Network for wildlife health surveillance in Europe

Diagnosis Card

Anthrax

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Etiology

Gram positive, endospore forming, rod shaped, obligate pathogen, aerobic or facultative anaerobe belonging to the Bacillaceae family.

Bacillus anthracis is genetically homogeneous bacteria; however, researchers have identified three major lineages A,B and C divided in 12 sub-lineages. Lineage A is widely present around the world, in contrast lineage B is restricted to Southern Africa (B1) and B2 in Europe & North America while lineage C is very rare.

Affected species (wildlife, domestic animals, humans)

All mammals and some birds can contract anthrax. Susceptibility varies widely and most clinical cases occur in wild and domesticated herbivores. Cattle, sheep and goats are considered to be highly susceptible while horses are a little less. Pigs, other omnivores and carnivores are more resistant to disease, but they may become ill if the dose is high. Birds are highly resistant but ostriches & rheas are susceptible. Serious zoonotic disease.

Epidemiological characteristics and disease course

Anthrax is a seasonal disease, with different pattern in different location The frequency of the disease tends to increase during dry summers that follow very wet springs.
It's an acute to peracute infection. Dying or dead animals from anthrax are the source of infection for other animals through the dissemination of sporulated forms. Sporulation requires the presence of free oxygen; it occurs primarily after an infected fresh carcass has been opened &/or body fluids have seeped into the soil. Climate, topography, other microbial life, certain chemicals and plant materials affect the spore survival.
Spores remain at the site of dead animal, but some are dispersed mechanically by run-off water, scavengers (mammals and birds), passive vectors such as blowflies, houseflies, biting flies, mosquitoes and humans.
Source of anthrax infection for human: direct or indirect contact with infected animals or occupational exposure to infected or contaminated animal products.

Clinical signs

Domestic and wild herbivores: peracute systemic disease is most common, and usually sudden death (less than 2h after onset of disease) may be the only sign. In the acute form, fever and excitement may be followed by depression, stupor, disorientation, muscle tremors, dyspnoea before fall dead. Bloody discharges from the nose, mouth and anus are sometimes seen. A number will be subclinically infected and will display positive serum titres, in the range is 2-10 seroconverters per clinical case.

Equids: typically develop acute disease. Common symptoms include fever, anorexia, depression, severe colic and bloody diarrhoea and may last for up to four days before death results. Swellings may be seen in the neck, sternum, lower abdomen and external genitalia.

Carnivores and suids: they have usually mild sub-acute to chronic infections characterized by oedematous swellings of the face, throat, neck and/or ventral parts of the body. Subclinical seroconverters are common.

Gross lesions

Necropsies in the field should be avoided, to prevent contamination of the surrounding area with spores. Carcasses should be incinerated.

Principal lesions in septicaemic animals are those of widespread oedema, haemorrhage and necrosis.

Domestic and wild herbivores: marked bloating soon after death. Incomplete rigor-mortis. Dark, thick blood that does not clot readily ooze from the body orifices. Splenomegaly (rarely develop by sheep and impala).

Equids: septicaemia lesions with the presence of swollen draining lymph nodes of the digestive tract.

Carnivores: Oedema around the throat, tongue, neck, stomach and intestine. Signs of septicaemia will be evident: petechiae and ecchymoses in the lymph nodes, serosal surfaces of the abdomen and thorax, in the epicardium and endocardium. Spleen is usually enlarged and has a 'blackberry jam' consistency. Hemorrhages and ulcers are also common in the intestinal tract. Peritonitis. The tonsils may be covered by diphtheritic membranes or ulcers.

Histological lesions

Microscopic findings in generalized cases are dominated by the presence of large numbers of bacilli in blood and other tissues.

Differential diagnosis

All cause of sudden death, haemorrhagic septicaemia and oedematous localized lesions should be considered, according to species and geographical area involved.

Criteria for diagnosis

The history is usually the first step in the diagnosis of anthrax. Blood or tissue smears and culture. Colony morphology of *B. anthracis* is quite characteristic.

Recommended diagnostic method(s) and preferred samples (incl. recommended amount and appropriate storage)

Isolation and identification of the agent:

Staining: from fresh tissue like blood or other fluids collected from vascularized regions of the carcass (nostrils, eye socket, any bloody material). The slide may be air dried and stained with the Polychrome methylene blue stain or Azure B Capsule Stain (M'Fadyean reaction) to check the presence of typical bacilli with methacromatic capsule. Smear should be done within hours of collecting blood. This method remains the simplest and most reliable method of suspecting the presence of capsulated *B. anthracis*. If a delay in reaching the laboratory is expected, the smear should be made on a slide immediately after collection and the blood should be collected on a dry swab.

Culture and identification of the agent: Confirmation should be done on clinical or environmental specimen through culture and molecular methods. Isolation using semi-selective blood containing media is proven to have high specificity but low sensitivity in fastidious samples like: putrefied carcasses, carcasses of species with low terminal bacteremia (pigs and carnivores), and environmental samples. Molecular confirmation should be done using conventional or real time PCR targeting chromosome and plasmids. Suitable targets for chromosome are *plcR, Ba813 and rpoB* while *pag* and *capC* are used for confirming the presence of the pX01 and pX02 plasmids respectively.

Storage: 2-8 °C

Guidelines on appropriate specimens from animals suspected of having died from anthrax

Circumstance	Specimen	Container	Other action
Fresh carcass	Blood from vein (0.1 ml) or, if opened (e.g. by scavengers), blood and fluid from body cavity or piece of highly vascularized tissue (usually ear clipping).	Small vial, or leave in syringe.	Use for smear and culture. The smear can be prepared on the spot. Test with antigen detection device if available
Putrefied carcass	Piece of highly vascularized tissue and swabs of vascularized regions (nostrils, eye socket, any bloody material). Bloody soil from under head or tail.	Swab tubes. For soils, sealable specimen container.	Culture animal specimens on Blood agar A or semi selective agar (Columbia agar with polymyxin, trimetoprim and sulfametoxazole) . Culture soil on semi-selective agar after treatments including washing fluid and temperature at 64°C per 30 minutes.
Very old carcass, hides, bones, soil around/under carcass, etc.	Swabs of nostrils, eye sockets. Nasal turbinates. Soil from where body fluids believed to have fallen.	Swab tubes. For soils & turbinates, sealable specimen	Culture on semi-selective agar

Immunological detection and diagnosis: An immunochromatographic test for anthrax as a rapid field, diagnostic test, which detects the PA component of the circulating anthrax toxin was developed by US-Naval Medical Research Center. At the moment is being evaluated in several field studies but is available in few countries. Immunological detection of *B. anthracis* cells can be performed in laboratory using direct fluorescent-antibody (DFA) assays on cell wall and capsule antigens.
Few laboratory capable of preparing and supplying monoclonal antibodies.
Serology: Enzyme immunoassays (EIA) have been proved to be useful in serology. These tests targeting the major virulence factors of B. anthracis (PA, LF, EF) can be used to test the level of antibodies after vaccination and to have a retrospective confirmation of diagnosis.

APHAEA protocol (for harmonization at large scale)

Make a thin smear of clinical material by rolling over the swabs or spreading a small drop of blood on a microscope slide, then smear with Polychrome methylene blue or Azure B Capsule Stain. The presence of long chains of bacilli with metachromatic capsule is suspicious of B. anthracis.

Spread the same material onto blood selective media and incubate at 37°C for 24 hours. The presence of white colonies of pasty consistency and non-hemolytic is suspicious of B. anthracis.
PCR tests should be performed for confirmation of any suspicious samples.

Laboratories that can be contacted for diagnostic support

Canadian Food Inspection Agency Lethbridge Laboratory Canada (mailto:Betty.Golsteyn-Thomas@inspection.gc.ca)

Harvey National Veterinary Services Laboratories USDA, APHIS, Veterinary Services United states of America (mailto:ginger.r.harvey@aphis.usda.gov)

Spatial Epidemiology & Ecology Research Laboratory, Emerging Pathogens Institute, University of Florida, 2055 Mowry Rd., Gainesville, FL 32611

(mailto:jkblackburn@ufl.edu)

Friedrich-Loeffler-Institut (FLI), National Reference Laboratory for Anthrax, Germany, Naumburger Str. 96 a D-07743, Jena, (Mandy.Elschner@fli.bund.de)

Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Anthrax Reference Institute of Italy, Foggia, Italy(mailto:a.fasanella@izsfg.it

Recommended literature

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