxin on macrophages (see 5.2) is probably a major factor. It is also possible that some action of the toxin which results in release of nutrients needed for multiplication is inhibited.

8.4.2 Animal vaccines

Most anthrax vaccines in use around the world today utilize the toxigenic, non-capsulating (pXO1+/pXO2-) *B. anthracis* strain 34F₂ isolated in 1937 (Sterne, 1937). Earlier Pasteur-type vaccines are still in use in at least one country (see Appendix V). The animal vaccines that use strain 34F₂ are essentially as originally formulated (Sterne, 1939) with approximately 10⁷ spores per ml suspended in 0.5% saponin in 50% glycerine-saline.

Animal vaccines against anthrax should be prepared in accordance with the *Requirements for Anthrax Spore Vaccine (Live – for Veterinary Use), Requirements for Biological Substances No. 13* (WHO, 1967) which points out the considerable differences in quality that can exist between anthrax vaccines. The glycerine and saponin are important to vaccine performance. Vaccine strains must be maintained carefully since uncapsulated *B. anthracis* variants may lose their immunogenic powers on subculture (Sterne *et al.*, 1939; Sterne, 1959). Since, in theory, the

vaccine is easy to prepare, many countries appear to be taking on their own manufacture; it is important that careful quality control be exercised to avoid resurgences of anthrax because inadequate vaccines are being used.

The protective effect of a single dose of strain 34F₂ vaccine is said to last about 1 year (Sterne, 1939) and annual boosters are recommended for livestock in endemic areas. The duration of the protection has never been systematically studied and this is an area of research that needs to be done.

The 34F₂ live spore vaccines appear to retain a degree of virulence for certain species such as goats (Sterne, 1939; Cartwright *et al.*, 1987) and llamas (Cartwright *et al.*, 1987) in which they must be used with caution (see Appendix V [A.V.1.1]).

8.4.3 Human vaccines

In China and in the countries of the former USSR, live spore vaccines are prepared for human use (Appendix V). In most other countries, live spore vaccines are not licensed for use in humans. In the UK and USA, non-living human vaccines developed respectively in the 1950s and 1960s are available (Turnbull, 1991). The UK vaccine is an alum-precipitated cell-free culture filtrate of strain 34F₂ while the US vaccine is an aluminium hydroxide-adsorbed cell-free culture filtrate of a non-capsulating, non-proteolytic derivative of bovine isolate V770.

The primary use of the human vaccines is for persons in at-risk occupations, particularly in industries concerned with processing of animal products from endemic regions. The duration of the protection afforded in man by vaccination is uncertain and, on empirical grounds, annual boosters are recommended for workers in at-risk occupations. In certain circumstances, post-exposure vaccination at the same time as administration of antibiotics may be appropriate (see 7.2.6).

Vaccination of humans who are not occupationally exposed to anthrax is inappropriate. For the general public, control of the disease should be done through control in livestock.

8.4.4 Prospective new vaccines

As covered in 8.4.1, the prerequisite for protection against anthrax is an adequate humoral immunity to the protective antigen (PA) combined with an appropriate cellular immune response. This has to be taken into account in the design of new vaccines. While cellular immune stimulation can be achieved by means of an effective adjuvant, the surest way of ensuring this combined humoral and cellular response is to immunize with live vaccines. The current animal vaccine (8.4.2) is a live vaccine and its greater efficacy over the non-living human vaccines (8.4.3) in animal protection studies is attributed to its greater stimulation of cellular immune responses in the recipients.

The live spore **animal** vaccine, however, has a number of disadvantages which need to be overcome: (a) although greatly attenuated in comparison to normal virulent *B. anthracis*, the

34F₂ vaccine strain retains a residual virulence (Welkos *et al.*, 1986) and is responsible for occasional losses among vaccinated animals; (b) its duration of effect is limited, and animals in enzootic areas must be immunized annually; (c) it has to be administered parenterally, and (d) efficacy and unwanted side effects can be greatly influenced by small faults in production. Items (b) and (c) become important in developing countries where appropriate equipment and manpower are limiting factors; item (d) has increasingly become a problem as "local" production has tended to replace manufacture by a few major companies or centres.

When first introduced for administration to workers in at-risk occupations, **human** anthrax vaccines were shown to be clearly associated with protection against the disease (Brachman *et al.*, 1962). That paper contains the only existing reference to anthrax in a fully vaccinated person; since then, there have been no other such cases on record. However, protection tests in animals have indicated that currently licensed human non-living vaccines (8.4.3) may have limited efficacy and the protection they afford is of uncertain duration. Being essentially simple culture filtrates, the vaccines are somewhat undefined and they are occasionally associated with complaints of side effects. The case for the development of new vaccines rests on these issues.

The aims for prospective new vaccines are that they should be:

- well-defined with well-characterized ingredients and mode of action
- effective against challenge with any anthrax strain
- safe, giving no dangerous side-effects in any species
- long-lasting
- easy to administer rapidly and by non-skilled persons
- orally administrable, especially for livestock and at-risk wildlife species
- cheap
- environmentally acceptable.

Future vaccines are likely to take one of three forms:

(A) Subunit: non-living vaccines consisting of purified PA with an effective, well-defined adjuvant. Recombinant PA from a *B. subtilis* construct could replace PA from *B. anthracis*, thereby enabling manufacturers to produce the vaccine without having to grow or handle *B. anthracis* or to separate PA from other toxin components. For reasons of cost and practicality, subunit vaccines would only be appropriate for human use. They would still need to be administered parenterally and would continue to require regular boosters.

(B) Genetically manipulated

- Deleted mutants: deletions from the gene for the PA have now been achieved (Singh *et al.*, 1989) such that this can no longer combine with lethal or oedema factors to form anthrax toxin. A live vaccine based on a strain carrying this deletion would lack the residual virulence associated with the existing live spore vaccine. Deletion of undesired toxin factors might also eliminate adverse reactions.
- Recombinant constructs: Constructs of *B. subtilis*, *Salmonella typhimurium*, baculovirus and vaccinia virus carrying the PA gene have been made and shown to be capable of eliciting protective immunity (Ivins *et al.*, 1990; Iacono-Connors *et al.*, 1991; Coulson *et al.*, 1994). Constructs of *Lactobacillus* species are being considered as potential oral vaccines.
- DNA: The possibility that DNA coding for the appropriate protective antigens, or subunits of those antigens, may replace the antigens themselves is true in the context of anthrax vaccines as it is for a number of other diseases.

(C) Oral: The ideal vaccine is one which is only required in a single oral dose. The constructs of *Salmonella* or *Lactobacillus* species referred to in the paragraph above have been chosen for study with this in mind. Other approaches include the use of microencapsulated PA, or modified PA, possibly with other immunogens or adjuvants, or microencapsulated DNA for the appropriate antigens.

When choosing the appropriate design for an oral animal vaccine, it is important first to consider the method by which the oral vaccine is to be administered. This must be such that most, if not all, the target animals or species actually receive the vaccine at the correct dose level. Livestock industries have extensive experience of oral vaccines (other than anthrax) for domestic herds. The experience on the oral vaccination of foxes, other wild carnivores and dogs against rabies may be used for designing a suitable vaccine and vaccine delivery system for anthrax in wildlife (Schumacher and Meslin, 1998). Some consideration in this case also needs to be given to the consequences if non-target animals or species take up the vaccine. Furthermore, the vaccine must remain active sufficiently long to ensure it is effective at administration and uptake by the proposed method, but it must not persist in such a way as to cause environmental contamination. It is clear that producing the ideal oral vaccine for animals poses difficult challenges.

8.5 Intersectoral issues: the question of eradication

As with any zoonosis, intersectoral cooperation is essential for effective control of anthrax. Public health officials should be notified by the veterinary authorities in the event of outbreaks in livestock so they become alert to the possibility of associated human cases. Likewise, medical authorities should notify veterinary health officials when a human case is encountered (see 9.2).

Cooperation between livestock officials and wildlife managers in enzootic zones is more complex. While the target of the former is eradication from his or her region, the disease is regarded in game management areas as an integral part of natural population control mechanisms. Action is seen as being necessary only when endangered species are at risk and is usually targeted specifically at that species; beyond this, control actions are regarded as constituting unwarranted interference with natural processes. In this instance, cooperation needs to take the form of joint efforts to prevent mingling of susceptible livestock and wildlife species, such as adequate and appropriate fencing, and to minimize other means by which anthrax may be transmitted from wildlife to livestock.

Despite the well-known longevity of anthrax spores, decline in numbers does occur through decay and/or dispersal at contaminated sites and eradication from livestock areas is feasible, given sufficient time together with unremitting efficient application of the control procedures covered in 8.1 to 8.4.

9 Surveillance

Surveillance, the systematic collection, collation and analysis of data and the dissemination of the information to those who need to know in order that action may be taken, should provide information throughout prevention, control and elimination/eradication. Control will not be cost-effective or efficient if surveillance is not an integral part of the management of the programme.

Most countries have laid down regulations for the reporting of infectious diseases including anthrax. The problems that exist generally stem from failure to implement regulations, rather than from their non-existence (see Appendix VII).

Clearly full implementation of the regulations depends on awareness and understanding of these regulations and on cooperation by all involved. Those involved are likely to have diverse standings within the community and include farmers/owners, community elders or members of local authorities, local and regional human and veterinary health workers and officials, local and regional clinical and veterinary laboratories, and officials of the ministries of health and agriculture.

The surveillance scheme given below can be adapted to accommodate the local veterinary and public health systems within any country. Routine cross-notification between the veterinary and health sectors should be the rule, as well as close collaboration during epidemiological investigations. However, well organised and reliable reporting systems of animal and human cases still remain a distant goal for most developing countries.

A surprising number of countries use humans as sentinels as a result of differential quality and availability of medical and veterinary diagnostic laboratories, and thereby missing the majority of the livestock cases and misemphasising the impact of the disease and direction of ongoing control programmes.

9.1 Public health surveillance of anthrax in humans

9.1.1 Specific objectives

Some of the specific objectives of a public health surveillance programme can be summarised as follows:

- (a) Identify the characteristics of the disease in the affected populations.
- (b) Formulate prevention and control programmes for the human health and veterinary sectors.
- (c) Evaluate prevention and control activities through the monitoring of subsequent incidence of the disease and carry out an evaluation of the cost-effectiveness of the programme.
- (d) Detect outbreaks and monitor changes in the epidemiological patterns of the disease, modifying control activities appropriately.
- (e) Ensure regular feedback of information to all groups involved.

9.1.2 Suggested case definition

The suggested case definition should be adapted to local needs and laboratory capabilities available for confirmation of diagnosis (WHO, 1997c).

Clinical description

An illness with acute onset characterised by several clinical forms as described in detail in section 4.4. These could be summarised in a concise case definition for surveillance purposes as follows:

- (a) Localised form:
 - cutaneous: skin lesion evolving over 2 to 6 days from a papular through a vesicular stage, to a depressed black eschar invariably accompanied by oedema that may be mild to extensive.
- (b) systemic forms:
 - gastrointestinal: abdominal distress characterised by nausea, vomiting, anorexia and followed by fever
 - pulmonary (inhalation): brief prodrome resembling acute viral respiratory illness, followed by rapid onset of hypoxia, dyspnea and high temperature, with X-ray evidence of mediastinal widening
 - meningeal: acute onset of high fever possibly with convulsions and loss of consciousness, meningeal signs and symptoms

Laboratory criteria for diagnosis

Laboratory confirmation by **one or more** of the following:

- Demonstration of *B. anthracis* in a clinical specimen by microscopic examination of stained smears of vesicular fluid, blood, cerebrospinal fluid, pleural fluid, stools, etc
- Isolation of *B. anthracis* from a clinical specimen (e.g. blood, lesions, discharges)
- Positive serology (ELISA, Western blot, toxin detection, chromatographic assay, fluorescent antibody test (FAT))

Note: It may not be possible to demonstrate *B. anthracis* in clinical specimens if the patient has been treated with antimicrobial agents.

Case classification

Suspected: A case that is compatible with the clinical description and has an epidemiological link to confirmed or suspected animal cases or contaminated animal products

Probable: A suspected case that has a positive reaction to allergic skin test (in non-vaccinated individuals)

Confirmed: A suspected case that is laboratory-confirmed

9.1.3 Reporting and information flow

The *local level* (health care centres, private clinics and physicians, and other health personnel) is the first point of official contact with the infected patient and the point at which surveillance data should first be collected. Suspected rather than confirmed cases may be reported from this level to higher levels. The tasks at this level are diagnosis and case management, including treatment and health education plus, resources permitting, case and outbreak investigation.

The *intermediate level* collates and analyses data from local levels. The tasks of the intermediate level are: case management which cannot be done at the local level; analysis of data from local levels; epidemiological investigations, tracing sources of infection from infected animals or animal products; monitoring of prevention activities; provision of laboratory support; feedback of information to the local level; and reporting to the central level.

The *central level* formulates national policies and allocates resources. It provides technical support (e.g. laboratory or epidemiological) to the intermediate and local levels as appropriate, and reports to WHO.

Mandatory notification should be required for all cases of anthrax, human and animal. The system must include zero reporting of human cases; this will avoid confusion over whether “no report” is equivalent to “no cases”. All cases or outbreaks should be investigated. If this is not possible, a representative sample of cases should be followed up carefully. Routine surveillance should be carried out, particularly among high-risk groups such as those who are

occupationally exposed (e.g., selected health care providers in areas with hide processing industry). Routine exchange of information on anthrax cases between public health and veterinary services should be in place.

Minimum data elements for case-based reporting are:

- case classification (e.g. suspected, probable or confirmed)
- clinical form of the case (cutaneous, gastrointestinal, pulmonary (inhalation), meningial)
- unique identification code
- other pertinent data, such as age, sex, geographical information, occupation, date of presentation, exposure history as well as race/nationality, date of clinical onset, treatment and outcome, if possible.

9.2 Veterinary surveillance of anthrax in animals

9.2.1 Specific objectives

Some of the specific objectives of a veterinary surveillance programme can be summarised as follows:

- (a) Evaluate the health state of the at-risk animal populations:
 - investigate common source outbreaks and any connections between infected herds/flocks and human cases
 - estimate the extent and geographical distribution of the disease in the animal population
 - identify high risk areas and animal populations
 - evaluate the need for interventions, establishing priorities and allocating resources
 - facilitate planning and communication in both human and animal health sectors
 - develop a database for regular communications to all persons and institutions directly involved in anthrax control and prevention (including the media).
- (b) Evaluate prevention and control activities by monitoring disease trends and measuring the impact of programmes (programme evaluation and cost-effectiveness).
- (c) Monitor changes in the epidemiological patterns of the disease to be able to modify control activities appropriately, by monitoring:
 - changes in the animal populations and geographical areas involved
 - incidence of anthrax in livestock in order to predict the possible occurrence in humans.
- (d) Ensure regular feedback of information to all groups involved.

9.2.2 Suggested case definition

The unit of reference is usually the infected herd or flock rather than the individual animal. An identification system for single animals or herd/flock identification should be in place for effective surveillance.

Clinical description (see also section 3.4 and 3.5)

In non-immunized cattle, sheep or goat anthrax is usually an peracute disease characterised by septicaemia and sudden death with bleeding from orifices and subcutaneous haemorrhages. Other reported symptoms in cattle, horses, sheep and some wild herbivores consist of fever, dyspnoea, agitation, convulsions followed by death. In pigs, carnivores and primates the main symptoms are local oedema and swelling of the face and neck. Failure of the blood to clot, absence of rigor mortis and the presence of splenomegaly are the most important necropsy findings.

Laboratory criteria for diagnosis

Internationally recognized standard diagnostic techniques and their interpretation for diagnosis of anthrax in animals are reported in section 3.5 and Appendix I as well as in the *Manual of Standards for Diagnostic Tests and Vaccine of the Office International des Épizooties* (OIE, 1996).

Case classification

Suspected: A case that is compatible with the clinical description. *In enzootic areas all sudden death should be regarded as suspected anthrax cases.*

Confirmed: A suspected case that is laboratory-confirmed. A diagnosis based on clinical signs may be difficult, especially when the disease occurs in a new area. Therefore a confirmatory laboratory examination should be done as described in section 3.5 and Appendix I.

9.2.3 Reporting and information flow

The *local level* (field veterinarians, veterinary assistants, etc.) is the first point of official contact with the infected herd or flock and the level at which the surveillance data should first be collected. The tasks at this level are herd or flock diseases management (e.g. implementation of hygienic measures, education, carcass disposal and site decontamination, etc.) and, if resources permit, case and outbreak investigation.

In those countries where a well established and effective surveillance system is not in place, it will be necessary to provide appropriate education at the owner farmer/village level in recognising the disease, and instruction on whom a report should be made to.

The tasks at the *intermediate level* can be summarised as follows: herd or flock disease management (e.g. carcass disposal and site decontamination) and epidemiological investigations which can not be done at the local level; analysis of data from local levels;

monitoring of prevention and control activities; provision of supportive laboratory tests; and feedback of information to the local level and reporting to the central level.

At the intermediate level, consideration should be given for the establishment of an "Anthrax Watch Team" consisting of (i) an Animal Health or Livestock Officer (ideally a veterinarian), properly versed in methods of diagnosis, specimen collection and subsequent control measures for anthrax, (ii) a laboratory technician trained in confirmation of the diagnosis, and (iii) personnel trained in carcass disposal and site decontamination. The Team officers should report to the central level (e.g. national Animal Health Center).

The *central level* analyses data from the intermediate level for epidemiological links, trends and achievement of control targets, formulate national policies and allocate resources. It provides technical support (e.g. laboratory or epidemiological) to the intermediate and local levels as appropriate, coordination of national prevention, surveillance and control activities, feedback of analysis data to the intermediate level, and possibly to the local level and reports to OIE.

It is implicit that appropriate national funding will be necessarily such that reporting by the owner/farmer should incur no penalty and that reporting does not involve uncompensated cost to the farmer/owner, the animal health officers or the laboratory as long as the appropriate and regulated procedures are followed. Anything that discourages reporting should be avoided and everything that encourages reporting should be embraced. Suggested approaches to ensuring this are:

- the reasons for discouraging or prohibiting owners from using sudden death carcasses for food and from using or selling the hides, bones, meat, etc. from such carcasses, unless it is established beyond doubt by the relevant veterinary officials that death was not due to anthrax or some other transmissible disease, should be carefully explained to owners by appropriate education programmes. It must be clear to them that butchering of anthrax carcasses presents a highly significant risk to those involved as well as seriously adding to ground contamination;
- compensation to the owner should be available for any carcass proven (i) to be a case of anthrax and (ii) to have been incinerated or disposed of by another officially approved method;
- quarantine should be kept as non-onerous as possible; it need not be longer than 21 days after vaccination of the affected herd has been carried out (or 21 days after the last case if that occurs after the vaccination campaign);
- animal health officers should be properly equipped with specimen collection materials, equipment and materials necessary for disinfection, clean-up and disposal and with antibiotics and vaccine plus syringes and needles. They should also have the necessary transport and suitable office back-up for reporting cases;

- the laboratory technician should be supplied with the appropriate equipment and materials for confirmation of diagnosis;
- appropriate educational literature for all persons involved from the farmer/owner to ministry levels should be produced. Every effort should be made to get local understanding and popular support for the policy of carcass destruction and herd vaccination;
- the reporting system should be based more on incentives than penalties although penalties for failure to report may be desirable. The reporting should not incur cost (e.g. forms, postage, etc.) to those required to make reports;
- treatment and vaccination of the remainder of the herd/flock and the services of the animal health officers in assisting with cleaning and disinfection should be free of charge.

9.4 Recommended data analyses, presentation, reports

Data are used to generate indicators which consist of synthetic information from which can be derived any particular aspect of the state of the disease (e.g. human disease incidence, herd/flock incidence, animal disease incidence, percentage of herds/flocks vaccinated, etc.) and its dynamics (e.g. percent variation of herd incidence in different years, percent of variation of vaccinated animals between consecutive years, etc.), together with information of the corresponding control programme in a population. Surveillance indicators from the veterinary and public health services are needed to monitor the performances of the prevention, surveillance and control activities. Data and indicators can be displayed for easy interpretation in graphs (e.g. number of suspected/probable/confirmed cases by age, sex, month and place), tables (e.g. number of suspected/probable/confirmed cases by age, sex, month and place) and maps (e.g. number of suspected/probable/confirmed cases by place). The use of mapping or other geographical information tools for surveillance of suspected endemic/epidemic and free areas is recommended.

9.5 Strategies of anthrax surveillance in animals

In countries or areas free of anthrax, the disease is treated as an exotic or foreign disease and measures to be adopted are based on primary prevention, mainly the control of imported animals and animal products (see appendix IV).

In enzootic countries or areas, whether or not animal vaccination is carried out, all suspected sudden deaths in animals should be investigated as possible cases of anthrax. Flocks or herds in direct or indirect contact with positive human cases should be monitored. The primary prevention measures mentioned above should also be in place. In enzootic countries or areas where animal vaccination has been discontinued particular emphasis must then be placed on continued surveillance.

In enzootic countries or areas without animal surveillance and control programmes the main strategy to protect the population should be health education, promoting primary prevention in humans (e.g. education to improve food and occupational hygiene), and specifically targeting high risk populations.

Appendix I Methods

A.I.1 General standard laboratory procedures for isolation, identification, diagnosis and confirmation of anthrax

Various procedures have been followed and described in texts over the years. Those found most satisfactory in the Anthrax Reference and Research unit, Centre for Applied Microbiology and Research, Porton Down, Salisbury, England, is shown schematically in A.I.1.4.1 and A.I.4.1.2 below.

Type of specimen

The approach taken will depend on the type of specimen being examined which, for the purposes of examination will fall broadly into (a) fresh specimens from untreated animals or humans, (b) specimens from treated animals or humans, and (c) specimens from old and decomposed animal carcasses or from animal products or environmental specimens.

A.I.1.1 Fresh specimens from untreated animals or humans

As said in section 3.5, few difficulties should be encountered in (i) identifying *B. anthracis* in M'Fadyean capsule-stained smears of from blood, lymph or oedematous fluid from untreated animals shortly before or after death from anthrax, or (ii) isolating *B. anthracis* from these types of specimen. Similarly, the bacteria should be readily visible in, or isolated from, vesicular fluid before treatment in humans, or, so long as no treatment was given, from body fluids near to death or at post-mortem.

A.I.1.2 Specimens from treated animals and humans

Treatment of an animal suffering from anthrax may sterilize the blood and tissues even though the animal may go on to die from the effect of the toxin. Similarly, cutaneous lesions in humans will be quickly sterilized by treatment but will continue to pass through their stages of evolution and resolution.

Residual forms of the capsulated bacilli may be visible in fluid smears from such animals or persons. Isolation generally will not be possible. Confirmation of diagnosis may be possible with a sensitive detection device for the toxin, such as that of Burans *et al.*, (1996). It is hoped this will come more widely available in the future.

A.I.1.3 Specimens from old or decomposed animal specimens or from animal products or environmental specimens

As said in section 6.1, the problem likely to be encountered with this group of specimens is that detection will frequently involve a search for relatively few *B. anthracis* amongst many other

Bacillus species, particularly *B. cereus*. The selective approach shown in the flow diagrams is necessary.

A.I.1.4 Suggested procedure for isolation and identification of *B. anthracis* and confirmation of diagnosis

See schematic flow diagrams A.I.1.4.1 and A.I.1.4.2

A.I.1.5 Molecular diagnosis

Newer technologies for diagnosis are being reported with increasing frequency for a number of diseases. The immunochromatographic Protective Antigen assay of Burans *et al.*, (1996) (see A.I.3.2 below) has already been alluded to. As well as being highly reliable and very sensitive in detection of the toxin in specimens from animals that have died of anthrax, it has the advantages of not requiring an expensive item of equipment such as the microscope, of having a built in control, of not requiring microbiological or veterinary/medical skills to use it and of being simple and portable.

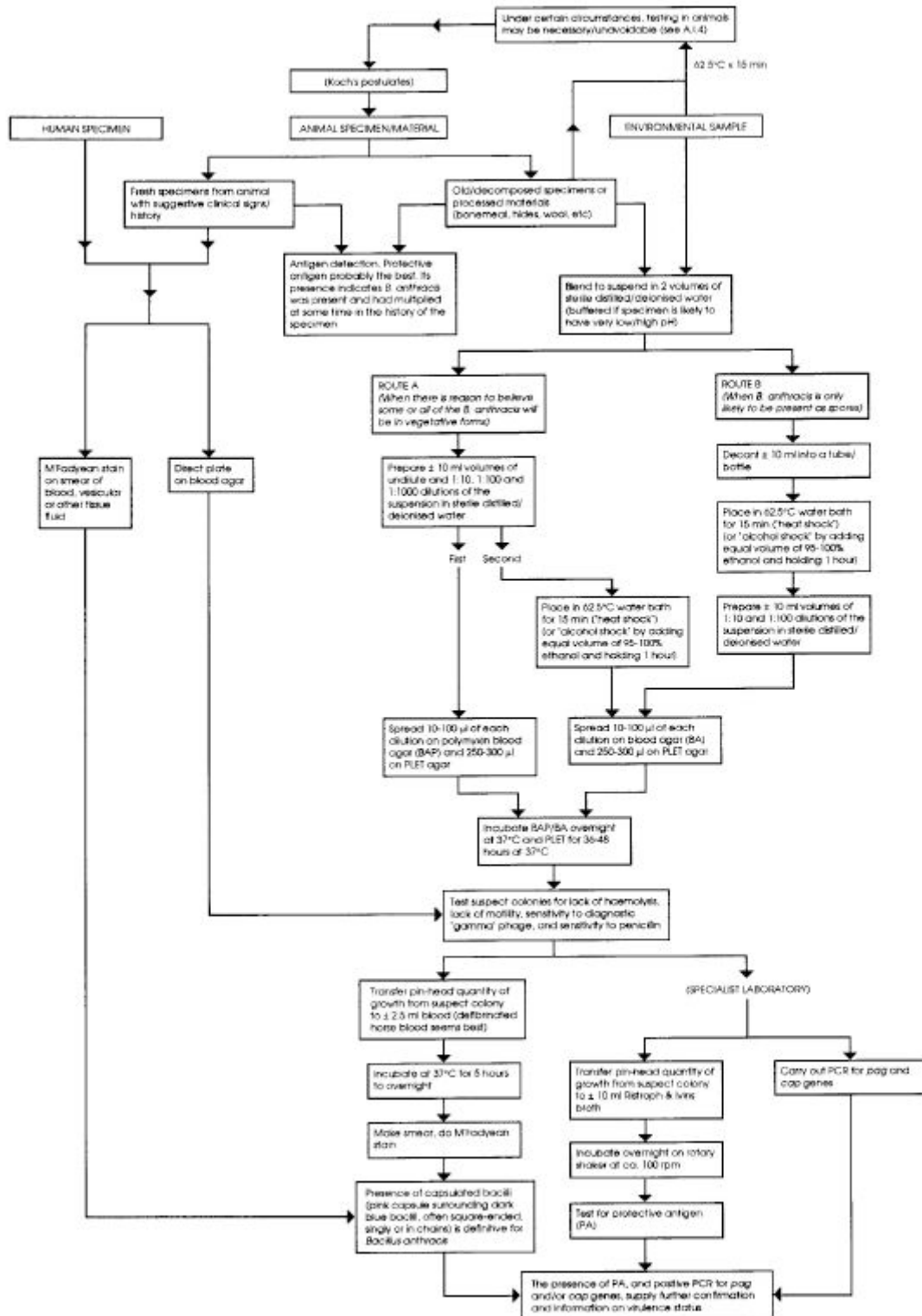
The polymerase chain reaction (PCR) has not yet become a standard method for direct diagnosis of anthrax, although, as covered below (A.I.6), it has become an important test for confirming the virulence of *B. anthracis* isolates.

A.I.2 Capsule visualisation

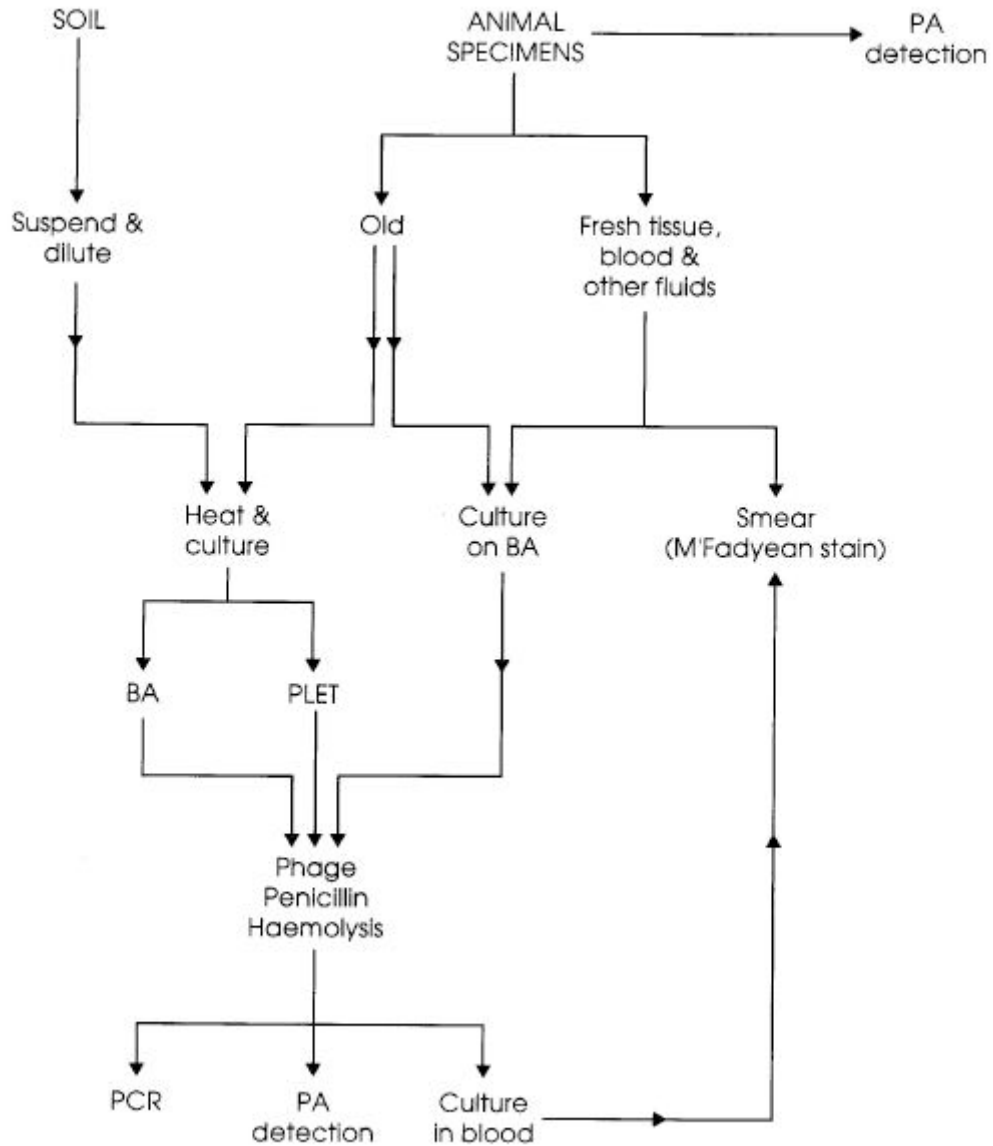
A.I.2.1 Polychrome methylene blue stain (M'Fadyean reaction)

Dating from 1903 (M'Fadyean, 1903), this probably remains both the simplest and most reliable method of confirming the presence of capsulated *B. anthracis*. [NOTE: the Gram stain is not suitable for looking for capsulated *B. anthracis*].

A.1.1.4.1 Flow diagram of suggested procedure for isolation and identification of B.anthraxis and confirmation of diagnosis



A.1.1.4.2 Simplified version of A.1.1.4.1 (following Route B for old animal specimens or environmental samples)



A.I.2.1.1 Staining theory

Polychrome methylene blue is a complex mixture of methylene blue and substantial amounts of other homologs, primarily azure A and azure B, which are produced by oxidation ("ripening") which takes place in methylene blue solution upon standing. Natural ripening takes a year or more to complete but can be hastened by addition of 1% K_2CO_3 to Loeffler's alkaline methylene blue.

Two alternative formulae (others probably exist) for Loeffler's alkaline methylene blue are given in the table below.

Agent	Formula A	Formula B
Methylene blue	0.3 g	1.5 g
95% ethanol	30 ml	100 ml
Dissolve the methylene blue in the ethanol		
KOH	100 ml of a 0.01% solution	3.3 ml of a 1% solution
Distilled/deionised water	-	330 ml

After addition of K_2CO_3 to 1% final concentration, distribute the stain in bottles to half-full. Shake at intervals to aerate the contents. Test periodically for capsule staining ability. Quality control is not complete, however, until the stain has been validated on specimens from anthrax cases in the field (see cautions in A.I.2.1.2 below).

Polychrome methylene blue is an ingredient of Wright's and Leishmann's stains. Both these are methanol solutions of a water-*insoluble* precipitate formed when eosin is added to a methylene blue solution. In Wright's stain, the methylene blue solution contains $NaHCO_3$ and is steamed before the eosin is added; in Leishmann's stain, the methylene blue solution is held for 12 hours at 65 °C followed by standing for 10 days. Giemsa stain is a deliberately formulated cocktail of the eosinates of methylene blue with pre-purified azure A and azure B.

Romanowsky in 1891 was the first to combine eosin with methylene blue so Wright's, Leishmann's and Giemsa stains are referred to as Romanowski stains. In theory, these stains, in having the ingredients of polychrome methylene blue, should be usable for M'Fadyean reaction staining; in practice, reports suggest that Giemsa stains give variable results, possibly reflecting the variable presence of certain active but undefined impurities present in true polychrome methylene blue. The experience of the Anthrax Reference and Research unit at Porton Down, UK, is that the capsule will probably not be rendered visible by Giemsa stain.

We propose that the best stain to use for normal diagnostic purposes in sudden death cases is true polychrome methylene blue - that is, the non-eosinated ripened Loeffler's methylene blue.

However, in the extensive experience of Sterne (1959), confirmed over the past few years by Dr Pauline M. Lindeque in the Etosha National Park, Namibia, the capsule loses its affinity for methylene blue during putrefaction and may no longer be visible with M'Fadyean's stain. Dr Lindeque (personal communication) finds that the Giemsa-type stains show up the characteristic square-ended shape of the anthrax bacillus better than M'Fadyean's stain and this becomes advantageous in partially decomposed carcasses as encountered frequently in the wildlife situation.

In the experience of Lindeque and Turnbull, a particularly reliable stain in the recent past has been CAM's Quick-stain (C.A. Milsch [Pty] Ltd, P.O. Box 943, Krugersdorp, 1740, Republic of South Africa).

A.I.2.1.2 Polychrome methylene blue staining procedure

- (a) make a thin smear of blood, tissue fluid, etc. by spreading a small (1-5 μ l) drop on a microscope slide with the edge of a coverslip (*remember this is potentially infective material; discard the coverslip into hypochlorite solution*). Air dry and fix by dipping in absolute, or 95% methanol or ethanol for 30-60 seconds and re-drying
- (b) put a large drop (approx. 20 μ l) of polychrome methylene blue on the smear, spreading with an inoculating loop to cover all parts of the smear. Leave for 30-60 seconds ("flooding the slide" with the stain is unnecessary and wasteful)
- (c) wash the stain off with water into hypochlorite solution. Blot, dry and examine. The bacteria can be seen as fine threads of varying length under the low power (10x) objective. Under oil immersion (100x objective), the capsule is seen clearly (pink) surrounding the blue-black, often square-ended bacilli. (*Discard the blotting paper and slide into containers destined for autoclaving or into hypochlorite solution when finished with*).

Cautions on negatives

Very little is known about the development and changing nature of the capsule with time, but it has been noted by Turnbull and Lindeque (unpublished), and in occasional other personal communications, that commercial polychrome methylene blue may fail to reveal the capsule in specimens from anthrax victims in the field while being able to do so with artificially induced capsules obtained by *in vitro* growth of the organisms in blood or on bicarbonate agar (see A.I.2.2 below). It is suspected that this is related to inadequate maturation of the stain. The consequences are considerable, however, and laboratories are urged to ensure new stocks are thoroughly tested for reliability before old stocks are finished and discarded.

A.I.2.1.3 India ink method

This, of course, is not a true staining method but highlights the capsule as a transparent halo around the bacillus. With good capsule preparations, such as blood from a freshly-dead animal

or smears of bacilli from mucoid colonies on bicarbonate agar grown under CO₂ (see A.I.2.2.2 below), it is satisfactory. It may be less sensitive when smaller numbers of anthrax bacilli are present or when the bacilli are dead and disintegrating as may be the case in specimens from old carcasses or from animals/humans that were treated before the specimens were collected.

Procedure

Premix a loopful of the blood or other tissue fluid with a small drop of India ink on a clean slide such that a thin layer results when a cover slip is placed on top and pressed down lightly. If the India ink is too dark, dilute appropriately with water.

As before, the bacteria can be found by scanning under low power (10x objective) and then examined under oil immersion (100x) for the presence of the capsule.

A.I.2.2 Induction of capsule formation

A.I.2.2.1 In blood

As indicated in the flow diagrams (A.I.1.4 above), capsule formation can be demonstrated by transferring a pin-head quantity of growth from a suspect colony to approximately 2.5 ml defibrinated blood (see next paragraph) in a sterile test tube or small bottle and incubating 5 hours to overnight. A smear is made from the blood and stained and examined as described above.

Theoretically blood from any species should do but, in our experience, there is great animal-to-animal variation and it is necessary to identify a source of reliable blood giving good capsule production. Defibrinated horse blood seems to be best. In the absence of a local source, this may be obtained commercially from Tissue Culture Services (TCS Biologicals Ltd, Botolph Claydon, Bucks MK18, 2LR, UK, phone 44 296 714071, fax 44 296 714806).

A.I.2.2.2 On agar plates

Plate the suspect colony onto nutrient agar containing 0.7% sodium bicarbonate (see Appendix II) and incubate overnight at 37 °C under CO₂ (a candle jar does very well for general purposes, but 20% CO₂, as supplied from a gas cylinder is best if it is necessary to be specific). Capsule-forming strains of *B. anthracis* form mucoid colonies (non-capsulating strains are rough). Smears of these can be stained and examined as described above.

A.I.3 Antigen detection tests

A.I.3.1 Ascoli precipitin test (thermostable antigen test)

This test dating from 1911 (Ascoli, 1911) was designed to detect *B. anthracis* antigens in the tissues of animals being utilized in animal by-products and thereby to reveal when these products contained ingredients originating from animals that had died of anthrax; i.e., its purpose is to supply rapid retrospective evidence of anthrax infection in an animal. Over the

years, it has been one of the most valuable tools for controlling anthrax in most European countries, and it remains in use, particularly in eastern Europe.

It needs to be borne in mind that this test is not rigorously specific for *B. anthracis*. The thermostable antigens involved are common to other *Bacillus* species so the test depends on the fact that the only *Bacillus* likely to have proliferated within and throughout an animal depositing extensive precipitating antigens in the tissues is *B. anthracis*.

The test is **not** suitable for detection of *B. anthracis* in environmental specimens; numerous other *Bacillus* species can be expected to occur in these. Application of the immunochromatographic assay of Burans *et al.*, (1996) (A.I.3.2 below) may be the replacement of the future.

Procedure

- (a) chop or slice the specimen into fine pieces or strips
- (b) boil approximately 2 g of the specimen for 5 min in 5 ml saline containing 1:100 (final concentration) acetic acid. Alternatively, soak in saline containing 0.5% phenol for 24-48 hours in a refrigerator
- (c) after cooling, filter through filter paper till completely clear
- (d) insert a few drops of the antiserum in the bottom of a small test-tube and carefully add some of the filtrate from (b) down the side of the tube to form a layer of antigen above the antiserum. (As an alternative to using a test-tube, and more economical on the antisera, capillary tubes can be used as in the Lancefield test for streptococcal grouping)
- (e) appropriate positive and negative specimen controls should be included.

Antiserum for the Ascoli test

(Commercially prepared donkey or mule serum is available from Bioveta plc, Komenského 212, 683 23 Ivanovice na Hané, Czech Republic, fax +42 507 932 84)

To prepare in rabbits, on days 1 and 14, the rabbits should be inoculated subcutaneously with the animal anthrax vaccine (Sterne strain 34F₂). On days 28 and 35, further subcutaneous injections of 0.05 ml of a suspension in physiological saline of a mixture of several strains of virulent *B. anthracis* should be administered. The viable count of this suspension should not exceed 100 000 colony-forming units/ml. After a further 10 days, a test-bleed will reveal the activity of the antiserum; if not adequate, further injections of the suspension of virulent *B. anthracis* should be administered at 7-10 day intervals.

If considerations of safety prevent the use of live virulent *B. anthracis*, the mixture of several strains of *B. anthracis* can be suspended to a final count of 10⁸-10⁹/ml in physiological saline containing 0.2% formalin. This is held until sterile (at least 2 weeks). After the vaccine strain

inoculations on days 1 and 14 as before, increasing doses of 0.1, 0.5, 1 and 2 ml of the formalized suspension should be administered intravenously at approximately 4-5 day intervals. A test bleed should be done 10 days after the last injection. Further 2 ml doses can be administered if the titre is not adequate initially.

A.I.3.2 Other antigen detection tests

Over the years numerous attempts have been made to identify truly anthrax-specific antigens (i.e. antigens not shared by other *Bacillus* species) and to design antigen-based detection tests for *B. anthracis*. Most of the resulting systems did not reach the point of being tried out extensively in the field or were only available to highly specialized laboratories.

Although there is evidence that a variety of anthrax-specific antigens do exist, the only ones that are at all readily isolated and purified are the toxin antigens. Capsular antigen is probably also fairly specific but is poorly immunogenic and, therefore, of limited use outside the specialized laboratory.

The highly specific immunochromatographic assay of Burans *et al.*, (1996) utilizes a monoclonal capture antibody to the anthrax protective antigen (PA) bound to a nitrocellulose membrane, and a second monoclonal antibody specific for a different epitope of PA bound to colloidal gold particles which become visible when they accumulate at the capture sites. The assay can detect as little as 25 ng/ml of PA and is performed in a few minutes without the need for special reagents. It therefore lends itself to direct diagnosis of cases of anthrax by detection of PA in the blood or body fluids, or to retrospective analysis of extracts from the types of sample of animal origin for which the Ascoli test was designed.

A.I.4 Isolation in animals

On account of increasing concern to eliminate the use of laboratory animals wherever possible, and of the increasing reliability and sophistication of alternative *in vitro* methods, the use of animals for isolation or confirmation of identity of *B. anthracis* can and should generally be avoided. It should be noted that EC Directive 86/609/EEC relating to protection of animals used for experimental and other scientific purposes pertains to members of the EU.

There still are occasions, however, such as those where potential legal disputes may be involved, when confirmation of the presence or the virulence of *B. anthracis* is necessary. In the absence of a selective enrichment system (see 6.1), inoculation of mice or guinea pigs, essentially as done more than a century ago by Pasteur, is still probably the most sensitive isolation method. Pending the development of equally sensitive conventional immunological or DNA-based techniques, animal tests may offer the only chance of (i) confirmation of diagnosis in certain situations such as in the case of individuals or animals that were treated before specimens were taken, or (ii) detection of the organism when present in very low numbers in environmental samples, or in environmental samples containing sporostatic chemicals.

Confirmation of identity or of virulence can be done by injecting light suspensions (approximately 10 000 colony-forming units/ml) into mice (0.05-0.1 ml subcutaneously) or guinea pigs (0.1-0.2 ml intramuscularly). Virulent *B. anthracis* will kill the animals at about 42-48 hours; M'Fadyean-stained blood smears examined at death will reveal large numbers of the capsulated bacilli which can also be isolated and confirmed bacteriologically.

In the rare situation in which it is necessary to use animals to isolate *B. anthracis* from soil or other environmental samples, the animals should be inoculated the day before with subcutaneous doses of mixed gas-gangrene antisera (extremely difficult to obtain, however) and anti-tetanus serum. Heated (62.5 °C for 15 min) soil extracts, as prepared for plating on selective or non-selective agar (see A.I.1.4.1), are then injected (0.05-0.1 ml subcutaneously in a mouse or up to 0.4 ml intramuscularly in a guinea pig [0.2 ml in each thigh muscle]). M'Fadyean-stained blood smears from any animals that die are examined for the presence of the typical capsulated *B. anthracis* which can also be isolated and confirmed bacteriologically.

A.I.5 Serology

Effective serological enzyme immunoassays (EIA) for confirmation of the diagnosis of anthrax have been designed now and have proved to be useful diagnostic, epidemiological and research aids (Turnbull *et al.*, 1992). The usual provisos for any serological confirmatory test apply, namely, that (i) two or more serum samples taken 2-4 weeks apart will give greater diagnostic reliability, (ii) if only one serum sample is collected, it will be of greater diagnostic value if collected more than a week after onset of symptoms, and (iii) negative or weak results be interpreted in the light of treatment the patient or animal may have received early on in the course of the infection. The last is particularly important in anthrax since, as covered under section 7 on treatment, antibiotic therapy rapidly kills infecting *B. anthracis* and, if carried out early enough in an infected individual, may prevent the elaboration of sufficient antigen to induce a detectable immune response.

Available immunoassays for anthrax are mostly based on antibodies to the toxin antigens, primarily the protective antigen component of the toxin (see Section 5 and A.I.3.2). Although not difficult to perform (any standard EIA methodology will do), they are, at present, confined to a few specialist laboratories capable of preparing the necessary purified toxin antigens.

It is hoped that an immunochromatographic assay for detecting anti-PA antibody, analagous to the assay for detecting PA (A.I.3.2), may become available in the foreseeable future for retrospective confirmation of diagnosis.

A.I.6 PCR

Template DNA for PCR from *B. anthracis* colonies can be prepared by transferring a loopful of growth from a young culture on nutrient agar to 25 µl of sterile deionised (or distilled) water

(SDW) and heating to 95 °C for 10 minutes. Following cooling to approximately 4 °C, and brief centrifugation, 5 µl of this is taken for the PCR reaction.

Suitable primers (Hutson *et al.*, 1993; Beyer *et al.*, 1996) for confirming the presence of the pX01 and pX02 plasmids are given in the following table:

Target	Primer ID	Sequence 5'-3'	Product size	Conc.
PA	PA 5 3048-3029	TCC TAA CAC TAA CGA AGT CG	596 bp	1 mM
	PA 8 2452-2471	GAG GTA GAA GGA TAT ACG GT		
Capsule	1234 1411-1430	CTG AGC CAT TAA TCG ATA TG	846 bp	0.2 mM
	1301 2257-2238	TCC CAC TTA CGT AAT CTG AG		

PCR can be carried out in 50 µl volumes using the above primers, 200 µM each of dATP, dCTP, dTTP and dGTP, 1.5 mM MgCl₂ and 2.5 units of amplitaq polymerase all in NH₄ buffer, followed by addition of 5 µl of the template DNA.

Alternatively, "Ready-To-Go™" beads are available from Pharmacia Biotech (Uppsala, Sweden, product number 27-9555-01). These are pre-mixed, pre-dispensed, dried beads, stable at room temperature, containing all the necessary reagents, except primer and template, for performing 25 µl PCR reactions. The template can be added in a 2.5 µl volume.

For strains of *B. anthracis* lacking both pX01 and pX02, the primers specific to the S-layer shown below (J.E. Bowen and C.P. Quinn, unpublished; kindly supplied by J.E. Bowen) can be included to confirm the presence of *B. anthracis* chromosomal DNA. It is probably advisable to run the S-layer primers alone rather than in multiplexed with PA and capsule primers.

Target	Primer ID	Sequence 5'-3'	Product size	Conc.
S-layer	Upper 391-413	CGC GTT TCT ATG GCA TCT CTT CT	639 bp	0.2 mM
	lower 1029-1008	TTC TGA AGC TGG CGT TAC AAA T		

The following PCR cycle can be used: 1 x 95 °C for 5 min; 30 x (95 °C for 0.5 min followed by 55 °C for 0.5 min followed by 72 °C for 0.5 min); 1 x 72 °C 5 min; cool to 4 °C.

After PCR 10% of the reaction volume of tracking dye (0.02% xylene cyanol, 0.02% bromophenol blue and 50% glycerol) is added to each PCR tube, and 10 µl loaded into wells on a 1% agarose gel in TBE (45 mM Tris-base, 45 mM boric acid, 1 mM EDTA pH 8.0, diluted 1 in 10) and the gel electrophoresed for 1 h at 80 V followed by staining in ethidium bromide solution for visualisation under UV light. A 1-kilobase ladder for size markers should be included in the outer wells of the gel.

Appendix II - Media and Reagents

A.II.1 Polymyxin blood agar

This is useful for testing unheated suspensions of old decomposed or processed animal specimens or environmental specimens and reduces or prevents growth of many Gram negative species.

Polymyxin B sulphate should be added to a level of 100 000 units per litre of medium to the cooled blood agar base at the same time as adding the blood.

A.II.1.1 Polymyxin-lysozyme-EDTA-thallos acetate (PLET) agar

PLET (Knisely, 1966) is the best selective agar currently available for isolation of *B. anthracis* from animal or environmental specimens contaminated with other organisms including other *Bacillus* species.

Difco heart infusion agar (or Difco heart infusion broth with other agar base) (Difco Laboratories, Detroit, MI 48232-7058, USA) is made up according to the manufacturer's instructions. EDTA (0.3 g/l) and thallos acetate (0.04 g/l) are added before autoclaving. (*Note: thallos acetate is poisonous and should be handled with care; avoid skin contact or inhalation of the powder while weighing out*). Heart infusion agars of other manufacturers or other nutrient bases, such as brain-heart infusion, can be made to work, but the optimal concentrations need to be determined; the concentrations recommended for normal use are unlikely to be optimal for use in PLET. A starting point for determination of optimal concentration is 25 g/l of dehydrated broth plus agar at the manufacturer's recommended concentration.

After autoclaving, the agar is cooled to 50 °C and polymyxin (30 000 units/l) and lysozyme (300 000 units/l) are added. (It is important that the agar is left at 50 °C for long enough to ensure that this temperature has been reached throughout the medium before the polymyxin and lysozyme are added). After swirling to ensure even suspension of the ingredients, the agar is poured into petri dishes.

Sporulation agar

Peptone USP	15.0 g
Yeast extract	3.0 g
NaCl	6.0 g
Dextrose	1.6 g
Agar	12.0 g
MnSO ₄	0.1 g
Water	1000 ml

Sterilize at 121 °C for 15 minutes, cool to 55 °C, and dispense into the appropriate containers (bottles, petri dishes, etc.) to set.

0.7% bicarbonate agar

The agar is prepared by reconstituting enough nutrient agar base powder for 100 ml of agar in 90 ml of water. Autoclave and cool to 50 °C in a water bath. Add 10 ml of a filter-sterilized (0.22-0.45 µm filter) 7 % solution of sodium bicarbonate. Mix and pour into Petri dishes.

The source of the nutrient agar may be important. If capsule formation is not obtained (see A.I.2.2.2), agar from another source should be tried. Oxoid nutrient agar (Unipath Ltd, Wade Road, Basingstoke, Hampshire RG24 8PW, UK, Fax +44 1256 463388) appears to be reliable.

A.II.1.2 Diagnostic gamma-phage

As reviewed elsewhere (Redmond *et al.*, 1996), anthrax-specific phages were first isolated in the 1950s. The specifically-named gamma phage was first reported in 1955 and became the standard anthrax diagnostic phage, although it is clear that it is just one of a family of closely related anthrax phages (Redmond *et al.*, 1996). On the rare occasion phage-negative *B. anthracis* or phage-positive *B. cereus* are encountered. The phage must, therefore be used in conjunction with the other tests indicated in 6.2 and A.I.1.4. With that proviso, it is a very useful and reliable test.

Phage suspensions may be obtained from central veterinary laboratories or central public health laboratories.

The phage can be propagated and concentrated by the following protocol:

Stage one

- i) Spread a blood agar (BA) plate of the Sterne vaccine strain of *B. anthracis*. Incubate at 37 °C overnight (o/n)
- ii) Inoculate approximately 10 ml of nutrient broth (NB) with growth from the BA plate and incubate at 37 °C for about 4 hours (until just cloudy), then refrigerate.
- iii) Spread 100 µl of the culture from (ii) on three pre-dried BA plates and incubate at 37 °C for 30-60 min.
- iv) Spread 100 µl of the phage suspension to be amplified over the same plates. Incubate at 37 °C o/n.
- v) Harvest the phage-lysed growth on the BA plate in 5 ml of NB followed by a second wash of 5 ml NB. Incubate at 37 °C o/n.
- vi) Filter (0.45 µm) and count by dropping 20 µl drops (3 drops per dilution) of 10-fold dilutions of the filtrate in saline onto lawns of the *B. anthracis* culture prepared as in (iii).

Stage two

This is essentially the same procedure as Stage one, only using the filtrate from (vi) to harvest the phage from the plates, i.e.:

- vii) Prepare 3 Sterne strain lawns on BA, as in (iii). Incubate at 37 °C for 30-60 min.
- viii) Spread 100 µl phage from (vi). Incubate at 37 °C o/n.
- ix) To 9 ml of filtrate from (vi), add 1 ml of 10x concentrated NB.
- x) Harvest the phage from (viii) with 5 ml of (ix), followed by a second 5 ml wash with the rest of (ix).
- xi) Add 10 ml of 1x NB.
- xii) Incubate at 37 °C o/n, filter and count.

Stage three

- xiii) Inoculate 100 ml of brain-heart infusion broth with approximately 2.5 ml of the culture from (ii). Incubate on a rotary shaker at 37 °C until just turbid.
- xiv) Add the 20 ml of filtrate from (xii) and continue incubation o/n.
- xv) The resultant filtrate is checked for sterility and titrated in ten-fold dilutions on lawns of the vaccine strain as in (vi) to determine the concentration of the phage. This should be of the order of 10^8 - 10^9 plaque forming units per ml.

Appendix III

Disinfection, Decontamination and Incineration of Carcasses

A.III.1 Introduction

This appendix is concerned with control of anthrax through targeting the reservoirs of *B. anthracis*, taking into account the chemical and physical decontamination procedures it is susceptible to, and detailing the practical details of these procedures.

Attention is drawn to the importance of handling the concentrated liquid disinfectants referred to with caution using gloves and aprons or overalls and goggles or eye shields to prevent contact with skin or eyes. Clean water for immediate washing or showering should be at hand for immediate washing or showering in the event of an accident while handling concentrated disinfectants. All containers of disinfectants should be properly and accurately labelled as to their contents. Peroxides may be explosive under certain circumstances.

Appropriate (chemical) respirators should be worn by personnel disinfecting or fumigating closed spaces (rooms, stables, etc.) and when opening up such places to ventilate them at the end of the disinfection or fumigation procedure.

Lists of approved disinfectants published periodically in some countries can be misleading when selecting the sporicidal disinfectant to use for *B. anthracis* and, as is clear from Section 8, procedures which are both practical and effective have yet to be worked out for numerous situations. Probably because *B. anthracis* is an obligate pathogen and depends for the continued existence of the species on the survival of the spores in the environment between infections of successive hosts, it appears to produce particularly tenacious spores. The results are that (i) only a few fairly formidable chemicals and procedures (Section 8.3.2) are capable of reliably killing anthrax spores, and (ii) information on the sporicidal activities of disinfectants based on other *Bacillus* species should be viewed with caution.

A.III.2 Disinfectants

The principal disinfecting agents for destruction of anthrax spores are formaldehyde, glutaraldehyde (at pH 8.0-8.5), hydrogen peroxide and peracetic acid (Dietz and Böhm, 1980; Böhm, 1990). Hypochlorites are sporicidal but are rapidly neutralized by organic matter and, therefore, while good for items like laboratory surfaces (not wooden ones) or glassware, or for water treatment, are unsuitable for disinfecting most environmental sites or materials. Hydrogen peroxide and peracetic acid are not appropriate if blood is present. The agents should have been tested for their sporicidal activity according to recommendations below and

validity test results of two independent laboratories should be included on the manufacturer's product information sheet.

As said in 8.3.3, for environmental protection and human and animal health hazard reasons, alternatives to formaldehyde as the recommended general purpose disinfectant have been sought. The information in this Appendix will need to be updated in the future when and if satisfactory alternatives have been identified.

If heat treatment or incineration of the contaminated material is possible, this should be done in preference to chemical decontamination and disinfection. For certain materials or animal by-products, irradiation with gamma rays or particle bombardment or fumigation with a gaseous disinfectant such as ethylene oxide may be appropriate (see also Sections 8.3.10-8.3.12).

A.III.2.1 Efficacy tests for sporicidal disinfectants

Useful information on the sporicidal efficacies of disinfectant solutions can be obtained from the Kelsey-Sykes capacity test (Kelsey and Sykes, 1969), which is now published as a British Standard, BS 6905:1987 (Estimation of the Concentration of Disinfectants used in 'Dirty' Conditions in Hospitals by the Modified Kelsey-Sykes Test). However, it is officially concerned with the bactericidal, rather than the sporicidal efficacy of a product. A US Association of Official Analytical Chemists method (AOAC Official Method 966.04 - Sporicidal Activity of Disinfectants) uses surgical silk sutures and porcelain "penicylinders". At present there is no European Standard method for sporicidal efficacy testing. A procedure based on the methods accepted and used in Germany (Anon, 1972; 1976), also aimed at testing sporicidal efficacy on surfaces rather than in liquid suspensions, was detailed in the previous edition of these guidelines (Turnbull, 1993).

A.III.2.2 Titration of available chlorine in hypochlorite solutions

Hypochlorite is a strong oxidising agent and will oxidize iodide ions to form elemental iodine. The iodine so formed may be titrated with standard sodium thiosulphate using starch solution as an indicator. The starch solution can be made by making a paste of 0.1 g of soluble starch with a little water and transferring the paste to 100 ml of boiling water. Boil for one minute. Allow the solution to cool and add 2 to 3 g of potassium iodide. The solution should be kept in a stoppered bottle.

- (i) The chlorine solution to be tested should be diluted to an estimated 10 000 ppm (see 8.3.4).
- (ii) Fill a clean 50 ml burette with 0.1M sodium thiosulphate solution.
- (iii) Accurately pipette 5 ml of the solution being tested into a clean flask and acidify with 5 ml of glacial acetic acid. Then add approximately 0.2 to 0.3 g of potassium iodide to the solution which now becomes orange in colour.
- (iv) Titrate the mixture by adding the sodium thiosulphate from the burette until the colour is pale yellow.
- (v) Add 5 drops of the starch solution and continue the titration until the blue colour of the starch is just detectable (it will look slightly pink now).
- (vi) Note the

burette reading and then continue to add the sodium thiosulphate dropwise. The burette reading immediately preceding the observation of a colourless solution is the end point.

Note the volume of sodium thiosulphate added from the burette and calculate the available chlorine in the test solution from the expression:

$$1 \text{ ml } 0.1\text{M sodium thiosulphate} \equiv 0.00355 \text{ g chlorine}$$

Correcting for the original dilution of the concentrated sample
and converting to a percentage,

$$\text{Available chlorine (\%w/v)} = \text{Titre} \times 0.00355 \times 10 \times 20.$$

A.III.3 Guidelines on incineration of carcasses

(Modified from MAFF, 1992)

The underlying physical principle that has to be addressed in designing an efficient incineration procedure is that material underneath a flame can remain cool so that contaminated materials (ground, soil, carcass remains, etc.) that remain below the flames during incineration will remain contaminated. A number of approaches can be taken to ensure incineration is fully effective and the one of choice depends on available resources and other circumstances. In some countries, portable incinerators with gas-fired jets at base level and 0.25 m above base level are available. Similarly flame guns are available in some countries; in directing the flames downwards, these provide a good way of ensuring complete and effective incineration.

The following suggestions are offered to cover the different circumstances which may be encountered. It should be pointed out that all the procedures described below take many hours for a large domestic animal, such as a cow.

A.III.3.1 Pit method

For a large animal, a pit about 0.5 m deep and exceeding the length and breadth of the carcass by about 0.25 m on each side should be dug. A trench approximately 0.25 m wide by 0.25 m deep should be dug along the length of the centre of the pit extending beyond the ends by about 0.75 m; this serves the purpose of allowing air for the fire under the carcass. The bottom of the pit and the trench should be covered with straw which is then soaked in kerosene.

Above the kerosene-soaked straw, place a few pieces of heavy timber (or other type of beams which will hold the carcass well above the bottom of the pit) across the pit and then scatter thin pieces of wood over beams and straw. Then add larger pieces of wood and, if available, coal, until the pit is filled top ground level. Saturate all the fuel with kerosene.

The carcass can then be drawn onto the pyre, preferably propped up so that it is lying on its back. Further kerosene should be poured over the carcass. The fire is started at either end of the longitudinal trench. Once the incineration is well underway (probably after about the first

hour), the pyre should be covered with corrugated iron or other metal sheeting in such a way as to reduce heat loss without cutting off the ventilation.

The approximate quantities of fuel that will be needed for a large domestic animal are 20 kg of straw, 10 litres of kerosene, and either 2 tonnes of wood or 0.5 tonnes of wood and 0.5 tonnes of coal. Note: it will be necessary to decontaminate the ground where the carcass lay and from where it was removed to the pit and also the ground, equipment, etc. contaminated during this moving process (see Section 8.3.8, 8.3.9).

A.III.3.2 Raised carcass method

This method may be appropriate when labour is scarce or the ground unsuitable for the construction of a pit.

Place straw over a 2 m by 1.5 m area. Place two wooden beams (approximately 2 m lengths of small tree trunks, railway sleepers, etc.) over the straw parallel to each other and about 1.25 m apart and aligned with the direction of the prevailing wind. Soak the straw with kerosene and cover with thin and thick pieces of wood and coal if available as in A.III.3.1. Place further stout cross-pieces of wood or other material across the two main beams to support the carcass. The fuel (wood or coal) is banked up either side (but not at the ends where the air must be allowed to enter under the carcass) of the carcass and fuel and carcass further doused with kerosene.

The fire can then be started and as before, when well underway, it should be covered with metal sheeting to retain heat but without inhibiting ventilation. Further fuel should be added if and when necessary.

Rather more fuel may be required than with the pit method. For a large domestic animal, an estimate is 0.75 tonnes coal + 0.5 tonnes wood or, if coal is unavailable, approximately 3 tonnes of wood, plus 20 kg straw and 20 litres of kerosene.

As with A.III.3.1, it will be necessary to decontaminate the site where the carcass lay before incineration and the ground and equipment contaminated in moving it from there to the pyre (see Section 8.3.8, 8.3.9).

A.III.3.3 Commercial incinerators

(see also section 8.1.3)

A.III.3.3.1 Down-directed blow torches

An example of the use of down-directed blow-torches for incineration of a carcass is shown in Figures K and L.

A.III.3.3.2 Portable incinerator

An example of incineration of a bovine anthrax carcass in a portable incinerator is shown in Figures G-J.

A.III.3.3.3 Centralized incinerators

Largely since the advent of the focus on BSE, commercial incinerators capable of taking whole bovine carcasses have now become available. It seems feasible for an anthrax carcass to be well-bagged in the same manner as if it was being taken to a rendering facility, and to be taken for incineration at one of these types of incinerator. While this would appear to be perfectly practicable, under the current legislation in certain countries, there would be problems obtaining movement orders permitting the transport of the carcass.

As with A.III.3.1 and A.III.3.2, it will be necessary to decontaminate the site where the carcass lay before removal and any equipment contaminated when bagging it.

Appendix IV

International Zoo-Sanitary Code

(OIE 1997b)

Chapter 3.1.1 – ANTHRAX

Preamble

For vaccine standards, reference should be made to the Manual (OIE, 1996).

Article 3.1.1.1

For the purposes of this *Code*, the *incubation period* for anthrax shall be 20 days.

Article 3.1.1.2

Veterinary Administrations of importing countries should require:

for domestic ruminants, equines and pigs the presentation of an *international animal health certificate* attesting that the animals:

1. showed no clinical signs of anthrax on the day of shipment;
2. were kept for 20 days prior to shipment in an *establishment* in which no case of anthrax was officially reported during that period; and/or
3. were vaccinated, not less than 20 days and not more than six months prior to shipment.

Article 3.1.1.3

Veterinary Administrations of importing countries should require:

for wild ruminants, equines and pigs the presentation of an *international animal health certificate* attesting that the animals:

1. showed no clinical signs of anthrax on the day of shipment;
2. were vaccinated not less than 20 days and not more than six months prior to shipment.

Article 3.1.1.4

Veterinary Administrations of importing countries should require:

for products of animal origin (from ruminants, equines and pigs) destined for use in animal feeding,
the presentation of an *international sanitary certificate* attesting that these products:

1. originate from healthy animals;
2. have been processed to ensure the destruction of both bacillary and spore forms of *Bacillus anthracis*.

Article 3.1.1.5

Veterinary Administrations of importing countries should require:

for products of animal origin (from ruminants, equine and pigs) destined for industrial use the
presentation of an *international sanitary certificate* attesting that these products:

1. originate from healthy animals;
2. have been processed to ensure the destruction of both bacillary and spore forms of *Bacillus anthracis*;
3. have been kept in areas where anthrax is not prevalent.

Appendix V - Vaccines

A.V.1 General

The history and theory of anthrax vaccines is covered in Section 8.4. This Appendix lists the names, addresses, telephone and fax numbers and other salient data that could be traced on available anthrax vaccines. The list is almost certainly not exhaustive and WHO would like to hear from any manufacturer not listed. Manufacturers are requested to inform WHO of any information that is not correct; corrections will be published in subsequent supplements or revisions of the guidelines.

Important note: The list is supplied for the benefit of users of the guidelines but does not represent endorsement of the products by the World Health Organization (WHO). While every attempt has been made to ensure the data given are correct, WHO does not guarantee the absolute accuracy of the information supplied in these lists. For confirmation of the data and for further information about the products, the reader should contact the relevant manufacturer directly.

A.V.1.1 Veterinary vaccines

For the most part, the veterinary vaccines appear to have been manufactured broadly in line with the WHO "Requirements for Anthrax Spore Vaccine" (WHO, 1967) and using spores of the 34F₂ "Sterne" strain suspended in glycerol with saponin added as an adjuvant essentially as first formulated by Sterne (1939). One vaccine is still formulated with the Pasteur strains dating from before Sterne's vaccine while at least two vaccines use alternative vaccine strains.

For further information, enquiries should be addressed to the manufacturers directly.

Cautions applicable to all these vaccines are:

- store in a refrigerator; do not freeze (repeated freeze-thawing will result in reduced inocula)
- antibiotic treatment will interfere with vaccine performance. Animals being vaccinated should not receive antibiotics for several days before or after vaccination (for example, if cattle are on anti-mastitis therapy, the vaccine may be rendered ineffective)
- animals should not be vaccinated later than 6 weeks before slaughter for human consumption
- Sterne himself (1937; 1939; Sterne *et al.*, 1939) noted that horses were slow to develop immunity following vaccination; some manufacturers recommend that the initial schedule in the horse should be two doses with a one-month interval (and a single annual booster thereafter)

- goats are well-known as being prone to severe reactions in response to the vaccine. One possible approach to vaccination of goats is an initial schedule of two inoculations one month apart with the first dose being one-quarter of the standard recommended dose ("pre-inoculation dose") and the second dose being the standard recommended dose. A single annual booster may be administered thereafter. One manufacturer recommends that injection of the vaccine in goats should be done in the tail-fold region (compared with the neck region in most species)
- injection should be made through an area of clean, dry skin
- pregnant animals should not be vaccinated unless the risk to them of acquiring anthrax is very high
- being a live spore vaccine, leftover vaccine vials, used syringes, needles and other contaminated items should be disinfected, autoclaved or incinerated after completion of the operation (see Section 8.3.9)
- in the event of accidental self-inoculation by the operator, gentle pressure should be applied to the wound to squeeze out any inoculum followed by thorough washing with soap and water. If saponin is included in the vaccine, there may be a painful local reaction at the site of inoculation. Medical advice should be sought if infection sets in.

A.V.2 Vaccine failures in livestock

Questions do arise from time to time regarding cases of anthrax which occur in herds which have been vaccinated, or about continuing cases after vaccination to control outbreaks. Usually it is not possible to identify the specific reasons for these vaccine failures, but the following points may be helpful:

Tests in guinea pigs show that:

- (a) *the antibody response in different individuals can vary.* Variable antibody titres are a feature in animals receiving a particular dose of a live spore vaccine.
- (b) *receiving the correct dose is important.* Very marked differences in titres are seen in groups receiving one million and ten million spores in a dose.
- (c) *protection tends to be less than 100% in animals if (i) they have only received a single dose, and (ii) the doses were less than 10^7 spores.*

Extrapolating this to livestock, circumstances which lead to the animals not receiving the correct dose are probably important. Examples of such circumstances in mass vaccination campaigns might be:

- the vaccine spores in the reservoir of the automatic syringe settle, so some animals get a reduced dose

- the reservoir containing the vaccine runs out and some animals, although injected, actually receive no vaccine
- the needle gets blocked, or the needles blunted, resulting in a reduced dose or no vaccine being administered
- some of the animals are fairly wild and move violently before delivery of the vaccine is complete

Other considerations that may lead to vaccine failures are:

- some or all of the animals are on antibiotics, for treatment or growth promotion
- the animals were too young at vaccination, *per se* or because the vaccine effect was neutralized by maternal antibody
- the potency of the vaccine itself has fallen for reasons beyond the control of the person or team carrying out the vaccination.

Enzyme immunoassay studies in the Anthrax Reference and Research Laboratory, CAMR, Porton Down, UK, on cattle with a range of vaccination histories have revealed a great variability in titres among animals with identical vaccination records; a few even exhibited low or negative titres despite multiple boosters (unpublished results). The reasons for this variability were not identified. It was not possible to establish a correlation, or lack of it, between low titre and succumbing to anthrax. However, the observation does serve to underscore the need to take considerable care to ensure all animals receive the correct dose in a vaccination campaign and also that all other conditions are favourable to optimal vaccine performance at the time of the campaign.

A.V.3 Manufacturers of live spore (veterinary) vaccines

MANUFACTURERS OF VETERINARY ANTHRAX VACCINES			
Country	Manufacturer	Description	Dose*
Australia	CSL Ltd. 45 Poplar Road Parkville Victoria 3052 Tel.: +61 3 9389 1372 Fax: +61 3 9389 1100	Strain: 34F2 Adjuvant: saponin	Sheep, goats, pigs: 0.5 ml s.c. Cattle: 1 ml s.c. Horses: 2 s.c. doses of 1ml separated by a 1-month interval
Bangladesh	Animal Husbandry Laboratory Mohakhali Dhaka Animal Husbandry Laboratory Cossilla		
Britain (UK)	Biological Products Unit Central Veterinary Laboratory New Haw Surrey KT15 3NB Tel.: +44 1932 357641 Fax: +44 1932 357701	Strain: 34F2 Adjuvant: saponin	Sheep, goats, pigs: 0.5 ml s.c. Cattle: 1 ml s.c.
China (People's Republic)	National Control Institute of Veterinary Bioproducts & Pharmaceuticals Ministry of Agriculture 30 Baishiqiao Road Beijing 100081 Tel.: +86 2568844 ext.261 Fax: +86 831 6545	Strain: 34F2 Adjuvant: ? (Contact manufacturer)	Small animals: 1 ml Sheep, pigs: 2 ml Cattle, horses; 2 ml
Colombia S.A.	Laboratorios Erma Avenida El Dorado N 90-31 A.A. 98835 Bogota D.E. Tel.: +57 1 295 0900 Fax: +57 1 410 2424 Laboratorios Probiol Ltda Diagonal 183 N 41-71 Apartado aereo 8001 Bogota Tel.: +57 1 671 1023 Fax: +57 1 671 1066 Laboratorios ERMA Av. El Dorado No. 90-31 Apartado Aereo 98835 Tel:/Fax: (+57-1) 2950900/4102410 Santafé de Bogotá. E-mail: Laberma@ col.1.telecom.com.co	Strain: 34F2 Adjuvant: saponin Strain 34F2 Adjuvant: (contact manufacturer) Sterne/Saponin	Sheep, goats, pigs: 1 ml s.c. Cattle, horses: 2 ml s.c. Sheep, goats, pigs, cattle, horses: 2 ml Cattle and horse: 20 x10 ⁶ Subcutaneous (2ml) Sheep and Goats: 10x10 ⁶ Subcutaneous (1ml) Annual revaccination 50ml flasks

MANUFACTURERS OF VETERINARY ANTHRAX VACCINES

Country	Manufacturer	Description	Dose*
Colombia S.A. cont.	LAVERLAM Carrera 5 No. 47-165 Salomnia Industrial Apartado aéreo 9985 Cali. Valle del Cauca Phone/fax: (+57-2) 4474411/4474409	Sterne/Alumini um hydroxide	Cattle: 20x10 ⁶ Subcutaneous (2ml) Other species: 10x10 ⁶ Subcutaneous (1ml) Annual revaccination 50ml flasks
	Laboratorios V.M. Autopista Norte kilómetro 19 Urbanización Industrial El Pilar Apartado aéreo 15453 Phone/fax: (+57-1) 6760901/6760823 Santafé de Bogotá.	Sterne/Saponin	Cattle and horse: 20x10 ⁶ Subcutaneous (2ml) Sheep and goats: 10x10 ⁶ Subcutaneous (1ml) Annual revaccination 10, 20, 50 and 100ml flasks
	VECOL Av. El Dorado No. 82-93 Apartado aéreo 7476 Phone/fax: (+57-1) 2633100/2638331 Santafé de Bogotá. E-mail: Vecol@insat.net.co	Sterne/Buffered glycerin	Cattle and horse : 15- 20x10 ⁶ Subcutaneous (2ml) Sheep, goats and pigs: 7- 10x10 ⁶ Subcutaneous (1ml) Annual revaccination 50ml flasks and box of ten, 10ml flasks
Czech Republic	Bioveta plc Komenského 212 683 23 Ivanovice na Hané Tel.: +42 507 933 21-4 Fax: +42 507 932 84 (or 94)	Strain: 34F2 Adjuvant: saponin Name of product: "Antraxen inj.ad us. vet."	Sheep, goats, pigs, cattle, horses: 1 ml s.c.
Ethiopia	National Veterinary Institute P.O. Box 19 Bebre - Zeit Tel.: +251 133 8388 Fax: +251 133 8844	Strain: 34F2 Adjuvant: saponin	Sheep, goats, pigs: 1 ml Cattle, horses: 2 ml
France	Rhone Mérieux 17 Rue Bourgelat BP 2006 69227 Lyon Cedex 02 Tel.: +33 7272 3000 Fax: +33 7272 3069	Strain: 34F2 Adjuvant: saponin	Sheep, goats, pigs: 1 ml Cattle, horses: 2 ml
Hungary	Phylaxia-Sanofi Veterinary Biologicals Co Ltd P.O. Box 68 1475 Budapest Tel.: +36 1 262 9505 Fax: +36 1 260 3889	Strain: 34F2 Adjuvant: saponin	Sheep, goats: 0.5 ml Cattle: 1 ml

MANUFACTURERS OF VETERINARY ANTHRAX VACCINES			
Country	Manufacturer	Description	Dose*
India	Institute of Veterinary Preventive Medicine Ranipet - 632 402 North Arcot Ambedkar District Tamil Nadu Tel.: +91 4172 22633	Strain: 34F2 Adjuvant: none	Sheep, goats: 1 ml injected s.c. in tail fold Cattle, pigs, horses, camels: 1 ml s.c. Elephants: 1 ml s.c. with second dose of 3 ml after 1 month
Indonesia	Pusat Veterinaria Farma Jalan Jenderal A. Yani 68-70 Kotak Pos WO.3 Surabaya 60231 Tel.: +62 31 816123 Fax: +62 31 814126	Strain: 34F2 Adjuvant: saponin	Sheep, goats, pigs: 0.5 ml s.c. Cattle, horses: 1 ml s.c.
Italy	Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata Via Manfredonia, 20 I-71100 Foggia Tel: +39 0881 786 111/786 300 Fax: +39 0881 786 362	Strain: Pasteur 2 Adjuvat: saponin Strain: Pasteur 1 Adjuvant: saponin	Cattle (> 6 months): 0.25 l, s.c. Cattle (< 6 months), sheep: 0.125 ml, s.c. Horse, goats: 0.125 ml, s.c.
Japan	The Chemo-Sero-Therapeutic Research Institute 668 Okubo Shimizu Kumamoto 860 Tel.: +81 96 344 1211 Fax: +81 96 345 1345	Strain: 34F2 Adjuvant: none	Cattle & horses only: 0.2 ml s.c.
Kenya	Cooper Kenya Ltd Wellcome Centre Kaptagat Road (off Waiyaki Road), P.O. Box 40596 Nairobi Tel.: +254 580612 Fax: +254 632123	Strain: 34F2 Adjuvant: ?	Sheep, pigs: 0.5 ml s.c. Cattle, horses: 1 ml s.c. (It is recommended that goats should not be vaccinated without first consulting veterinary surgeon)
Korea (South)	Choong Ang Animal Disease Laboratory Seo TaeJeon P.O Box 312 408-1 Sa Jung Dong Choong-Ku TaeJeon Tel.: +82 42 581 2991 Fax: +82 42 581 5856	Strain: 34F2 Adjuvant: saponin	Sheep, goats, pigs: 1.0 ml Cattle, horses: 2.0 ml
Nepal	Biological Products Div. Central Animal Health Centre Veterinary Complex Tripureswor Kathmandu Tel.: + 977 211 335	Strain: 34F2 Adjuvant: saponin	All species: 1 ml

MANUFACTURERS OF VETERINARY ANTHRAX VACCINES

Country	Manufacturer	Description	Dose*
Netherlands	Institute for Animal Science and Health P.O. Box 65 (Edelhertweg 15) 8200 AB Lelystad Tel.: +31 320 238238 Fax: +31 320 238 050	Strain: 34F2 Adjuvant: saponin	Small animals: 0.5 ml s.c. Sheep, goats, pigs, young foals and calves: 0.5 ml s.c. Adult cows, horses: 1 ml s.c.
Pakistan	Vaccine Production Laboratories Brewery Road Quetta	Details not obtained	
România	Institutul National de Medicina Veterinara "Pasteur" 77826 Sos. Giulesti 333 Sector 6 R-7000 Bucuresti Tel.: +40 1 220 6486 Fax: +40 1 220 6915	Strain: 1190 R – Stamatin Adjuvant: saponin	Cattle (> 2 months): 0.5 ml s.c. Sheep, pigs (> 2 months): 0.2 ml s.c. Horses (>6 months): 0.2 ml s.c. Goats (>2 months): 0.1 ml s.c.
South Africa (Republic of)	Onderstepoort Biological Products Private Bag X 07 0110 Onderstepoort Tel.: +27 12 529 9111 Fax: +27 12 546 0216 Telex: 322088 SA	Strain: 34F2 Adjuvant: saponin (Al[OH] ₃ for goats)	All species: 1ml s.c.
Turkey	Etlik Hayvan Hastalıkları Arastırma Enstitüsü 06020 Ankara Tel.: +90 4 321 1200 Fax: +90 4 321 1755	Strain: 34F2 Adjuvant: saponin	Sheep, goats, pigs: 0.5 ml s.c. 2-6 month old lambs, kids: 0.25 ml s.c. Horses, mules, donkeys, cattle, camels: 1 ml s.c. 2-6 month old horses, donkeys, mules, calves: 0.5 ml s.c.
Russian Federation	All Russian Institute of Veterinary Virology and Microbiology Pokrov Biological Plant 601121, pos. Volginskiy, Vladimirskaya oblast Tel.: +7 09243 6-7110	Strain: 55	Four versions to meet different needs Doses vary with different versions of the vaccine. (Further information from Dr V. Gavrilov at given address)
Uruguay	Interifa SA, Rhône Mérieux José Ma. Penco 3427 11700 Montevideo Tel.: +598 29 70 91 Fax: +598 23 78 52	Strain: 34F2 Adjuvant: saponin	Sheep, goats, pigs: 1 ml s.c. Cattle, horses: 2 ml s.c.

MANUFACTURERS OF VETERINARY ANTHRAX VACCINES			
Country	Manufacturer	Description	Dose*
Uruguay <i>cont.</i>	Laboratorio Prondil S.A Barros Arana 5402 C.P. 12200 P.O.Box 15147 Dto.5 Montevideo Uruguay Tel : +598 25 13 32 54 Fax: +598 25 13 32 52 E-mail: pron@netgate.com.uy	Anthrax only Strain : 34F2 Adjuvant: saponin	1. Sheep, goats, pigs: 0.5 ml s.c.
		« Blanthrax » Combination anthrax and blackquarter (pending registration) « Supervax » Combination anthrax, blackquarter, botulism C,D (S. African strains)	Cattle, sheep, goats only: 2 ml s.c. Cattle, sheep, goats only : 2 ml s.c.
USA	Colorado Serum Co. 4950 York Street Denver, CO 80216-0428 Tel.: +1 303 295 7527 Fax: +1 303 295 1923	Strain: 34F2 Adjuvant: saponin	All domestic farm animals: 1 ml s.c. Booster recommended 2 to 3 weeks after first dose in heavily contaminated areas
Zambia	Central Veterinary Research Institute P.O. Box 33980, Lusaka Tel.: +260 1 216031/211389/233445 Fax: +260 1 2334444/236283/252608 E-mail: cvri@zamnet.zm	Strain: 34F2 Adjuvant: saponin	Sheep, goats, pigs: 0.5 ml Cattle, horses: 1 ml

*s.c. = subcutaneous

No reply was received from a number of these companies/institutes to a questionnaire sent to obtain confirmation of this information, but we have reason to believe they manufacture and/or distribute the vaccine in their regions. Other bodies included in the previous edition of this document (Turnbull, 1993), but for whom no direct or indirect information was available, have not been included in this table.

A.V.4 Manufacturers of human anthrax vaccines

MANUFACTURERS OF HUMAN ANTHRAX VACCINES			
Country	Manufacturer	Description	Dose*
Britain (UK)	(For UK Department of Health) CAMR Porton Down Salisbury, Wiltshire SP4 0JG Enquiries about obtaining vaccine: WITHIN UK to: Central Vaccine Unit Department of Health Room 715, Wellington House 133-155 Waterloo Road London SE1 8UG Tel.: 071 972 4477 OUTSIDE UK to: Porton Vaccines Porton Down Salisbury, Wiltshire SP4 0JG Tel.: +1980 610896 Fax: +1980 610896	Alum precipitated filtrate of strain 34F2 culture	3 doses of 0.5 ml i.m. at intervals of 3 weeks followed by a fourth dose after an interval of 6 months. Reinforcing doses of 0.5 ml i.m. should be given annually.
China (People's Republic)	Lanzhou Institute of Biological Products 118 Yan Chang Road Lanzhou, Gansu 730046 Tel.: +86 931 8888311 Fax: +86 931 417199	Live spore suspension of strain A16R	Single dose by scarification of a 20 μ l (approx.) droplet in skin.
Russian Federation	Institute of Microbiology 010024 Kirov Oktyabrskiy Prospect 119 Tel.: +7 62 84 08	Live spore suspension of strain STI (Sanitary Technical Institute), Nikolai Ginsberg 1940	Initial 2 doses 21 days apart and single annual booster doses. Administered by scarification of one or two 10-20 μ l droplets in skin, or s.c. 0.5 ml
USA	Michigan Biologic Products Institute 3500 N. Martin Luther King Jr Blvd, P.O. Box 30035 Lansing, MI 48909 Tel.: +1 517 335 8450 Fax: +1 517 335 9486	Aluminum hydroxide-adsorbed filtrate of strain V770 culture	3 s.c. doses, 0.5 ml each, given 2 weeks apart followed by 3 additional s.c. injections, 0.5 ml each, at 6, 12 and 18 months. Subsequent booster injections of 0.5 ml at one-year intervals.

*s.c. = subcutaneous; i.m. = intramuscular

Appendix VI

Contingency Plan for the Prevention and Control of Anthrax

(modified from Whifford 1987)

A.VI.1 Action in the event of an outbreak of anthrax in livestock

An exemplary control programme was instituted by the Department of Natural Resources and Environment, Victoria, Australia, in response to an outbreak of anthrax in cattle during the first half of 1997 affecting some 83 farms in the Goulburn Valley. This has now been established as an Australian standard and would appear to serve well as a global model for anthrax control. The following summary, based on the respective report (unpublished) of the Chief Veterinary Officer (Dr A.J. Turner), Victoria, is included here by kind permission.

On each affected farm, the following measures must be applied:

1. The carcasses of infected cattle are to be either burnt at the site of death and the ashes buried deeply, or wrapped in double thickness plastic, to prevent spilling of body fluids, and removed to a more suitable site (e.g. a quarry) where they are burnt and the ashes buried. (Consideration may be given to removing the carcasses to suitable commercial incinerators or rendering plants)
2. The site where the animal died is to be disinfected with 5% formaldehyde after disposal of the carcass.
3. All other animals in the affected herd are to be vaccinated.
4. Affected properties are to be quarantined for at least 20 days after the last case or 6 weeks after vaccination, whichever is later.
5. Any milk collected from a cow showing signs of anthrax within 8 hours of milking is to be destroyed, along with any other milk that may have been mixed with the suspect milk.
6. Any movement of susceptible livestock, or risk items (hides, skins, carcasses, etc.) that have left the property in the 20 days before the first anthrax case, are to be traced, and appropriate action taken if necessary.

7. As far as possible, vehicles should remain on made roads in infected and vaccinated farms; where vehicles have to enter the pasture fields, the vehicle should be disinfected before exiting the property by washing down with water and detergent to remove mud followed by a disinfectant wash.
8. People entering infected properties are required to wear protective clothing and footwear which are disinfected before leaving the property.
9. All cattle on neighbouring properties should also be vaccinated and the property quarantined for the appropriate period to cover the withholding period for the vaccine. Generally three weeks is regarded as a satisfactory time period, but other considerations, such as the requirements of countries to which meat from such farms is to be exported, have to be taken into account in deciding the appropriate period.
10. A buffer zone, 20-30 km wide, is to be established around the infected property (-ies) within which all cattle and exposed sheep are vaccinated and quarantined.

A.VI.2 Other actions in the event of a case, or cases, of anthrax in livestock

The appearance of infection in animals from a previously uninfected environment (usually a farm) may be dealt with in the following ways:

1. Identify, isolate and remove apparently healthy animals, and monitor these carefully for signs of illness (see 5 below).
2. Decontaminate soil, bedding, unused feed, manure, etc., or disinfect premises that may have become contaminated by exudations from the dying or dead animals (see Sections 8.3.1/2/5-8).
3. Avoid any unnecessary ante- and post-mortem operation in animals on the premises (see Section 8.1).
4. Destroy carcasses and their parts by burning, removal for rendering or, as a last resort, (see Section 8.1) deep burial after disinfection, preferably with formalin solution.
5. Vaccinate all susceptible animals if there is reason to believe they continue to be exposed to the source of the incident. Where high exposure is suspected or certain, more rapid protection may be obtained by antibiotic therapy. Antibiotics and vaccine should not be administered simultaneously (see Section 7.1 and Appendix V);
6. Control scavengers and possible vectors, such as flies, rodents and birds.
7. Carry out an epidemiological investigation to detect the source of infection (history of site, feed, disturbance of the environment, etc.) to identify the source of the incident.

8. Take proper measures to avoid the contamination of water and soil and to prevent the spread of the infection to other farms and environments. Disturbed soil thought to be related to the incident should be fenced off and, ideally, not used for grazing again, at least until well overgrown again with vegetation, preferably of the type that inhibits access by susceptible animals.
9. Alert public health authorities.

A.VI.3 Guidance on actions relating to milk from herds/flocks in which anthrax has occurred

Action to be taken on milk from a herd or flock experiencing cases of anthrax infection occasionally presents a dilemma for health authorities. Wasteful destruction of large quantities of milk, and consequent financial losses, can be avoided by considering the following:

1. Animals with anthrax infection do not usually discharge the infecting organism in their milk. Secretion of milk would normally be expected to cease with onset of bacteraemia and illness. The organism would only gain entry to the milk secreting glands through breakdown of blood vessel walls at terminal stages of the disease, long after secretion of milk has stopped. Milk from other healthy animals in the herd/flock poses no risk of carrying anthrax organisms.
2. In the unlikely event of an exception to the rules given in 1. above occurring, and anthrax organisms reaching the milk before secretion has ceased, it must be remembered that
 - the organisms are in vegetative form,
 - they will have no opportunity to sporulate as a result of having minimal aeration and, where commercial processing follows, of rapid cooling to refrigeration temperatures,
 - the vegetative forms die quite rapidly in the milk (Bowen and Turnbull, 1992) and will be killed immediately by pasteurisation,
 - any anthrax organisms reaching the bulked milk will have been considerably diluted; the infectivity of anthrax organisms for humans by the oral route is very low. Vegetative forms will be killed by the gastric juices.
3. We are unaware of any instances on record of human anthrax cases resulting from handling or consumption of milk from herds/flocks experiencing anthrax and processed dairy products have never been associated with human anthrax.

Action on the part of farmers/owners and public health authorities aimed at reducing the minimal risk to zero risk should consist of:

- milking only health animals; animals showing signs of illness should be set aside for appropriate treatment and the milk from those animals, and utensils used in its collection, should be sterilised,
- ensuring hygienic practices are in place which prevent the environment of the premises becoming contaminated with anthrax spores and, further, prevent milk becoming contaminated from the environment,
- ensuring all milk is rapidly cooled to 4 C or less within 4 hours of milking and is held at this temperature until processed at a licensed dairy plant,
 - pasteurisation of all milk before processing for human or animal consumption

If the veterinary inspector is satisfied these requirements have been met, milk from healthy animals in herds/flocks in which cases of anthrax have occurred need not be excluded from processing and bulked milk containing such milk need not be condemned.

A.VI.4 Precautions for exposed personnel

Persons who have to handle animals known to be, or suspected of being infected with anthrax, or carcasses from such animals, or parts from such carcasses, should take the following precautions. They should:

1. be vaccinated against anthrax if their exposure is frequent and if the human vaccine is available (see Appendix V);
2. avoid all blood-spilling operations (slaughtering included) on infected or suspect animals/carcasses;
3. use protective clothing such as strong gloves, boots, coveralls, etc., as appropriate, to avoid direct contact with infected/contaminated materials. Cuts, abrasions, or other lesions should be properly dressed before putting on the protective clothing. The equipment used must be adequately disinfected or appropriately destroyed (see Section 8.3.9);
4. avoid any contact with other persons (family included) or animals, without first changing clothing, washing hands, and taking appropriate disinfection measures (see Section 8.3.9);
5. report to a physician any suspicious symptoms appearing after contact with infected animals or materials.

Where there is a risk of aerosolisation of spores, further precautions should be considered, such as damping down the material, possibly with 5% formalin, wearing of face masks, etc.

Appendix VII

Model Country Programme

A.VII.1 Background

At the creation of the "WHO working group on anthrax control and research" in November, 1990, a long-term 3-phase "model country programme" was formulated to address the need for improved surveillance and control of anthrax throughout the world (WHO, 1991). The purpose of this was to work out the design and method of implementation in practice of a surveillance and control programme in certain "model countries" and then to put this programme forward as a template to guide other countries in formulating their own national policies with regard to anthrax surveillance and control.

In the event, following a meeting of the working group in Mongu, Western Zambia, 23-28 September 1992, the Department of Veterinary and Tsetse Control Services in Zambia, with the aid of the Dutch Government sponsored Livestock Development Project Phase II, took up the challenge in the Western Province. A series of templates for national anthrax control programmes has now been constructed for the benefit of other countries on the basis of the experience gained in Western Province. This has been demonstrated at Southeast Asia and Central Asia inter-regional WHO-sponsored anthrax workshops in Kathmandu, Nepal, 9-11 March 1997 (Joshi, 1997) and Almaty, Kazakhstan, 5-6 October 1997 (Cherkasskiy, 1997).

In general, the problems in controlling anthrax in many African countries, and possibly a number of countries in other parts of the world, stem from:

- the custom within many communities of butchering, eating and distributing meat from sudden death animals and utilising or selling their hides, bones, etc.
- lack of cooperation by farmers/owners over reporting sudden deaths and over vaccination
- failure or long delays in diagnosis due both to poor communication and inadequate local laboratory training/facilities
- frequent failure to implement policies on disposal of carcasses and subsequent disinfection and decontamination as a result of shortage of veterinary staff, lack of fuel or unavailability of disinfectants.

The appropriate regulations and guidelines in regard to surveillance, reporting and control measures for anthrax are generally in place in these countries; the problem lies with the implementation of these regulations and guidelines. The templates given below, therefore, in specifying the classical actions involved for an effective control programme, do not add

anything which is new or revolutionary, but rather supply a means for veterinary and public health authorities to examine the shortfalls occurring in practice in their countries, and which are the cause of the continuing incidence of anthrax they are experiencing.

Every country suffering from frequent incidents or outbreaks of anthrax will have its own underlying related problems. In the templates which are given below, the problems faced by veterinary and public health authorities in Western Province, Zambia, are inserted to supply examples of how the templates might be used by other countries when analysing their own problems for the purpose of formulating their control programmes and policies.

The templates are designed to enable a simple comparison between classical, or textbook, approaches to control and what actually occurs in reality in a country. Action to reduce the discrepancy between the ideal and the real will lead to reduced incidence of anthrax.

A.VII.2 Templates for control problems illustrated with the experience gained in Western Province, Zambia

The situation in Western Province, Zambia

In Western Province, cattle are often kept by people who are not the actual owners, whether for long term (mafisa), or temporary caretaking in the uplands (bulisana), or in the short term for ploughing or manuring (kutulisa). In the event of an animal dying, the owner must be presented with the hide or head as identification. Thus cattle that have died unexpectedly are dragged to the village or they are skinned or butchered on the spot and the meat, hide and head transported to the village.

Efforts to advise the local communities on the dangers of such behaviour meet resistance as it is thought burying or burning the carcass is a waste. It is recognized that the obviously affected, swollen spleen represents serious danger and this is fed to dogs or buried. However, in view of the suddenness of illness and death, the meat looks good and is not associated with high risk of illness. Of many people who eat such meat, usually few get disease and very few actually die. Indeed, if 50 people ate the meat and 2 died, then the community may not accept that the meat was responsible for the two deaths. The value of the meat, therefore, is seen to greatly outweigh the risks of members of the community contracting the disease.

Burying requires extra labour and burning not only requires extra labour but also wood as well which is not available in some areas where outbreaks occur. For these reasons anthrax carcasses are almost always opened up and the meat consumed. Since the affected communities are frequently in remote and inaccessible places, reports reach veterinary services much later and hence there is little supervision of the disposal of carcasses.

In the following templates, the blank boxes on the right are for the benefit of the user of this document in assessing the surveillance and control situation within his or her country or area.

Template 1: Surveillance

Ideal	Western Province situation	Our situation
All unexplained livestock deaths or suspected cases must be investigated with laboratory support	The term "all" is unachievable, although it should be possible to achieve many more	(This space for user of template)
Constraints	Western Province situation	Our situation
	<ul style="list-style-type: none"> • Distances involved • Remoteness in some cases • Lack of transport • Delays in specimen delivery • Laboratory materials unavailable 	(This space for user of template)
Importance	<ul style="list-style-type: none"> • Confusion as to cause of death is avoided • Demonstrates that control measures are working 	

Additional notes: Every unexpected death in livestock should automatically result in a blood smear for examination by veterinary authorities at the earliest opportunity. A person of appropriate standing in each community could perhaps be recruited to implement this; he/she should be given adequate instruction, including on safety issues. If vaccine is available, the individual concerned should be offered this.

Since it is a frequent experience that smears may fail to reveal anthrax bacilli, especially if the carcass was not fresh at the time of making the smear, laboratory culture should be regarded as an essential back-up procedure for diagnosis. This should be made possible by provision of the appropriate equipment, materials and instruction at least at the District Veterinary Laboratory level.

The development of "at-the-site" test systems not requiring expensive equipment such as a microscope should be regarded as an urgent priority. As discussed elsewhere in this document, this has now been done (Burans et al., 1996) and awaits the stage where it can be fully evaluated in the field and made widely available.

Template 2: Reporting

Ideal	Western Province situation	Our situation
Reporting and information must be efficient, involving <ul style="list-style-type: none"> • keeping good records • mandatory reporting of deaths 	Reporting does occur, but <ul style="list-style-type: none"> • efficiency needs to improve • cooperation between the local established social structure (Barotse Royal Establishment) and the veterinary public health officials may be the way to improve this 	(This space for user of template)
Constraints	Western Province situation	Our situation
	<ul style="list-style-type: none"> • the size of the areas being monitored • the remoteness of some of the villages • the inaccessibility of many of the communities • shortage of basic materials at the veterinary assistant level 	(This space for user of template)
Importance	<ul style="list-style-type: none"> • Success of control efforts cannot be monitored without good reporting 	

Additional note: A concerted effort should be made to ensure that hospitals and human clinics inform veterinary officials upon seeing a case of anthrax.

Template 3: Disposal

Ideal	Western Province situation	Our situation
After confirmation as being a case of anthrax, a carcass should not be opened and should be burnt (or, the less preferred option, buried)	<p>Many carcasses are butchered and sold for human consumption. The meat and hides are seen as good and their value is seen as outweighing risks of serious illness</p> <p>Burning is not feasible due to shortage of fuel</p>	(This space for user of template)
Constraints	Western Province situation	Our situation
	<ul style="list-style-type: none"> • attitudes of stock owners • tradition of keeping head, meat and hides of animal that has died by caretakers to present to owners when they return from absence • lack of understanding why only some of those handling an anthrax carcass or eating the meat acquire the disease 	(This space for user of template)
Importance	<ul style="list-style-type: none"> • avoidance of environmental contamination • avoidance of spread of anthrax 	

Additional notes: Options on the best official approach to dealing with anthrax carcasses in developing country situations range from leaving it unopened for several days to allow putrefaction to kill the anthrax bacilli within the carcass to a mandatory rendering policy with many other approaches in between. Research is needed to determine under what conditions rendering policies might be established and also on practical and effective incineration procedures appropriate to the local circumstances.

Template 4: Disinfection

Ideal	Western Province situation	Our situation
Disinfectants should be held in reasonable quantities at field stations. Veterinary assistants and stock owners should be trained in their use	At this point in time, this is not possible	(This space for user of template)
Constraints	Western Province situation	Our situation
	<ul style="list-style-type: none"> • Lack of availability of suitable disinfectants • Cost • Hazards posed by the appropriate disinfectants • The types of premises where anthrax carcasses are handled 	(This space for user of template)
Importance	<ul style="list-style-type: none"> • Disinfection is a useful control measure • It should be introduced when possible in endemic areas where this is currently not possible 	

Additional notes: Provision, supply and storage of appropriate types of disinfectants are clearly a problem for developing countries principally because these have to be imported at unacceptable cost. Research is needed on the design of disinfection procedures applicable to the economic and other conditions pertaining in such countries.

Template 5: Vaccination

Ideal	Western Province situation	Our situation
<ul style="list-style-type: none"> · A vaccine meeting acceptable standards should be available • Field officers should have the necessary storage facilities • A contingency stock should be readily available 	<ul style="list-style-type: none"> • Variable and inadequate vaccination coverage • Intermittent supply of the vaccine • Limited storage facilities at field level • Inadequate duration of vaccine campaigns 	(This space for user of template)
Constraints	Western Province situation	Our situation
	<ul style="list-style-type: none"> • Perceived cost • Free vaccination → retroactive immunisation → perceived failure → loss of confidence in vaccination on the part of the stock owner 	(This space for user of template)
Importance	Vaccination is the hub of anthrax control, at least in endemic areas	

Additional notes: For maximum success, vaccination as a control measure must be applied TOGETHER WITH other control measures and must be continued for a full specified period (frequently 3 years is the period specified).

It makes sense in many endemic regions to use combined vaccines, such as Black Quarter/ Anthrax vaccine.

Vaccines are generally available (see Appendix V). Problems largely relate to farmers' attitudes (Dietvorst, 1996a), the cost and the logistics of carrying out and evaluating the effectiveness of vaccination campaigns. Examples of the sort of resistance on the farmers' part to vaccination and the rationale for this resistance are well covered by Dietvorst (1996a). As a first example, vaccination initiated in response to an outbreak has led to the situation in which the vaccine has been administered to animals already infected and which have died shortly after. This results in, at best, loss of faith in the vaccine and, at worst, a belief that the vaccine killed the animal(s). A second example is a belief that animals must be rested for two weeks after vaccination; frequently farmers feel they are unable to cease work for this period.

Template 6: Education

Ideal	Western Province situation	Our situation
Educational material on anthrax should be supplied to field or other appropriate stations for distribution in the affected community	Successful. Booklet, poster, radio programmes have been prepared and have reached the relevant communities (see Figure 4) Needs to be continued, expanded (more issues) and extended (more affected people reached)	(This space for user of template)
Constraints	Western Province situation	Our situation
	<ul style="list-style-type: none"> • Distances involved and inaccessibility of some communities • Printing and paper costs • Dissemination costs 	(This space for user of template)
Importance	The relevant individuals gain a better understanding of the rationale AND the limitations of control actions	

Additional notes: Inherent in all the control measures is the underlying need for educational programmes and materials. Of foremost importance is education of the farmer/owner to recognize and report suspected anthrax and take proper action over the disposal of the carcass. Appropriate education must also be available for instruction of veterinary, medical and other officials in confirmation of diagnosis and correct action thereafter.

The clear need for informational broadsheets, manuals, videos and films for disseminating information at courses, seminars and village meetings has been addressed in an exemplary manner in Western Zambia (Dietvorst, 1996a), where posters, booklets and a radio play (Dietvorst, 1996b) have been developed (Figure 4).

Intersectoral cooperation

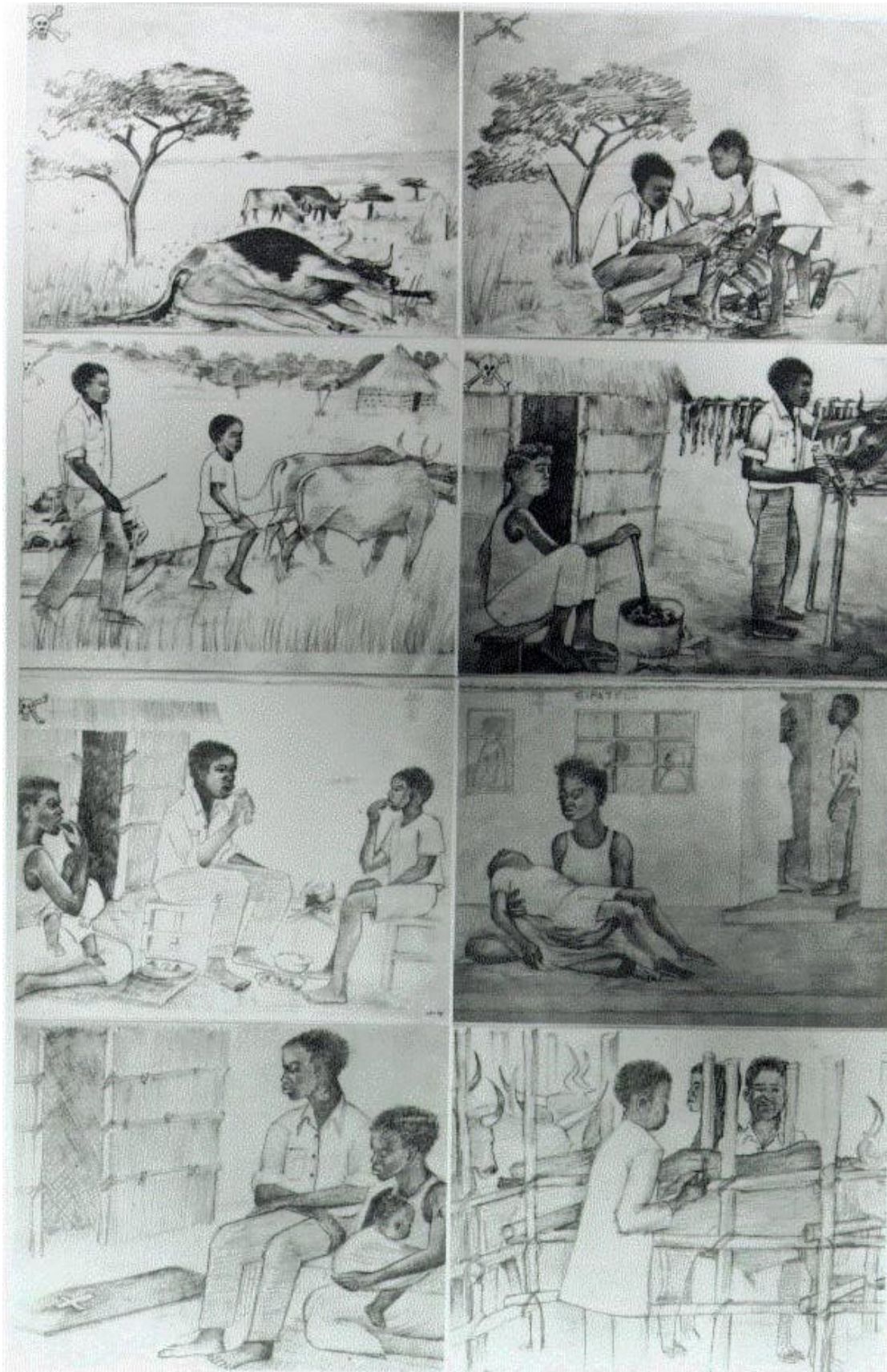
Good communication and cooperation (including sharing laboratory facilities and knowledge) between veterinary, medical and wildlife services are essential to control of anthrax. Such communication and cooperation falls short of ideal in many countries at present.

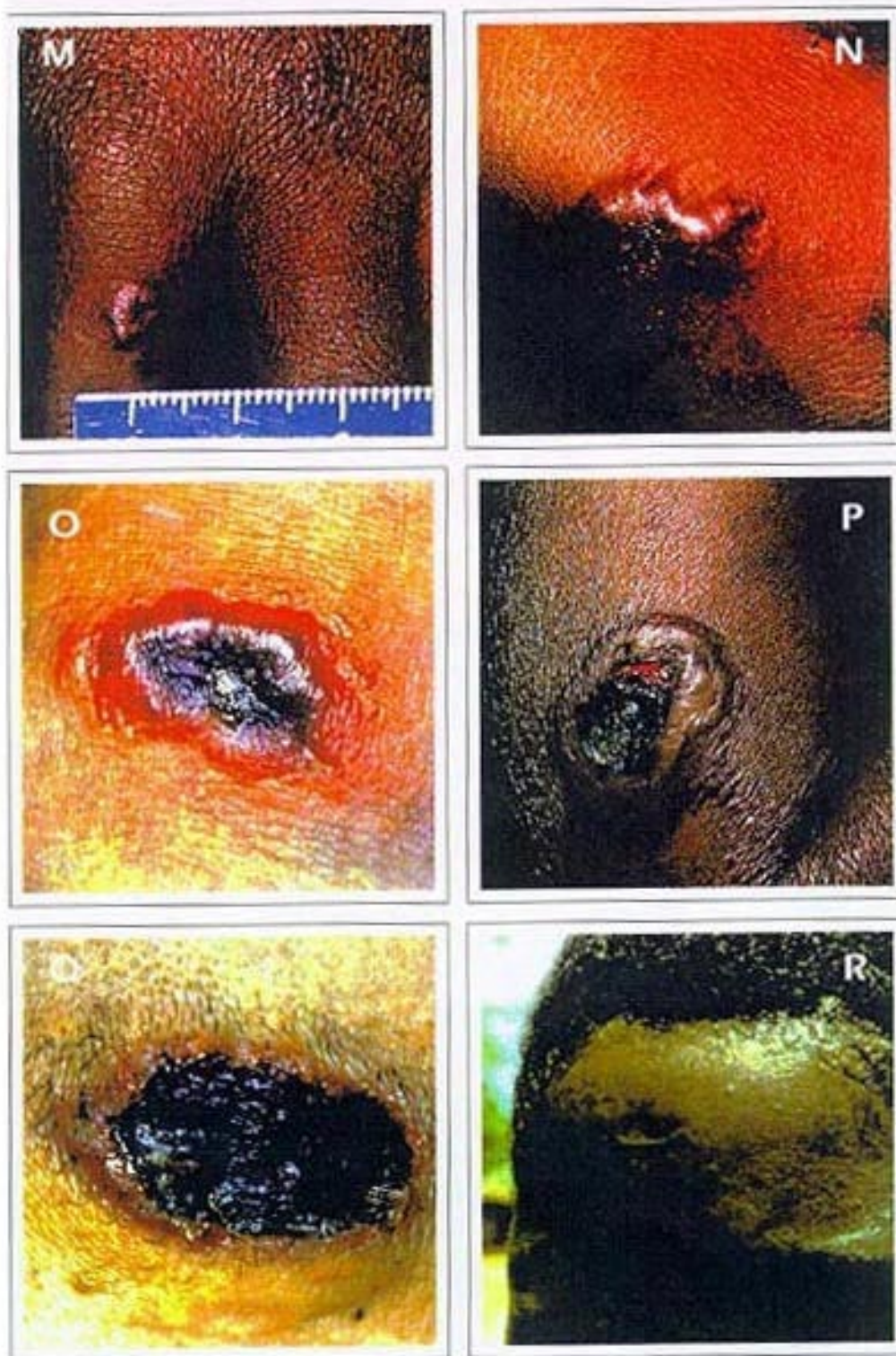
Figure 4

The pictures feature in a book and on a poster, designed to convey in an interesting manner to those likely to encounter anthrax, the important points about the disease which they should be aware of and how it can be prevented. The points are made by telling the story of the problems the Liseli family suffered following the death of one of their cows from anthrax.

Drawings by Lou Sifuniso, text by Désirée Dietvorst (Dietvorst, 1996b). Reproduced by kind permission of the Livestock Development Programme, P.O. Box 910428, Mongu, Zambia, and RDP Livestock Services B.V., Zeist, The Netherlands.

One of Mr and Mrs Liseli's cows has died unexpectedly of anthrax (top left). Meat is precious and the carcass looks good, so Mr Liseli decides to cut it up so that he can take it to the village (top right). The meat looks fine and smells all right, so Mr Liseli and his son, Likando, lift the pieces onto their sledge and take them back to the village (row 2, left). Once at the village, Mrs Liseli takes some of the meat and starts to prepare dinner while Mr Liseli hangs strips of the meat and other parts of the carcass out to dry (row 2, right). They enjoy a nice dinner (row 3, left), though Sespio, Likando's younger brother, is too small to eat meat. A few days later, Mr and Mrs Liseli fall ill and have large sores, Mr Liseli on his neck, Mrs Liseli on her arm. Likando is even more ill. They do not know what has made them ill and they decide to go to the hospital (row 3, right). Despite treatment, Likando dies (bottom left). In the meantime, the veterinary assistant has come to visit the farmers. He explains that anthrax can spread quickly when carcasses are opened and the soil becomes infected with spores. He tells them that the carcasses should be buried very deep in the ground so that dogs and hyenas cannot dig them up again and that their animals can be vaccinated against anthrax to prevent more of them getting the disease and dying (bottom right).





G



H



I



J



K



L





Appendix VIII

Packaging, Labelling and Documentation for Transport

(extracted from WHO 1997b)

Because of the distinction of risks between infectious substances and diagnostic specimens, there are variations to the packaging, labelling and documentation requirements. The packaging requirements are determined by the UN and are contained in ICAO and IATA regulations in the form of Packaging Instructions (PI) 602 and 650. The requirements are subject to change and upgrade by these organisations. The current packaging requirements are described below. UN-approved packaging systems are available commercially.

Basic triple packaging system

The system consists of three layers as follows.

1. Primary receptacle. A labelled primary watertight, leak-proof receptacle containing the specimen. The receptacle is wrapped in enough absorbent material to absorb all fluid in case of breakage.
2. Secondary receptacle. A second durable, watertight, leak-proof receptacle to enclose and protect the primary receptacle(s). Several wrapped primary receptacles may be placed in one secondary receptacle. Sufficient additional absorbent material must be used to cushion multiple primary receptacles.
3. Outer shipping package. The secondary receptacle is placed in an outer shipping package which protects it and its contents from outside influences such as physical damage and water while in transit.

Specimen data forms, letters and other types of information that identify or describe the specimen and also identify the shipper and receiver should be taped to the outside of the secondary receptacle.

Hand carriage of infectious substances is strictly prohibited by international air carriers, as is the use of diplomatic pouches for that purpose.

The maximum net quantity of infectious substances which can be contained in an outer shipping package is 50 mL or 50g if transport is by passenger aircraft. Otherwise, the limit per package is 4L-4Kg for transport by cargo aircraft or other carriers. Primary receptacles exceeding 50 mL in combination packing must be oriented so the closures are upward, and labels (arrows) indicating the "UP" direction must be placed on two opposite sides of the

package. The passenger aircraft quantify limits do not apply to blood or blood products for which there is no reason to believe they contain infectious substances, when in receptacles of not more than 500 mL each and with a total volume of not more than 4L in the outer package.

Hazard labels for dangerous goods

For all dangerous goods to be shipped by airfreight, specific hazard label(s) must be affixed to the outside of each package. The following hazards labels are of importance for culture collections or other institutions shipping biological substances.

Hazard labels for infectious substances and for genetically modified microorganisms which meet the IATA definition of an infectious substance:

Figure 1

Name:	Infectious Substance
Minimum dimensions:	100 x 100 mm
For small packages:	50 x 50 mm (black and white)

Labelling of the outer package for shipment of infectious substances must include the elements listed hereafter.

1. The International Infectious Substance Label.
2. An address label with the following information:
 - the receiver's (consignee) name, address and telephone number
 - the shipper's (consignor) name, address and telephone number
 - the UN shipping name (Infectious Substances Affecting Humans or Animals as the case may be) followed by the scientific name of the substance
 - the UN Number (Humans - UN2814, Animals UN2900)
 - temperature storage requirements (optional).

If the outer package is further packed in an overpack (with dry ice for instance) both outerpack and overpack must carry the above information, and the overpack must have a label stating "INNER PACKAGES COMPLY WITH PRESCRIBED SPECIFICATIONS".

- 3. Required shipping documents – these are obtained from the carrier and are fixed to the outer package:
 - the shipper’s Declaration of Dangerous Goods (Figure 2 is one example)
 - a packing list/proforma invoice which includes the receiver’s address, the number of packages, detail of contents, weight, value (note: state that there is “no commercial value” as the items are supplied free of charge)
 - an airwaybill if shipping by air.
- 4. An import and/or export permit and/or declaration if required.
- 5. If the outer package contains primary receptacles exceeding 50 mL in combination at least two “Orientation Labels” (arrows) must be placed on opposite sides of the package showing correct orientation of the package.

Requirements for Air Mail

Infectious substances and diagnostic specimens may be shipped by registered air mail. The basic triple packaging system is used with the same requirements as for other means of conveyance.

The address label must display the word “LETTRE” and the green Customs Declaration Label for Postal Mail is required for international mailing. Diagnostic specimens are to be identified with the violet UPU “PERISHABLE BIOLOGICAL SUBSTANCES” label. Infectious substances are to be identified with the International Infectious Substance label (see Figure 1). Infectious substances must also be accompanied with a shipper’s Declaration of Dangerous Goods form (see Figures 2a and 2b).

Because of local/international restrictions, prior contact should be made with the local post office to ascertain whether the packaged material will be accepted by the postal service.

Appendix IX – References

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