A GUIDE TO THE DIAGNOSIS, TREATMENT, AND PREVENTION OF ANTHRAX

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## CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foreword</td>
<td>11</td>
</tr>
<tr>
<td><strong>1. Introduction</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>2. Epidemiology</strong></td>
<td>1</td>
</tr>
<tr>
<td>2.1 Etiologic agent</td>
<td>1</td>
</tr>
<tr>
<td>2.2 Distribution</td>
<td>2</td>
</tr>
<tr>
<td>2.2.1 Livestock</td>
<td>2</td>
</tr>
<tr>
<td>2.2.2 Man</td>
<td>3</td>
</tr>
<tr>
<td>2.3 Host</td>
<td>3</td>
</tr>
<tr>
<td>2.4 Transmission</td>
<td>4</td>
</tr>
<tr>
<td><strong>3. Anthrax in animals</strong></td>
<td>5</td>
</tr>
<tr>
<td>3.1 Clinical manifestations in animals</td>
<td>5</td>
</tr>
<tr>
<td>3.2 Postmortem findings in animals</td>
<td>6</td>
</tr>
<tr>
<td>3.3 Pathogenesis in laboratory animals</td>
<td>7</td>
</tr>
<tr>
<td><strong>4. Anthrax in man</strong></td>
<td>7</td>
</tr>
<tr>
<td>4.1 Susceptibility</td>
<td>7</td>
</tr>
<tr>
<td>4.2 Clinical manifestations, pathogenesis and lesions</td>
<td>7</td>
</tr>
<tr>
<td><strong>5. Diagnosis</strong></td>
<td>8</td>
</tr>
<tr>
<td>5.1 Diagnosis in animals</td>
<td>8</td>
</tr>
<tr>
<td>5.2 Diagnosis in man</td>
<td>10</td>
</tr>
<tr>
<td><strong>6. Prevention</strong></td>
<td>11</td>
</tr>
<tr>
<td><strong>7. Treatment of anthrax in man and animals</strong></td>
<td>13</td>
</tr>
<tr>
<td><strong>8. Selected references</strong></td>
<td>14</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>15</td>
</tr>
<tr>
<td><strong>Annex 1</strong></td>
<td>16</td>
</tr>
<tr>
<td>Disinfection in animal husbandry for controlling anthrax</td>
<td></td>
</tr>
<tr>
<td><strong>Annex 2</strong></td>
<td>18</td>
</tr>
<tr>
<td>International zoo-sanitary code</td>
<td></td>
</tr>
<tr>
<td><strong>Annex 3</strong></td>
<td>20</td>
</tr>
<tr>
<td>Sources of human and animal anthrax vaccines</td>
<td></td>
</tr>
<tr>
<td><strong>Annex 4</strong></td>
<td>22</td>
</tr>
<tr>
<td>World distribution of anthrax in livestock</td>
<td></td>
</tr>
<tr>
<td><strong>Annex 5</strong></td>
<td>23</td>
</tr>
<tr>
<td>Contingency plan for the prevention and control of anthrax</td>
<td></td>
</tr>
<tr>
<td><strong>Annex 6</strong></td>
<td>25</td>
</tr>
<tr>
<td>Outline of eight-hour short course to train persons to diagnose anthrax in animals</td>
<td></td>
</tr>
</tbody>
</table>
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FOREWORD

This manuscript was prepared with a great deal of input, revision, and critical evaluation by Dr Max Sterne. In my opinion, this man is one of the few living scientists who has a thorough understanding of the organism B. anthracis and the epidemic disease it produces. From his work in South Africa in the 1930's developing a safe and effective anthrax vaccine in livestock, to his later work in England at the Wellcome Laboratories with the clostridial diseases, he has probably contributed more than any other individual to the understanding, prevention, diagnosis, and control of these diseases.

In July 1965, I was privileged to meet Dr Sterne to prepare the final version of this manuscript after several exchanges of correspondence with him over an approximately two-year period. The time spent with Dr Max Sterne was likened to sitting at the feet of the master scholar as he reminisced about his experiences with anthrax. It is my opinion that, with the unsselfish support and encouragement given by Dr Max Sterne, this manuscript more adequately fulfills the purpose for which it was written.

I extend heartfelt appreciation to this great scientist.

Howard W. Whitford, DVM, PhD
1. Introduction

Anthrax is a disease of mammals, including man, that has probably existed since man first began to domesticate livestock. During the course of history, it has caused devastating epidemics and epizootics in many wild and domestic animals and birds. Ruminants are considered to be more susceptible than carnivores; some carnivores, such as domestic mink, are quite susceptible. Serious outbreaks may occur in wild and exotic animals and pose management problems in game parks and wildlife reserves. Domesticated ostriches as well as wild and domesticated elephants are quite susceptible to anthrax. Anthrax, also called charbon, malignant carbuncle, splenic fever, milzbrand, and woolsorters disease, among others, is caused by a gram positive, spore-forming, aerobic rod-shaped bacillus named Bacillus anthracis. The words "anthrax", and "anthracis" refer to the Greek word for charcoal, which aptly describes the colour of the cutaneous lesion caused by the organisms in man. B. anthracis has played an important role in the development of the modern sciences of bacteriology and immunology through the work of Pasteur, Koch, and others, during the latter part of the nineteenth century. More recently, the disease, and the organism causing the disease, has been extensively studied to the point that control, and even eradication, are within the realm of possibility.

2. Epidemiology

2.1 Etiologic agent

Bacillus anthracis is a gram positive, aerobic, spore-forming rod-shaped bacillus typical of the genus Bacillus. The vegetative cell measures from 1 to 1.5 microns wide to 4 to 10 microns long. The colony morphology of B. anthracis after 18-24 hours incubation at 37°C on nutrient agar is a large (1 cm), rough, ground glass-appearing colony. Filamentous outgrowths on the edge curl back toward the parent colony in the same direction giving an appearance described as "medusa head". On solid media that has red blood cells added, the colonies of B. anthracis show virtually no hemolysis at 24 hours incubation, and only slight or greenish hemolysis on prolonged incubation. This becomes important in separating B. anthracis from the ubiquitous B. cereus that is often recovered from clinical specimens as a saprophyte or contaminant.

Smears prepared from recently dead clinical specimens and stained with Romanovsky type stains (Giemsa, Wright, Leishmann) reveal a red-mauve capsule (see Diagnosis). The capsule is not formed when the bacilli are cultivated aerobically on nutrient agar, as evidenced by the rough colony appearance.

Other characteristics that are consistent with B. anthracis are:

1. non-motile;
2. negative production of urease;
3. delayed positive citrate reaction;
4. hydrolysis of starch;
5. production of gelatinase;
6. reduction of nitrate; and
7. acid production in peptone-free glucose.

Vegetative cells of B. anthracis are no more resistant than other vegetative bacteria and are susceptible to bactericidal products of saprophytic bacteria and molds. The vegetative cells sporulate to become resistant to heat, cold, chemical disinfectants, and desiccation. Ambient temperatures of greater than 15°C along with exposure to atmospheric oxygen are required for vegetative cells of B. anthracis to sporulate. B. anthracis does
not sporulate inside an unopened carcass – an important consideration to prevent the dissemination of spores into the environment. These spores can survive for many years in dry soil, hide, hair, wool, bristles, bone meal and dried blood. There is little evidence to support the theory that \textit{B. anthracis} spores vegetate in a natural environment in the absence of an animal host.

Several variant or dissociative forms of \textit{B. anthracis} have been described. Most have been induced by manipulating media composition and/or environmental conditions. When grown on nutrient agar in air, the colony type is rough and nonencapsulated. However, if serum and bicarbonate are added to the medium and the atmospheric carbon dioxide is increased, the colonial morphology assumes an encapsulated form and appears smooth and mucoid. Sterne found that upon further incubation, rough outgrowths from the parent smooth colony would appear. This mutation produced organisms incapable of forming a capsule. Although the capsule, made up of \textit{d}-polylactic acid, is innocuous when separated from the bacterial cell, it apparently serves as a defense mechanism against the host's immune mechanisms and stimulates a response that protects the animal from challenge with virulent \textit{B. anthracis}.

This nonencapsulated, spore-forming, immunogenic variant of \textit{B. anthracis} is the basis for the Sterne vaccine developed for animal use in the late 1930's and is the safest, most effective vaccine currently available for the prevention of anthrax in livestock. This vaccine is not recommended or available for use in man (see Prevention).

Toxins of \textit{B. anthracis} have been experimentally produced in vitro and separated into three components:

- Factor I, also called edema factor;
- Factor II, the protective antigen; and
- Factor III, the lethal factor.

All three are serologically distinct and produce no lesions when injected separately into laboratory animals. However, when the lethal factor and protective antigen are given in combination, the animal dies.

Survival of \textit{B. anthracis} spores can be quite long under controlled circumstances. Viable spores have been recovered from cultures stored for 60 years or more in a laboratory environment. The survival of spores in a natural environment is probably limited by microbial activity of soil saprophytes to not more than 3 to 4 years.

The Gruinard Island experience, in which \textit{B. anthracis} spores were released from ordnance devices being tested for biological warfare weapons during World War II, is an enigma. Viable anthrax spores were still being detected in 1979, some 37 years after the initial experiments. The climate on the island, located off the west coast of Scotland, is wet and cold; the soil quite acid (pH 4.2 to 4.7) and therefore not conducive to soil microbial activity. This, coupled with the massive amount of \textit{B. anthracis} contamination originally placed on the island (estimated to be $4 \times 10^{14}$ spores) from laboratory manipulated organisms, may explain the longevity of the spores in this somewhat artificial situation.

\subsection*{2.2 Distribution}

\subsubsection*{2.2.1 Livestock}

The organisms \textit{B. anthracis} and the disease caused by the bacillus have been worldwide in distribution. During the 19th century and first half of the 20th century, the disease was rampant in the livestock population throughout the world. During this same period, the disease in human beings was also common, especially in countries where sanitary science was not well developed and/or consumption of contaminated meat occurred due to scarcity of other sources of meat protein. In recent years, since 1950, the incidence of the disease in livestock and man has dropped markedly probably due to these three factors:
(1) the availability of a reliable, safe vaccine;

(2) the liberal use of antibiotics, namely penicillin; and

(3) implementation of strict quarantine laws in the more developed countries.

In Europe, the recent incidence (1970's) of anthrax in livestock is confined to the countries of Bulgaria, Greece, Italy, Romania, Spain, Turkey, USSR, and Yugoslavia. The other countries of Europe are virtually free of the disease. In the United States, the disease in livestock is very rare, being confined to five states representing 21 laboratory-confirmed cases of anthrax during 1981-1984. The states are as follows (cases/year): Idaho (1-1981), Louisiana (1-1984), North Dakota (2-1981, 1-1982); South Dakota (5-1981, 1-1982), and Texas (2-1981, 1-1982, 6-1983, 1-1984). During 1985, in the United States, *B. anthracis* was isolated from sheep, horses, and deer in separate, minor outbreaks in Texas. Anthrax was also reported during 1985 in cattle in Arkansas and Alabama, where the disease had not been diagnosed since the 1950's. Many Central and South American countries have livestock losses due to anthrax. In 1982, only Belize, Honduras, and Panama had no cases of anthrax reported.

The incidence of anthrax in livestock and contaminated animal by-products was quite common from some Eastern Mediterranean and Asian countries during the first half of the 20th century. However, recent reports are somewhat lacking. Africa and Egypt would also fall into this category. In 1984, the Jakarta (Indonesia) Post reported that anthrax-infected cattle and buffaloes were dying in the Sulawesi Province.


### 2.2.2 Man

The incidence of human anthrax had dropped markedly during the last quarter century. In 1958, Glassman estimated that between 20,000 and 100,000 cases of human anthrax occurred worldwide. During the period 1970 to 1979, Velimirovic reported that 8,580 (averaging 953/yr.) cases occurred in Europe, but that the incidence had dropped to 429 cases/year during the 5-year period from 1979-1984. The last death in the United States from anthrax occurred in 1976 in a person who used imported mohair from Pakistan. A case of anthrax was also reported in 1974 in an American who contracted the disease from hide used to cover a drum made in Haiti. The last human (non-fatal) case occurred in the United States in 1980.

Recently, sporadic reports of human anthrax have occurred throughout the world. Sverdlovsk oblast in USSR had sporadic incidence of human anthrax in 1979. During the period between November 1978 and October 1980, 9,711 cases of human anthrax, with 131 deaths, were documented in three provinces of Zimbabwe, Africa.

### 2.3 Host

A wide variety of wild and domestic animals and man are susceptible to anthrax. Herbivorous mammals are the usual victims, with cattle, sheep, goats and horses commonly affected. Domesticated elephants are also highly susceptible. Less frequent, but sometimes extensive outbreaks have occurred in pigs, dogs, and commercial mink, as well as in wild and exotic animals, such as elephants, deer, large cats, and ostriches, located in zoological gardens, game preserves, and in the wild. Even cold-blooded animals such as fish, amphibians, and reptiles may be experimentally infected if kept warm.


Mice, guinea pigs, and rabbits are the laboratory animals most susceptible to anthrax. Experimental inoculation with *B. anthracis* usually causes death in 24 hours or less, and rarely do the animals survive for more than 96 hours. Infant rats are also highly susceptible, but acquire a marked natural resistance with age.

2.4 Transmission

Since anthrax is predominantly a disease of warm-blooded mammals and some birds (ostriches), it is generally accepted that it is through them that *B. anthracis* is perpetuated in nature. An animal ingests soil-borne *B. anthracis* spores, becomes sick, and dies of anthrax. A few spores may be deposited into the soil through bloody discharges from natural body openings. If the carcass is opened by predators or carrion-eating birds (or man), many spores may be deposited at the primary site, while secondary foci of spores may be made elsewhere by faecal deposits of vultures, death of predatory mammals, or mechanical transmission by insects or man. Horses may live several days and develop edematous foci on dependent portions of the body after becoming infected by biting flies. Subsequently, flies cluster and feed on these edemas and carry the infection to a susceptible host, thus causing minor epizootics. Surface waters accessible to livestock and wildlife may become a source of *B. anthracis* transmission. The contamination may be introduced by defecation and vomition by vultures, or by sick and dying livestock depositing spores into the water with subsequent concentration of the contamination by evaporation during dry seasons.

Historically, human anthrax has been separated into the following categories based on the transmission of the disease:

**Industrial anthrax** refers to transmission of disease via animal by-products, or during the processing of animal by-products into finished goods. The most common sources of industrial anthrax are hides, wool, hair, bristles, bone meal, blood meal, or meat by-products. Ivory from elephant tusks has also been incriminated. Factory workers handling such animal by-products are a high-risk group. The site of infection is usually the skin (cutaneous anthrax) or the respiratory system (inhalation anthrax).

**Agricultural anthrax** refers to those cases in man caused by direct contact with the infected animal in the field. Farm workers, ranchers, veterinarians, and veterinary laboratory personnel are the most likely to come in contact with the disease from these sources. The cutaneous form is most often seen.

A third category involves **food-borne, water-borne and insect transmission** of the disease to human beings.

**Food-borne anthrax** refers to infection in individuals who skin, butcher, and/or consume meat from the carcasses of anthrax-infected animals. Factors associated with these outbreaks include the following:

1. They are always preceded by, and are concurrent with, anthrax in domestic, meat-producing animals (usually cattle);
2. Contaminated surface water sources (used for watering livestock and for bathing by man);
3. There is a large population of flying biting insects that mechanically transmit spores from animal to animal and animal to man;
4. The sites of infection with this type of anthrax, in man, are exposed skin surfaces (head, neck, arms) and the gastrointestinal system (intestinal anthrax);
(5) a period of civil strife which can curtail activities of health personnel (veterinarians, medical doctors, and health authorities) to control the disease in its early stages.

3. Anthrax in animals

3.1 Clinical manifestations in animals

Three different forms of anthrax in domestic animals are recognized:

(1) peracute or apoplectic;
(2) acute;
(3) subacute to chronic.

Ruminants are more likely to exhibit the peracute and acute forms, equines the acute form and carnivores and omnivores the subacute to chronic form.

In the peracute form, the animal is rarely observed to be ill until immediately before death. The most frequent signs, if seen, are pyrexia of up to 42°C, muscle tremors, dyspnea, and mucosal congestion, which is followed shortly after by collapse, terminal convulsions and death. Within a few hours after death, a bloody discharge from natural body openings is often observed. This is due to blood-clotting inhibition by the toxins of B. anthracis.

The acute form has a more prolonged prodromal period. In cattle, signs may be seen up to 48 hours prior to death. Depression, listlessness, and anorexia are sometimes preceded by a short period of excitement. Fever, rapid deep respiration, increased heart rate, congested and hemorrhagic mucous membranes, and ruminal stasis may also be observed. Abortions and reduced milk production are seen in cows, and the milk may become blood-stained or deep yellow in colour. Edema of the tongue, and edematous swellings along the region of the throat, sternum, perineum, and flank may also be seen.

In the horse, the acute form may show variable clinical signs depending on the mode of exposure. If spores are ingested, enteritis, colic, high fever, depression and death within 48 to 96 hours may occur. If the spore is introduced through the skin, by a biting insect for example, a hot, painful edematous subcutaneous swelling appears at the site and spreads to the throat and dependent parts of the axilla, abdomen and groin. Dyspnea due to laryngeal swelling may become apparent. The course of the disease may last from one to three days with some animals surviving for a week or longer.

In pigs, a subacute to chronic form occurs when the spores are ingested. The organisms tend to localize in the regional lymph nodes of the pharynx where severe swelling can occur, and death follows due to occlusion of the airway. In cases where this does not occur, the disease may progress to a fatal bacteremia, although some animals recover after a few days of illness. An intestinal form, with enteritis, constipation or diarrhoea, is also seen in swine.

Wild and domestic carnivores usually have a clinical course similar to that of pigs: a subacute to chronic infection. The infective organisms are either localized in the oropharynx and throat or are passed down the gastro-intestinal tract causing an intestinal form of the disease. Dogs that ingest spores may have carbuncular lesions of the tongue and fowl, and tumescence around the head, lips, or in the throat region. A severe acute gastroenteritis may occur in some cases. Many of the carnivores appear to have natural resistance to anthrax and recovery is not uncommon.

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Severe outbreaks of anthrax have been reported in domestically reared mink. Mink which have ingested B. anthracis spores usually show signs of depression, anorexia, muscular weakness, and death one or two days later. The diet of mink raised for fur production is conducive to the maintenance of anthrax spores since it consists mostly of unprocessed meat and meat by-products that are kept frozen until fed to the animals.

An interesting case involving extensive death losses due to anthrax in captive large cats (cougars and jaguars) has been reported. The cats were fed infected horse meat from animals dying of anthrax. The source of the infection was traced to contaminated saddle pads made with imported goat hair.1

3.2 Postmortem findings in animals

Anthrax has rather consistent, if not classical postmortem lesions, but as with most biological entities, there is variability and overlapping with the lesions of other infectious and non-infectious diseases.

In ruminants such as cattle, sheep, and goats, the following are characteristic findings:

1. a rapidly decomposing carcass;
2. blood-tinged serosanguineous to bloody fluid that exudes from natural body openings;
3. an incomplete rigor mortis;
4. unclotted intravenous blood which is dark and has a sticky, thickened consistency;
5. an enlarged, hemorrhagic spleen;
6. signs of septicemia as evidenced by small petechial hemorrhages throughout the body;
7. other less consistent gross lesions may be found, such as mucosal hemorrhages and inflammation of the gastro-intestinal tract around lymphoid follicles and Peyer's patches; drops of blood oozing through the skin; free blood in the colon with no apparent break in the mucosa; and edematous swellings in the subcutaneous tissue, alimentary tract, and around the lymph nodes. Blood-tinged urine has also been reported as a rare finding.

A similar postmortem picture may be seen in equines, but a different set of lesions has been described in a number of cases in horses. The postmortem lesions may consist of edematous infiltration of the subcutaneous and intramuscular tissues with no involvement of the digestive tract or the large parenchymatous organs.

In pigs, the postmortem lesions may resemble septicemic anthrax as described in ruminants. However, more often, there is extensive edema around the pharynx, mesenteric, and other lymph nodes. The nodes may also be hemorrhagic. If the focus of infection is in the gut, the intestinal wall may have a thickened necrotic appearance in a short segment, or more rarely, several feet of intestine may be involved. Peritonitis is often observed, as well as a thickened edematous mesentery with excessive peritoneal fluid.

Carnivores, such as dogs, show a severe inflammation of the tongue, throat, stomach, and intestines. Occasionally, the lips, gums, and jowls are also involved, especially if infected blood has been ingested.

3.3 Pathogenesis in laboratory animals

Following subcutaneous injection of viable spores into laboratory animals, the anthrax bacilli begin to germinate very quickly and, in about two hours, an edematous swelling begins to form at the injection site. Encapsulated vegetative bacteria occupy the lesion. After further bacterial multiplication, a zone of reaction characterized by altered capillaries and a collection of neutrophils, endothelial cells, fibrin, extravasated blood, and dilated lymphatics becomes apparent. Edema begins to spread out from the site of inoculation and the bacteria migrate by way of the lymph channels to regional lymph nodes. The organisms continue to multiply in the lymph nodes, and are continually released into the bloodstream. The spleen eventually becomes dark and swollen and usually contains large numbers of bacilli, although the appearance may be the same with relatively few organisms present. At approximately six hours before death, 50% of the bacilli are localized in the spleen. Shortly before, and at the time of death, 70-80% of the bacteria are in the bloodstream.

Historically, prior to the discovery of lethal toxins elaborated by B. anthracis, there were two popular theories, both incorrect, that attempted to explain the cause of death of animals having anthrax. One was that the considerable bacillosis causing a physical blockage of the capillaries with the resultant stoppage of blood flow; the other was that the bacteria multiplied and competed with the host for blood-bound oxygen causing anoxia and death. Although the exact mechanism as to how the toxins of B. anthracis cause death has not been fully elucidated, several theories have been postulated which include:

1. interference with carbohydrate metabolism and mineral imbalance causing hyperglycemia and hypermagnesemia;
2. direct damage to the central nervous system;
3. damage to the respiratory centre of the central nervous system with resultant anoxia due to respiratory collapse;
4. increased vascular permeability resulting in fluid loss.

4. Anthrax in man

4.1 Susceptibility

Man is somewhat resistant to anthrax when compared to other animal species, probably being slightly more sensitive than pigs and dogs, but considerably more resistant than ruminants. The disease caused by B. anthracis in man can be rather severe with mortality rates of 10%. Historically, the European plague or "black bane," presumed to be anthrax, and which occurred in 1613 A.D., killed 60,000 people. As recently as 1983, it was estimated that, worldwide, 20,000 to 100,000 people annually suffered from anthrax.

4.2 Clinical manifestations, pathogenesis and lesions

Three major clinical forms of the disease in man have been described, depending on the mode of entry and location of the infection. These are:

1. cutaneous;
2. inhalation;
3. intestinal anthrax.

Meningitis and/or septicemia/toxemia may be complications of any of the three forms.

Cutaneous anthrax accounts for about 95-98% of human cases. This occurs when the infectious organism enters through a cut, insect bite, or abrasion of the skin; there is no evidence that it penetrates the intact skin. During an incubation period of 1-7 days (1-5 most often), the organism germinates, multiplies, and produces toxin which causes a small papule to develop. This lesion is usually red, with the appearance of a pimple or insect
bites, and may be pruritic. As the lesion develops into a fluid-filled vesicle, extensive edema may evolve in adjacent tissues. Satellite vesicles may appear near the initial lesion and vesicular fluid, initially clear, becomes dark and bluish-black. When the vesicle ruptures, there is necrosis beginning at the centre, eventually developing into a typical black eschar. The lesion is relatively painless unless pressure is applied. Malaise and low-grade fever are experienced along with occasional tenderness of lymph nodes due to lymphadenitis. About 7-10 days after onset, the eschar dries to form a scab, which eventually looses and falls off, leaving a lesion that granulates and heals with a distinct scar remaining. Approximately 90% of the cases in man heal uneventfully; in about 10% of the cases, progressive extension of the infection into the regional lymph nodes and rapidly developing septicemia often results in death if not treated. The most prevalent infection sites of the cutaneous anthrax are the arms, face, and neck.

Inhalational anthrax (also called mediastinal anthrax) occurs when viable spores are deposited in the alveoli of the lungs. There is some evidence that this form may also result from cutaneous lesions on the chest, neck or upper arm. The spores are phagocytized and carried to the mediastinal lymph nodes where germination, multiplication, and toxin production take place. Toxin and bacteria are then taken up by the circulation via the efferent lymphatics producing a rapidly fatal toxemia and bacteremia. Early clinical symptoms mimic influenza, then the patient may show some improvement, only to be followed by severe respiratory distress. In some cases there is mediastinal edema with stridor (harsh respiratory sounds) and respiratory insufficiency. Untreated cases approach 100% mortality. Pulmonary lesions are minimal and may be due to a secondary pneumonia extending from the mediastinal nodes. The most common gross lesions seen at autopsy are mediastinal lymph nodes that are necrotic, hemorrhagic and edematous.

Intestinal anthrax is caused by eating flesh from animals dying from the disease. After ingestion of spores, penetration of the intestinal mucosa occurs with multiplication of organisms and toxin production in the submucosal tissues. The site of infection in the intestinal mucosa is demarcated as an ulceration due to the production of necrotizing toxins. The lesion is analogous to the eschar produced by cutaneous infection. The gut lesion is usually located at the terminal ileum or cecum and may be of sufficient size to cause hemorrhage into the gut lumen. The clinical course that accompanies intestinal anthrax is manifested 2 to 5 days following ingestion of the contaminated meat. Initial signs of nausea, vomiting, anorexia, and fever are followed by abdominal pain, meteasesis, and, occasionally, bloody diarrhea. The leukocyte count may be moderately elevated with a left shift and increased numbers of immature forms. The disease often progresses to generalized toxemia, shock, cyanosis and death. Mortality rates range from 25%–50%.

Meningeal anthrax may occur as a sequel to any form of anthrax. It has been estimated that approximately 5% of cases of cutaneous anthrax develop the meningeal form. This form of the disease approaches 100% mortality unless high doses of antibiotics are given promptly by the intrathecal route. Death usually occurs 2–4 days after symptoms of meningitis are apparent.

5. Diagnosis

5.1 Diagnosis in animals

Since anthrax can cause a rather acute death in livestock, the differential diagnosis should encompass those diseases which also cause acute death and may present a carcass that mimics one dying of anthrax. Those diseases and conditions commonly associated with acute death include:

(1) infection with various clostridial organisms or toxins (botulism);
(2) metabolic conditions such as bloat, lactic acidosis or magnesium deficiency;
(3) lightning strikes;
(4) ingestion of toxic substances such as heavy metals, plants containing high concentrations of nitrates/nitrites or HCN (prussic acid), or other toxic plants;

(5) snake bite;

(6) peracute babesiosis.

If the clinical signs indicate the possibility that the animal died from anthrax, it is best that the animal not be necropsied. The unopened carcass rapidly becomes anaerobic due to proliferation of putrefactive bacteria which, in turn, does not allow the anthrax bacillus to sporulate, making the vegetative bacteria susceptible to the adverse conditions created by the decomposing carcass. It has been shown experimentally that recovery of the anthrax organism from guinea pig cadavers is very difficult 48 hours after death if kept at ambient temperatures of 25-30°C. After 72 hours, it is virtually impossible to recover viable anthrax bacilli from an unopened carcass at temperatures above 30°C.

In places where anthrax is of rare incidence and the disease is not initially suspected, a necropsy may occasionally be inadvertently performed. If this should happen, the pathologist should take necessary measures to prevent B. anthracis exposure to himself and the environment. The use of rubber gloves and protective clothing is necessary while performing the necropsy. Fresh, unfixed specimens should be considered potentially infectious, and should be kept in closed containers and handled carefully. All animal parts, instruments, clothing, and premises should be properly disinfected, autoclaved, or incinerated to prevent spread of contamination. (Annex 1.)

In areas where laboratory facilities are not readily available for isolation and identification of B. anthracis, a quite reliable field test for diagnosis in dead ruminants can be made by obtaining a drop of blood via puncture of the lip and preparing an air-dried blood film on a glass slide. This slide is stained with a Romanovsky stain (Wright’s Giemsa, Leishmann) and when observed microscopically, a mauve-red capsule is seen surrounding the anthrax bacillus. This test will also show babesia, anaplasma, theileria and trypanosomes, thus aiding in a differential diagnosis should these organisms be present.

If a laboratory facility having bacteriology capability is available and isolation and identification of B. anthracis is desired, in addition to the air-dried blood smear, a refrigerated sample of blood collected via jugular venipuncture may be submitted. Removing an ear and submitting to the laboratory for anthrax diagnosis is very unsatisfactory. If the animal has been opened for necropsy, the spleen and other solid organs will yield B. anthracis when cultured, if postmortem autolysis is not advanced.

When received at the laboratory, the slide is stained with Romanovsky stain, as previously described. Gram stains of blood smears or tissue impressions are not reliable as a diagnostic aid, as several spore-forming, gram positive rods of the genus Bacillus and Clostridium resemble B. anthracis. Isolation of B. anthracis may be accomplished on any nutrient agar; however, an agar containing blood cells will produce non-hemolytic, rough, ground glass colonies after 8 to 12 hours incubation at 37°C.

Once isolated, a reliable confirmatory test is lysis by specific bacteriophage.\(^1\) This test is performed by streaking a small area on a nutrient agar plate with the suspected isolate. A drop of the phage suspension is placed in the centre of the streaked area. After 6-8 hours incubation at 37°C, an area of lysis (no growth) can be observed on the culture plate where the phage was dropped, with growth of the organism at the periphery of the lytic area. This becomes more evident with overnight incubation. Some laboratory adapted strains of B. anthracis have been shown to be resistant to lysis by bacteriophage, but field isolates are apparently susceptible. The Sterne strain, used for B. anthracis vaccine is sensitive to bacteriophage lysis and can be used for positive control. This is

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a vaccine that is in every way related to the pathogenic bacteria, with the exception of capsule formation.

Other tests have been described, some of which are listed below for information, but which are not essential for the diagnosis of anthrax. However, this does not preclude their usage to the interested individual.

A modified "string of pearls" test is easily done by streaking a suspect colony on nutrient agar plate that has no blood or dyes added. Over the streaked area, a cover glass is placed and adjacent to the cover glass a 10-unit penicillin disc. After 2-3 hours incubation at 37° C, the plate is placed on a microscope, a drop of oil applied to the cover slip, and observed under oil immersion (100X). If *B. anthracis* is present, as one moves the objective toward the penicillin disc, one observes slightly swollen bacilli, then grossly swollen bacilli, then rounded bacilli resembling pearls in a necklace. Laboratory animal inoculation and recovery of *B. anthracis* from dead, inoculated animals is very reliable. The problem of procurement of the animals, proper housing, and potential hazard to laboratory personnel due to dissemination of viable spores in the laboratory must be taken into consideration.

Contaminated specimens can present a problem, as far as diagnosis is concerned. Several procedures have been published to circumvent the problem of gross contamination. Some involve destruction of vegetative bacteria by heating and subsequent recovery of *Bacillus anthracis* through germination of surviving spores. Others involve the addition of inhibitors to media to prevent overgrowth of gram negative enteric bacteria. The use of phenylethanol blood agar allows the growth of *B. anthracis* while inhibiting the growth of the gram negative bacteria. However, even using this medium, contamination may still be a problem. At least three media have been formulated specifically for the isolation of *B. anthracis* from contaminated specimens, including soil. One medium contains hematin and lysozyme as inhibitors, another uses polymyxin B and propidil, and a third, called PLIT medium, has polymyxin B, lysozyme, dihydroxy-diaminatetraacetate (EDTA), and thallous acetate added as inhibitors.

An outline of an eight-hour short course to train persons to diagnose anthrax in animals is given in Annex 6.

5.2 Diagnosis in Man

Anthrax may be suspected in persons engaged in agricultural activities, veterinarians, and rendering plant employees who develop "malignant pustules" on exposed parts of their bodies, subsequent to coming in contact with carcasses contaminated with *B. anthracis*. Employees of animal by-product processing establishments such as wool carpet manufacturers, fertilizer plants, bone meal and bone charcoal plants, tanneries, animal source brush manufacturers, etc., should be considered at risk. Persons who purchase curios made of animal parts, as well as persons who use wool and mohair as hobbies, may occasionally be exposed to anthrax. The Zimbabwean major epidemic of anthrax (1978-1980) emphasizes the potential risk of the disease in man when a combination of civil unrest, disease in livestock, biting flies, and human protein deprivation occurs.

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Persons who fall into the above categories and develop a suspicious fluid-filled pustule surrounded by inflammation on exposed parts of the body should be tested for anthrax.

Cutaneous anthrax is not particularly difficult to diagnose if a history of exposure is noted and a typical eschar is present on exposed skin surfaces. B. anthracis can be cultured from the lesion, especially in the area immediately below the necrotic centre. Antibiotic therapy almost always results in uneventful recovery (see Treatment).

If the cutaneous lesion is on the upper torso, head, neck or upper arm, it is not unusual to have mediastinal edema and respiratory obstruction. Respiratory failure commonly causes death in individuals having cutaneous lesion on the upper part of the body. Mediastinal edema is not confined to inhalation anthrax.

The antemortem diagnosis of mediastinal or intestinal anthrax in man is somewhat more difficult owing to the rapidly fatal course following initial symptoms. Laboratory testing on samples such as throat swabs, sputum, or stool from patients is often not feasible. It is, therefore, essential that early diagnosis and prompt antibiotic and steroid therapy be instituted in order to arrest these forms of the disease. Diagnosis in persons having died of anthrax is the same as that previously described for diagnosis in animals, including isolation and identification of the causative organism.

6. Prevention

The control of anthrax in domestic animals has been best achieved by establishing an immune population through vaccination. Pasteur pioneered the use of live attenuated bacterial vaccines using B. anthracis in 1881. The original vaccine consisted of a two-stage vaccination programme. The primary inoculum was prepared by incubating virulent vegetative B. anthracis at 42-43°C for 15-20 days which attenuated the pathogenicity to the point that it killed only mice and young guinea pigs but not adult guinea pigs or rabbits. The second inoculum was prepared by incubating only 10-12 days at 42-43°C and was less attenuated than the primary inoculum, being able to kill mice, adult guinea pigs and a certain proportion of rabbits. Pasteur proved the efficacy of his vaccine by vaccinating sheep, goats, and cattle with the primary vaccine and, 11 days later, with the secondary vaccine followed by the challenge two weeks later of the vaccinated and unvaccinated controls. All vaccinated animals except one sheep (which died of an unrelated illness) survived the challenge, whereas the unvaccinated sheep and goats were dead two days later and the unvaccinated cattle showed edema and fever.

Only slight modifications of Pasteur's original vaccine were made during the next 50 years. In 1937, Sterne developed an avirulent spore vaccine using nonencapsulated variants. These mutants are apparently permanent and do not revert to virulence through succeeding generations by becoming encapsulated. The discovery of this vaccine has outmoded the old Pasteur vaccine and, through the use of the Sterne vaccine, several major outbreaks have been brought under control within a week to 10 days after vaccination. The major disadvantage of the nonencapsulated vaccine is that the immunity conferred is relatively short-lived and annual revaccination is recommended to keep animals protected. (See WHO requirements for manufacturing anthrax spore vaccine, live for veterinary use).

Preventive methods to minimize the spread of B. anthracis should be practised and in most developed countries are defined by law and administered by animal health officials. Proper disposal of carcasses, manure and bedding of animals having died of anthrax is very important to prevent dissemination of virulent organisms. The fact that B. anthracis does not sporulate in the absence of oxygen, and that vegetative organisms are rapidly destroyed in competition with putrefactive bacteria, lends credence to the practice of not performing a necropsy on an animal suspected of dying of anthrax. The recommended practice for disposal of an anthrax-infected carcass is to either incinerate the carcass or bury it deeply (at least 2 metres), and cover with a layer of quicklime (anhydrous calcium oxide) and a top layer of soil. (Annex 1.) This procedure is accompanied by vaccination of


healthy animals, and isolation and antibiotic therapy of animals showing illness. Concurrent use of vaccine and antibiotics on the same animal is clearly contraindicated since the antibiotic will inactivate the vaccine.

Often, where outbreaks of anthrax occur, urgent action is needed to prevent further outbreaks. A contingency plan for the prevention and control of anthrax is given in Annex 5.

Fomites that have become contaminated with B. anthracis should be disinfected using hot lye (NaOH), cresol, or hypochlorite. Creaseline disinfectant at a concentration of 300g/litre (4 oz./gallon) of water, liquified phenol (USP 87% phenol) at 10g/litre (1 lb./12 gallons) of water, and chlorinated lime (USP 30% chlorine) at 10g/litre (1 lb./12 gallons) of water, have also been recommended for disinfection. The disinfectants are applied after organic material has been removed from surfaces and walls and when the ambient temperature is above 15.5°C. (Annex 1.)

Eradication of anthrax in domestic animals through the use of vaccination can be, and has been achieved. Sterne did this in a region of South Africa where there were approximately 2 million cattle and anthrax was enzootic. The major obstacles to be overcome in eradication of the disease are purely economic, e.g. cost of the vaccine and cost of the labour required to administer the vaccine. Most developed countries are content to accept the loss of a few cases of anthrax in livestock rather than to embark on a costly eradication programme.

The prevention and control measures to be taken have been formulated in the International Zoo-Sanitary Code by the International Office of Epizootics, Paris, France, 1982 (see Annex 2).

The prevention and control of anthrax in free living game animals presents inherent difficulties not encountered in domestic animal populations. Diagnosis by microscopic examination of stained blood smears of all dead, wild animals should give an early diagnosis of anticipated epizootics in enzootic areas. Prompt disposal of all carcasses dying of anthrax by burning, or possibly deep burial, should be practiced. Since water holes are potential sources for B. anthracis spores to be concentrated, measures to provide alternate water supplies may be considered until rainy seasons flush and dilute the contamination. Methods to control predators, biting flies, and carrion eaters may also need to be considered.

Vaccination of free-living game has been practised to a limited extent. Vaccination by dart gun from aircraft and herding animals into enclosures has been described.

Controlled studies to evaluate practical methods to vaccinate free-living game against anthrax by the oral route through feed or water are lacking.

Preventive measures for man have been limited to vaccines for use in "at risk" persons, such as workers in wool or hide processing plants, certain laboratory personnel, and some veterinarians. A non-living vaccine was developed by Gladstone at the Lister Institute in the United Kingdom. Sterne found that this vaccine produced as good immunity in guinea pigs as had its live vaccine (unpublished data). A vaccine developed in the U.S.A. is a soluble cell wall antigen prepared from microaerophilic cultures of a nonencapsulated strain of B. anthracis, absorbed with aluminum hydroxide. Although its use is believed to reduce the incidence of anthrax, its efficacy in guinea pigs is 20 times less than the Sterne vaccine. In the USSR, at least two living vaccines have been developed and have been used in human beings.

Information on requirements for anthrax spore vaccine for veterinary use is given in the

1 USP = United States Pharmacopoeia.
reference contained in footnote 1 on page 11; sources of human/animal anthrax vaccines are given in Annex 3.

It has been postulated that a non-immunized person may inhale up to 1,300 B. anthracis spores during an 8-hour period without ill effects. In mills processing imported goat hair, industrial air samplers have been used to detect the level of spore contamination. If spore counts in the air exceed safe levels, they can be reduced by increasing the ventilation throughout the mills by installing exhaust fans in the ceilings.

Since cases of industrial anthrax are due to contaminated animal by-products, elaborate methods for disinfecting these products without affecting the quality have been developed. In the United Kingdom, a government wool disinfecting station was established at Liverpool in 1921. It used the following process on all imported wool:

(1) a 10-minute exposure to 0.5% sodium carbonate at 38°C;
(2) 10 minutes in a 2% formaldehyde solution at 42°C;
(3) 10 minutes in a 1.8% formaldehyde solution at 42°C;
(4) wash or rinse for 10 minutes in a constant flow of 38°C clean water with less than 0.25% formaldehyde;
(5) dry and cool;
(6) bale.

In South Africa, the requirements for disinfecting contaminated bone meal were as follows:

(1) steam sterilized at 2,800g/cm² (40 pounds/sq. inch) for 2 hours in a digester of not more than 4 tons capacity; or

(2) treatment of broken bone with benzene vapour at 95 to 115°C for not less than 4 hours, followed by 2 hours of treatment with live steam at 5,600g/cm² (80 pounds/sq. inch); or

(3) treatment of broken bones for at least 8 hours with benzene vapour at 95 to 115°C.

Other methods of eliminating B. anthracis contamination varies from the use of 3% peracetic acid for sterilizing contaminated mink pelts and dielectric heat on contaminated burlap bags.

7. Treatment of anthrax in man and animals

Prompt antibiotic therapy in both man and animals usually results in dramatic recovery of the individual infected with anthrax. B. anthracis is highly susceptible to penicillin and the tetracyclines. The treatment of choice for uncomplicated cutaneous anthrax in man is procaine penicillin G at a dosage of 10,000 U per kilogram body weight per day intramuscularly at 6 hourly intervals for 5 to 7 days. If the patient has extensive lesions or is showing systemic illness, intravenous procaine penicillin G at doses up to 10 million units is recommended. The dosage for tetracycline is 15 mg per kilogram body weight per day at 6 hourly intervals, administered orally for 5 to 7 days. In people showing mediastinal edema, high doses of steroids are also indicated. If meningitis is suspected, intrathecal penicillin may be efficacious.

In animals (cattle and horses), the recommended dosage of procaine penicillin G is 6 million units intramuscularly daily. Oxytetracycline given intravenously at 5 g daily in divided doses is also recommended. Other antibiotics such as chloramphenicol and erythromycin appear to be less effective, but can be utilized if penicillin or tetracycline are not available.
SELECTED REFERENCES


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The following WHO staff participated in the elaboration of this guide:

Dr K. Bügel, Chief, Veterinary Public Health, Division of Communicable Diseases
Dr T. Fujikura, Veterinary Public Health, Division of Communicable Diseases
ANNEX I

DISINFECTION IN ANIMAL HUSBANDRY

Introduction

This annex lays emphasis on the reservoir of anthrax, its mode of transmission to man and to other animals, the sensitivity of the aetiological agent to chemical and physical means of disinfection, as well as the practical methods for its application.

It is worthwhile remembering that various concentrated liquid disinfectants mentioned should be handled with caution using gloves and aprons to avoid contact with the skin (burns or allergic sensitization) and mucosal membranes (goggles should be worn to protect the eyes). Clean water for washing or showering should be available close by when handling concentrated disinfectants. Proper labelling is recommended to avoid absorption of concentrated or diluted disinfectants.

Appropriate respirators must be worn by personnel who have to enter rooms disinfected with gas prior to ventilation.

In some countries lists of approved disinfectants are published frequently, e.g. in the United Kingdom by the Ministry of Agriculture, Fisheries and Foods. These provide approved dilution rates for use in respect of different infectious diseases of animals, including those of a zoological nature.

It should be emphasized that, for controlling anthrax, the concentrations of disinfectants and their exposure times refer only to situations in which adequate cleaning has already been undertaken.

Anthrax

The reservoir of anthrax is a complex of animals and environment polluted with spores that survive for many years. On account of the high resistance of anthrax spores, disinfection becomes very difficult, and the contact time of all disinfectants should therefore be increased. In spite of the great significance of soil in anthrax epidemiology, there are, as yet, no suitable effective means in disinfection practice to ensure decontamination of soil foci on large territories.

The soil surface can be disinfected by one of the following disinfectants: 10% caustic soda solution, 4% formaldehyde solution, 5% clarified slaked lime solution, 10% neutral calcium hypochlorite solution, 15% basic calcium hypochlorite or sodium dichloroisocyanurate solutions (by active chlorine).

The usage of these solutions as follows: 4% formaldehyde - 5 litres/m², other disinfectants - 10 litres/m² with an exposure time of 24 hours.

At temperatures below 0°C, hot (50-60°C) neutral calcium hypochlorite solution is used containing 15% active chlorine prepared with 15-20% sodium chloride solution at a rate of 10 litres/m².

Soil, at the site of animal death, or where autopsy of anthrax-infected animals was mistakenly carried out, should be carefully burnt, followed by washing with slaked lime solution containing 5% active chlorine at a rate of 10 litres/m². Subsequently, the soil should be dug to a depth of at least 25cm, and mixed with dry slaked lime containing not less than 25% active chlorine at a rate of 1 part of slaked lime to three parts of soil. The soil should then be moistened with water.

To disinfect animal houses, preliminary disinfection is essential even before cleaning. The following disinfectants can be used for this purpose: 10% hot caustic soda solution, 4% formaldehyde solution, chlorine-containing disinfectant solutions (calcium hypochlorite, containing 5% active chlorine, 7% hydrogen peroxide solution or 2% glutaraldehyde solution).

Disinfection with the above-mentioned solutions is conducted twice with an interval of two hours between each application at a rate of 1 litre/m² for animal premises. When using hydrogen peroxide and glutaraldehyde, the surface should be treated twice with an interval of two hours between each application at a rate of 1 litre/m².

After the final disinfectant application the house should be closed for 3 hours followed by ventilation.

For disinfection of surfaces at low temperatures (below 0°C), 8% solutions of basic calcium hypochlorite or neutral calcium hypochlorite and 12% solutions of sodium dichlorisocyanurate are used. These solutions are prepared with hot (50-60°C) 15% and 20% sodium chloride solutions, and are used at a range of low temperatures from 0°C to -15°C and -15°C to -30°C, respectively.

Contaminated bedding, manure, and unused feed should be burned or treated with disinfectant and buried deeply at a suitable site.

Overalls, brushes, scrapers, buckets and other small implements should be decontaminated by dipping into 1% activated chloramine solution, or 4% formaldehyde solution for 4 hours or subjected to boiling in 2% calcined ash for not less than 90 minutes.

For articles, leather and rubber footwear and other articles which are likely to be damaged by any of the disinfection methods described above should be subjected to formaldehyde fumigation involving the use of 250 ml of formalin per m³ of the chamber volume, at 58-59°C with an exposure time of 3 hours.

Milk produced from affected or suspected cows should be disposed of after decontamination by adding lime containing not less than 25% active chlorine at a rate of 1 kg per 20 litres of milk. Milk can be considered as decontaminated after such treatment for 6 hours.

Trailers and vehicles can be disinfected by hot solutions of 3% cresylic disinfectant or 1% 3-phenylphenate.

In the case of anthrax outbreaks, all contaminated carcasses (necropsy must be avoided) must be incinerated or buried deeply.

It is extremely important that animal by-products such as hides, wool and hair are disinfected. Formaldehyde for wool, and hydrochloric acid and salt (2.5% and 15% respectively) for the disinfection of hides are recommended. Hair can be disinfected by boiling or autoclaving (120°C for 20 minutes). It can also be disinfected by dry heat at 95°C for 24 hours or by steaming for 6 hours.
ANNEX 2

INTERNATIONAL ZOO-SANITARY CODE
Amended edition, 1982
International Office of Epizootics, Paris, France

ANTHRAX

Article 2.1.5.1.

For the purposes of this Code, the maximum incubation period for Anthrax shall be 20 days.

Article 2.1.5.2.

On the application of the measures provided for in this Code, Veterinary Administrations of importing countries should require:

For animals for breeding or rearing or slaughter,

the presentation of an international zoo-sanitary Certificate attesting that:

1) on the day of their exportation, they showed no clinical signs of Anthrax;
2) for 20 days before their exportation they were in an establishment in which no case of Anthrax was officially declared during that period;
3) and/or, they had been vaccinated with an officially controlled vaccine over 20 days and less than six months before their exportation.

Article 2.1.5.3.

On the application of the measures provided for in this Code, Veterinary Administrations of importing countries should require:

for wild ruminants, equine animals and porcine animals,

the presentation of an international zoo-sanitary Certificate attesting that:

1) on the day of their embarkation, they showed no clinical signs of Anthrax;
2) they had been vaccinated with an officially controlled vaccine over 20 days and less than six months before their exportation.

Article 2.1.5.4.

On the application of the measures provided for in this Code, Veterinary Administrations of importing countries should require:

for products of animal origin (domestic or wild ruminants, porcine animals and equine animals) destined for use in animal feeding,

the presentation of an international sanitary Certificate attesting that:

1) these products originate from healthy animals;
2) the products have been subjected to treatment which is adequate for the destruction of both bacillary and spore forms of Bacillus anthracis.

* See Appendix 5.1.3.
Article 2.1.5.3.

On the application of the measures provided for in this Code, Veterinary Administrations of importing countries should require:

for products of animal origin (domestic or wild ruminants, porcine animals and equine animals) destined for industrial purposes,

the presentation of an international sanitary Certificate attesting that:

1) these products originate from healthy animals;

2) the products have been subjected to treatment likely to destroy both bacillary and spore forms of Bacillus anthracis;

3) the products originate from areas where Anthrax is not prevalent.

APPENDIX 5.1.3.

NORMS CONCERNING THE PRODUCTION AND CONTROL
OF VACCINE AGAINST ANTHRAX

Vaccines against Anthrax referred to in Articles 2.1.5.2. and 2.1.5.3. should be prepared in accordance with the Requirements for Anthrax Spore Vaccine (Live - for Veterinary Use), Requirements for Biological Substances No. 13, World Health Organisation, Technical Report Series, No. 361, 1967, and should in particular conform to those requirements in respect of control of source materials, production methods and precautions, freedom from contamination, safety, immunogenicity, identity, number of cultivable spores and stability.
ANNEX 3

SOURCES OF ANTHRAX VACCINES FOR HUMAN USE

Manufacturer: MICHIGAN DEPARTMENT OF PUBLIC HEALTH
3500 North Logan
P.O. Box 30035
Lansing
Michigan 48909
USA

Telephone: (517) 373-1290
Telex: Telegraphic address:

Type of vaccine: Anthrax vaccine, Aluminium Hydroxide adsorbed

Proprietary name: Liquid

Available packaging: 5 ml vials (10 doses)

Delivery time: Immediately, upon request

Recommended storage temperature: 2°C - 8°C

Shelf life: 12 months

Stability under tropical conditions: 12 months

Other remarks:

Manufacturer: INSTITUT PASTEUR
13 Place Pasteur
Tunis
Tunisia

Telephone: 283-022; 283-023
Telex: Telegraphic address: BP74
1002 TUNIS BELVEDERE

Type of vaccine: Formaline inactivated anthrax vaccine

Proprietary name: Anthrax vaccine

Available packaging: 2 x 25 dose ampoules

Delivery time: Immediate

Recommended storage temperature: + 4°C

Shelf life: 6 months

Stability under tropical conditions: Stable

Other remarks:
Manufacturer: Tbilisi Research Institute for Vaccines and Ser a
L. Gotua 3
380042 Tbilisi
USSR

Telephone: 37 11 15
Telex:
Telegraphic address:

Type of vaccine: Anthrax vaccine, live, dried
Proprietary name: "Sti" anthrax vaccine, live, dried
Available packaging: 5 x 20 dose ampoules + 5 x 1 ml of diluent
Delivery time: 10 weeks
Recommended storage temperature: 4°C
Shelf life: 2 years
Stability under tropical conditions: For all information write to V/O "Medexport"
Kakhovka 31, 121200 Moscow, USSR

Sources of Anthrax Vaccines for Veterinary Use

Vaccines are available from the following institutions:

1. Lauer, Dakar-Kanh, Senegal
2. Rhone-Mérieux, 17 rue Bourgelat, 69002 Lyon, France
3. Indian Veterinary Research Institute, Izatnagar, India
4. Kenya Veterinary Research Department
Kenya Agriculture
5. Laboratoire Central Vétérinaire, Bamako, Mali
6. Wellcome Foundation Ltd., Berkhamstead, Herts, United Kingdom
7. National Veterinary Institute, Debre-Žeit, Ethiopia

For further information, please contact:

Animal Production and Health Division
Food and Agriculture Organization of the United Nations (FAO)
Via delle Terme di Caracalla
00100 Rome
Italy
## World Distribution of Anthrax in Livestock

<table>
<thead>
<tr>
<th>Regions</th>
<th>Bovine</th>
<th>Equine</th>
<th>Ovine</th>
<th>Swine</th>
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<td>7/45</td>
<td>18/45</td>
<td>6/45</td>
</tr>
<tr>
<td></td>
<td>(60.0%)</td>
<td>(15.6%)</td>
<td>(40.0%)</td>
<td>(13.3%)</td>
</tr>
</tbody>
</table>

| AMERICAS         | 17/34  | 8/34   | 9/34  | 7/34  |
|                  | (50.0%) | (23.5%) | (26.5%) | (20.6%) |

| SOUTH-EAST ASIA  | 8/12   | 2/12   | 7/12  | 3/12  |
|                  | (66.7%) | (16.7%) | (58.3%) | (25.0%) |

| EUROPE           | 19/34  | 4/34   | 12/34 | 2/34  |
|                  | (55.9%) | (11.8%) | (35.3%) | (5.9%) |

| EASTERN MEDITERRANEAN | 8/23   | 3/23   | 10/23 | 2/23  |
|                       | (34.8%) | (13.0%) | (43.3%) | (8.7%) |

| WESTERN PACIFIC    | 4/20   | 1/20   | 0/20  | 1/20  |
|                    | (20.0%) | (5.0%) | (-)   | (5.0%) |

| Total             | 83/168 | 25/168 | 36/168 | 21/168 |
|                   | (49.4%) | (14.9%) | (33.3%) | (12.5%) |


** Number of countries where anthrax occurred/number of countries reported.
ANNEX 5

CONTINGENCY PLAN FOR THE PREVENTION AND CONTROL OF ANTHRAX*

Contingency plans may prove necessary to protect persons in charge of handling suspect or infected animals and their parts, or to protect animals in a previously non-exposed environment.

Persons who have to handle suspect or infected animals should take the following precautions:

1. to be vaccinated against anthrax if their exposure is frequent and if the human vaccine is available;
2. to avoid all blood-spilling operations (slaughtering included) in infected or suspect animals, be they alive or dead;
3. to use protective equipment such as strong gloves, masks, etc., to avoid contact both direct and by inhalation with infected and suspect animals, or their parts. The equipment used must be adequately disinfected and if possible, destroyed;
4. to avoid any contact with other persons (family included) and with animals, without first changing clothing and taking appropriate disinfection measures;
5. to report to the physician any suspicious symptoms appearing after contact with infected material.

The appearance of infection in animals from a previously uninfected environment (e.g. farm, etc.) may be dealt with in the following ways:

1. to identify, isolate and remove apparently healthy animals;
2. to decontaminate soil that may contain infective material. To avoid any unnecessary ante- and post-mortem operation in animals;
3. to destroy carcasses and their parts by burning or deep burial with quicklime;
4. to vaccinate all susceptible animals; in cases of high exposure more rapid protection may be obtained by specific immune serum or by antibiotics;
5. to control scavengers and possible vectors, such as flies, rodents and birds;
6. to perform an epidemiological investigation to detect the source of infection (animal, feed, environment) to avoid further spread;
7. to take proper measures to avoid the contamination of water and soil and the spread of the infection to other farms and environments.

Anthrax may create special problems in case of natural disasters. Attention should be paid to avoid human infection through feeding on infected animals or handling infected animals and animal parts, and to avoid infection of animals and the contamination of water and soils. The contingency plans described above and the "Guidelines on Veterinary Public Health in Disaster Situations" (in preparation by the WHO Collaborating Centre for Research and Training in Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy) may provide some guidance for these situations.
ANNEX 6

OUTLINE OF EIGHT-HOUR SHORT COURSE TO TRAIN PERSONS TO DIAGNOSE ANTHRAX IN ANIMALS

Day before

Streak blood agar plates with Sterne vaccine. (Optional) Inject laboratory animals with virulent B. anthracis.

Morning

(Optional) Set up Phage and String-of-Pearls (see pp 9-10 of text)

1. Thirty to forty-five minute lecture of basic bacteriology with visual aids to teach the concept of microbes and how they exist in nature, how they are transmitted, cause disease, protectionary precautions, etc.

2. One-hour orientation of use of microscope.

3. Two-hour practice of preparing slides and staining techniques. Gram and Dif-Quik (or other Romanovsky stain).

(Optional) Examine String-of-Pearls test.

Break for lunch

Afternoon

4. Forty-five minute lecture with visual aids of clinical signs and post-mortem lesions of anthrax in animals.

5. One-hour practice or lecture to learn specimen collection and slide preparation from animal source (mice or guinea pigs).

6. Lecture with visual aids of proper carcass disposal, disinfection of premise and personal hygiene.

(Optional) Examine Phage test.

Materials needed

Microscopes
Slides
Stains
Audio-visual equipment

Optional equipment

Incubator
Tryptose agar plates
Blood agar plates
Sterne's vaccine
Mice or guinea pigs
Burner, bacterial loops, swabs