Anthrax

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DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

Clinical signs and pathology

The incubation period of anthrax under natural conditions is not known, but probably ranges from one to 14 days. Under experimental conditions, it varies considerably, and is influenced by the virulence of the organisms, the resistance of the animal, the route of infection and the number of infecting organisms. Some animals suffering from anthrax develop a fever but its severity is extremely variable. It appears to be characteristic for a species, and may depend on the number of infecting organisms. The fever usually declines before death.

Three different manifestations of anthrax are recognized: peracute or apoplectic, acute, and subacute to chronic forms. Cattle, sheep, goats and some wild ruminants, such as kudu, roan antelope and impala, mainly manifest the peracute and acute forms. Horses, donkeys and zebra suffer from the acute to subacute form. Pigs, carnivores and immunized animals (i.e. immunized animals challenged with high doses of infective material, or animals with low-grade immunity) usually contract the subacute to chronic form.

In peracute anthrax, the course of the clinical disease is usually less than two hours. The majority of animals are found dead without having shown signs of illness. In those in which clinical signs occur, pyrexia (depending on the species), restlessness or anxiety, muscle tremors, dyspnoea, congestion of mucous membranes, ruminal stasis, collapse, and rarely terminal convulsions may be observed. Blood-stained fluid sometimes exudes from the nostrils, mouth and anus. Experimentally, impala and most wild ruminant species under natural conditions rarely show clinical signs until immediately before death when they are afebrile and manifest pronounced dyspnoea due to lung oedema, convulsions, and paddling movements of the legs. Terminally, they usually show opisthotonus with the fore legs rigidly extended. Similar clinical signs are regularly seen in wild ruminants naturally dying of the disease.
Anthrax carcass (zebra) with blood from nostrils and mouth. The upper eye has been removed by pied crows

A fatal case of anthrax in a kudu bull (Tragelaphus strepsiceros). Note the extensor rigidity and that the animal exhibited paddling before death as evidenced by marks left in the soil

The course of acute anthrax is usually less than 72 hours. A rise in body temperature is an inconsistent feature. At first, affected animals remain standing with their heads hanging and their eyes staring; they are depressed, lag behind the others, walk about listlessly, and lie down frequently. Respiration is laboured and rapid, and scattered small haemorrhages may occur in the visible mucous membranes and skin. The appetite is lost, rumination is suppressed and acute digestive disturbances may set in. Some animals may develop diarrhoea, which is usually haemorrhagic. In lactating cows, milk production decreases and the small amount of milk still secreted is either blood-stained or yellow. Pregnant animals may abort. Oedema of the tongue and subcutaneous oedematous swellings in the region of the throat (mostly carnivores) and ventral parts of the thorax, abdomen and perineum may occur, usually at the sites where the infection due to the bites of stable or horse flies took place. Horses that become infected orally, develop enteritis, colic, a high fever, and depression. They generally die within two to four days of infection or appearance of the first clinical signs.

In the subacute to chronic form of the disease, the course usually extends for more than three days before recovery or death occurs. The most frequent sign is an oedematous swelling of the face, throat and neck following primary infection of the pharynx, pharyngeal tissues and regional lymph nodes. The swelling in the pharyngeal region may become so extensive that it interferes with respiration (and may result in asphyxia) and the ingestion of food and water. The infection may progress to a more acute stage, or it may remain localized for some time, or the animal may recover. Recovery has been witnessed especially in carnivores and pigs, which have some degree of natural resistance to anthrax. Serological evidence also seems to indicate that herbivores may on occasion suffer subclinical anthrax infection.
In humans, anthrax manifests in one of three clinical forms: cutaneous, pulmonary or intestinal. Meningitis and septicaemia may develop during the course of any one of these. The cutaneous form accounts for more than 95 per cent of reported cases. The most prevalent sites of cutaneous anthrax are the exposed areas of the body, such as the head, face, neck, arms, hands and legs. Cutaneous lesions in humans are characterized by the development of a papule surrounded by a zone of hyperaemia and oedema of adjoining tissues. Central necrosis of the lesion occurs with the formation of an eschar, and is followed by ulceration. The cutaneous lesion is generally not painful, and, despite the designation “malignant pustule”, there is an absence of pus and suppuration at all stages. The subsequent course depends greatly on the site of the lesion. In approximately 90 per cent of cases, cutaneous lesions regress and spontaneous recovery occurs, but in about ten per cent of cases the infection spreads to the regional lymph nodes and develops into a fatal septicaemia.

Pulmonary anthrax (woolsorter’s disease or mediastinal anthrax), occurs when dust particles laden with anthrax spores are inhaled and deposited in the alveoli where they are phagocytosed by macrophages and transported to the regional lymph nodes in which they vegetate and the bacilli multiply. From there bacteria may enter the blood and initiate a rapidly fatal septicaemia. This form usually manifests as an acute atypical pneumonia or influenza followed by signs of cardiac failure. Virtually all untreated cases are fatal.

Intestinal anthrax is caused by eating uncooked or underdone B. anthracis-infected meat. Although this route of infection is considered most unlikely in developed countries, it is not uncommon in developing countries. The nature of the clinical signs depends on where the lesion is located. Involvement of the oropharynx results in fever, necrotic pseudomembranous mucosal lesions, cervical lymphadenopathy and marked oedema of the surrounding tissues extending some distance from the lesion. The neck and pharyngeal regions may be markedly swollen. Involvement of the intestines causes nausea, vomition, anorexia, fever, abdominal pain and a haemorrhagic enteritis.

The principal lesions in septicaemic anthrax in animals comprise edema, hemorrhage, and necrosis. Depending on the route of infection, host susceptibility and virulence of the bacteria, lesions vary at necropsy. Thus in some cases, only lesions consistent with septicemia are evident, whereas in others localized necrotizing lesions are found. Septicaemia and bacteraemia are generally consistent features of anthrax, although the numbers of bacilli encountered in the blood may vary considerably, depending on the species.

In ruminants, in which the course of the disease is usually acute or peracute, the most consistent changes encountered during a necropsy include evidence of rapid post-mortem decomposition of the carcass (resulting in marked bloating soon after death); incomplete development of rigor mortis; oozing of blood or blood-stained fluid from the natural body openings such as the nose, mouth and anus; dark-red, poorly-clotted blood; petechiae and ecchymoses throughout the carcass; degenerative changes in parenchymatous organs; extensive pulmonary oedema; excessive amounts of blood-tinged serous fluids.
in the peritoneal, pleural and pericardial cavities; oedema and haemorrhage in individual lymph nodes; and an enlarged pulpy spleen, the red pulp being blackish-red, soft and sometimes semifluid.

A blood clot that was expelled from the rectum of an African buffalo (*Syncerus caffer*)

As suggested by the names “splenic disease” and “miltsiekte”, severe splenomegaly is considered by many to be the most characteristic change at necropsy, but it is not a consistent feature and its absence does not rule out the possibility of anthrax. Certain species such as sheep, horses, pigs and impala rarely develop splenomegaly.

Splenomegaly
Other less frequent macroscopic lesions include localized inflammation and haemorrhages in the mucosa of the gastrointestinal tract, particularly in or in close proximity to lymphoid follicles and Peyer's patches, and free blood in the colon; drops of blood exuding through the skin; localized oedema in the subcutaneous tissue, wall of the gastrointestinal tract and around lymph nodes; haematuria; and congestion of subcutaneous blood vessels imparting a “fiery” appearance to the carcass. Most wild ruminants that have died from anthrax manifest a vasogenic brain oedema and the presence in the larger blood vessels of poorly-formed and disintegrating post mortal blood clots, containing numerous encapsulated bacteria.

Pigs may suffer from septicaemic, pharyngeal or intestinal forms of the disease. At one time the pharyngeal form was the most common but, with changing regulations regarding the feeding of food waste to pigs, this has become rare. In those which have died from the subacute to chronic disease resulting from primary invasion of *B. anthracis* through the mucous membrane of the pharynx, the cause of death may be the result of asphyxia following severe oedematous swellings of the mucosa and submucosa of the pharynx and glottis, and of the peripharyngeal and subcutaneous connective tissues of the throat and neck regions. A diphtheric to necrotic pharyngitis and tonsillitis, or eschar development at the site of entry of the organisms may be apparent while the regional lymph nodes are enlarged to several times their normal size and are bright to dark-red due to severe lymphadenopathy resulting from oedema, hyperaemia, haemorrhage and necrosis. In more chronic cases, lesions resembling tuberculous granulomas may occur in affected lymph nodes. Evidence of septicaemia is often absent from such cases.

In those animals in which primary infection has occurred lower down the gastrointestinal tract, localized lesions may be present at the site of entry of the organism. The capsulated bacilli which may be difficult to detect in blood smears are present in relatively large numbers (± $10^5$ cfu/ml) in the oedematous mesenteric fluid. Horses, donkeys and zebra usually suffer from septicaemic anthrax. With insect transmission, horses also manifest localized swellings at the sites of the insect bites, usually on the lower parts of the thorax and abdomen, shoulders, legs, perineum and around the external genitalia, with, in the latter stages of the disease, the development of the septicaemic form.
Severe inflammatory oedema of the soft tissues of the head, tongue and throat, stomach and intestines are characteristic features of anthrax in carnivores. In the African lion, localized lesions with no or late onset of septicaemia are often seen. These lesions are of variable severity, but are usually outspoken and localized in the tissues of the face, oral cavity and the regional lymph nodes. The changes are invariably characterized by localized necrotic glossitis or stomatitis to locally extensive necrotic cellulitis of the lips and the face accompanied by severe oedema causing severe swelling of the tissues of the head.
Laboratory confirmation

The history—including clinical manifestations and necropsy findings (if by mistake the carcass has been opened)—is usually the first step in the diagnosis of anthrax and should lead to further confirmation procedures.

In stained smears of blood or tissue fluid obtained from infected animals, the organisms appear truncated, measure 1.0 to 1.5 by 3.0 to 10.0 mm, commonly occur singly or in short chains and are surrounded by a well-developed capsule. The capsule shows up well with polychrome methylene blue (M’Fadyean reaction stain) and reasonably well with Wright’s and Giemsa stains. The staining reaction is so typical that demonstration of the capsule by staining blood smears is accepted as confirmation of anthrax. The absence of bacilli does not necessarily exclude the possibility of anthrax.

If, after the examination of a blood smear, anthrax is still suspected, suitable samples (blood, soft tissue or bone, and even soil underneath) should be collected for bacteriological examination and submitted on ice to a laboratory.

*Bacillus anthracis* grows readily on artificial media. Best results are obtained on media that contains serum or blood. In contaminated specimens selective isolation techniques are necessary. The PLET medium of Knisely is generally recommended and is sensitive to concentrations as low as three spores per gram of soil. An isolate with the characteristic colonial morphology that is non-haemolytic or only weakly haemolytic, non-motile, sensitive to anthrax-specific gamma-phage and penicillin, and able to produce a capsule *in vitro* in defibrinated blood can be diagnosed with confidence as *B. anthracis*. Because of humane considerations, *in vivo* tests are not recommended.

The polymerase chain reaction (PCR) and effective serological enzyme immunoassay (EIA) are useful diagnostic, epidemiological, and research aids but presently are confined to a few specialist laboratories. A very promising development for future portable on-the-spot diagnosis is an immunochromatographic protective antigen assay. Although recent developments in molecular biology have made genotyping of *B. anthracis* isolates possible, it is still a specialist procedure.

Differential diagnosis

In general, all causes of sudden death, especially in herbivores, and causes of localised subcutaneous edematous swellings, especially in horse and pig families and carnivores, resemble anthrax.