सत्यमेव जयते

GOVERNMENT OF MEGHALAYA
OFFICE OF THE ASSISTANT DIRECTOR (DISEASE INVESTIGATION)
ANIMAL HUSBANDRY & VETERINARY DEPARTMENT
MEGHALAYA : : SHILLONG

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No. AD(DI)/ Anthrax-176/2023-24/ 2720-39 **Dated Shillong, the 12th January 2024.**

From : Dr. P. Blahwar
Assistant Director (Disease Investigation),
A. H & Veterinary Department,
Meghalaya, Shillong.

To :
1. The District A.H. & Veterinary Officer
Shillong/Nongstoin/Mawkyrwat/Nongpoh/Jowai/Khliehriat/Tura/
Resubelpara/Baghmara/Ampati/Williamnagar

2. The Deputy Director IDP, Upper Shillong/ RPBF Kyrdemkulai

3. The Sub-Divisional A.H. & Veterinary Officer
Sohra/Mairang/Amlarem/Dadenggre

4. The Manager Cattle farms/Pig farms/ Sheep & goat farms.

Subject : Standard Operating Procedure (SOP) for Anthrax in Livestock

Madam/Sir,

With reference to the subject cited above, I am enclosing herewith the Standard Operating Procedure (SOP) for Anthrax in Livestock. It is requested that the same may please be circulated amongst the officers under your jurisdiction for their information and necessary action incase of outbreak/suspected cases of anthrax.

This is for favour of your kind information and necessary action.

Enclosed:- As Stated

Yours faithfully

Assistant Director (Disease Investigation)
A. H & Veterinary Department
Meghalaya, Shillong.

Memo No. AD(DI)/Anthrax-176/2023-24/ **Dated Shillong, the 12th January 2024**

1. The Director, A.H & Veterinary Department Meghalaya, Shillong, for kind information.
2. The Joint Director (AHP) A.H & Veterinary Department Meghalaya, Shillong, for kind information.

Assistant Director (Disease Investigation)
A.H. & Veterinary. Deptt.
Meghalaya, Shillong

STANDARD OPERATING PROCEDURE (SOP) FOR ANTHRAX IN LIVESTOCK

Anthrax is a zoonotic disease caused by the bacterium *Bacillus anthracis*. This organism is an aerobic, or facultatively anaerobic, Gram positive, large capsulated bacterium that produces spores upon exposure to the environment through different body fluids of a dead carcass, enabling long survival. The vegetative cells do not last long outside the host body without sporulation. An unopened carcass over 72 h inhibits sporulation of the vegetative cells, resulting in the end of their life cycle. Once sporulated, they are resistant to the hostile environment, including heat, cold, desiccation, chemical disinfection, salting hides, pH, and irradiation. Anthrax affects farm animals and wildlife; occasionally, an outbreak occurs in humans and is distributed globally. Among the farm animals, particularly cattle, sheep are more prone to infection than goats and horses.

Transmission:

Spores of *B anthracis* can remain viable in soil for many years. Grazing animals may become infected when they ingest sufficient quantities of spores from the soil. In addition to direct transmission, biting flies may mechanically transmit *B anthracis* spores from one animal to another. Feed contaminated with bone or other meal from infected animals can serve as a source of infection for production animals, as can hay muddy with contaminated soil. Raw or poorly cooked contaminated meat is a source of infection in pigs, dogs, cats, mink, wild carnivores, and humans.

Clinical Signs and symptoms: (As per World Organisation for Animal Health)

It is difficult to determine the incubation period after field infection, but it is usually 3–7 days following oral challenge. In naturally infected cattle, the incubation period is 1–14 days or more. The clinical course of anthrax in animals may be peracute, acute, subacute, and chronic. When an anthrax outbreak starts, the peracute form is most often seen in cattle, sheep, and goats. The acute and subacute conditions most often are found in cattle, sheep, and horses. The chronic form of anthrax is common in swine and reported in cattle, horses, dogs, and cats.

Ruminant animals are often found dead with no indication that they had been ill. In **this acute form**, there may be high fever, muscle tremors and difficult breathing seen shortly before the animal collapses and dies. Unclotted blood may exude from body openings and the body may not stiffen after death. **Subacute form** may be accompanied by progressive fever, depression, inappetence, weakness, prostration and death.

In horses and (on occasions) in ruminants there may be digestive upsets and colic, fever, depression and sometimes swelling. These symptoms may last for up to four days before death results.

In carnivores when the animal feeds on an infected source there may be an intestinal form of the disease with fever and cramps from which animals sometimes recover.

Procedure for sampling from carcasses

In case of reported suspected cases, the following should be followed while collecting samples:

• Caution should be exercised if suspected of Anthrax as it is Zoonotic in nature.
• Appropriate PPE should be used before sampling
• Follow the table in Annexure-I for appropriate sample collection
• Label sample identification number corresponding to that in the sample submission data sheet (Annexure-II)
• Pack properly in a sterile sample container and store one set at room temperature and another set in ice box before transporting to the laboratory.
• Prepare blood smear from the unclotted blood which oozes out from the natural orifice, heat fixed it and place it in a slide box before transporting to the laboratory for microscopic examination.

Procedures for collection of environmental samples for examination of B. *anthracis*

• Exposed surfaces (e.g. concrete floor, dirt floor) should be swabbed with moistened swabs and sent to the laboratory for examination.
• Water sample should be collected by means of a syringe without needle or in a sterile container
• Feed samples should be collected with sterile spoons or other suitable sterile collecting devices into small sterile containers.
• Soil samples should be collected with sterile spoons or other suitable sterilized tools into sterile, sealable containers (e.g. screw-capped container)
• All the above environmental samples should be properly packed (double-bagged) and submit to the laboratory for examination.

Standard Operation procedure for disposal of anthrax carcasses by burial

Procedure: Select an appropriate site for carcass burial

• Due consideration should be given not to contaminate water sources, residential areas, livestock facilities, pastures and other establishments in the vicinity. Preferably it should be away from any footpaths or roads leading to the site.

• Prepare a pit with sufficient width to accommodate the carcass with a minimum depth of 2 meters considering the size of the carcasses.
• Wear apron, face masks, goggle and gloves before handling the carcass.
• Drop the carcass into the pit.
• Cover the carcass with soil, 400 mm is suggested and add an unbroken layer of lime (calcium carbonate) or calcium hypochlorite (bleaching powder)
• Do not put lime directly on to the carcass (it will slow decomposition process).
•Close the pit with sufficient soil and make a heap over the site.
• Then put a layer of lime over the soil
• Secure the disposal site by fencing (if possible) and place a notification mark.

Standard Operating Procedure for disinfection and decontamination of contaminated premises and materials.

Procedure:

• Prepare 1% hypochlorite solution in a bucket.
• Spray and wash barn utensils, tools and equipments with the above solution thoroughly. Dry them for reusing.
• Bury the beddings with carcasses if it is in small quantities. Burn it in a pit if in larger quantities.
• Contaminated premises should be disinfected thoroughly with the 1% hypochlorite spray @ 1-1.5 lts/sq. mts. Allow contact time of 2-3 hrs.
• Contaminated laboratory materials can be disinfected by immersing them in 1% hypochlorite solution for at least 30 minutes.
• Disposable items, including used PPEs must be incinerated/burnt in a pit

Control measures:

- Prompt disposal of dead animals, feces, bedding, or other contaminated material by cremation (preferable) or deep burial.
- Disinfection and decontamination of contaminated materials, sheds, equipments and environmental decontamination.
- Rigid enforcement of quarantine (after vaccination, 2 weeks before movement off the farm, 6 weeks if going to slaughter)
- Isolation of sick animals.
- Use of insect repellents
- Control of scavengers that feed on animals dead from the disease
- General sanitary procedures by people who handle diseased animals, both for their own safety and to prevent spread of disease
- Ring Vaccination

Suggestive line of treatment:

1. **Antimicrobial therapy**

The commonly recommended doses of penicillin G sodium/potassium are 20,000 international units/kilogram (IU/kg), 12 hourly, intravenous (IV), and procaine penicillin is 22,000 IU/kg intramuscular (I/M), 12 hourly for the first 2 days. After 2 days, penicillin G sodium/potassium is 22,000 IU/kg, 24 hourly, IV, and procaine penicillin is 44,000 IU/kg, I/M, 24 hourly, following 3 days (Source: Constable P, Hinchcliff K w, Done S, Gruenberg W. *Veterinary medicine*. 11th. Elsevier; 2016. Anthrax; pp. 1–2235). The drug oxytetracycline given 10 mg/kg daily at IM or IV is effective. In the initial period of therapy, the daily dose should be divided and given for 12 hours. The penicillin drug combined with streptomycin is also curative (Source: WHO. Geneva, Switzerland: WHO Press; 2008. Anthrax in humans and animals; pp. 1–198).

2. **Supportive therapy**

The animal should be administered with analgesic, anti-inflammatory and antipyretic agent as necessary. The fluid therapy may be given where necessary. The remaining animals in the outbreak areas should be regularly checked 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 with long acting antibiotics (as mentioned above). *As preventive measures, the remaining unaffected herds in the outbreak areas should also be given long-acting antibiotic injection followed by vaccination after 7-10 days.*

3. **Vaccination of livestock**

In an outbreak situation ring vaccination using Anthrax Spore vaccine (live) may need to be applied to a distance of 1 km beyond an infected property or the vaccination program can be decided based on risk assessment and geographical terrain of the outbreak area. It has been observed that the pastures associated with anthrax continued to give rise to cases for up to three years after the index cases. *Therefore, if there has been an outbreak on a farm, the stock should be revaccinated annually for at least three years to prevent further cases*. Vaccination is not recommended if there has been no outbreak in a particular area for consecutive three years after the last outbreak or in areas where there has been no history of any anthrax outbreaks. Antibiotics should not be administered for at least one week after the vaccination (Source: MSD Manual). While vaccinating with the spore vaccine, once the vial is opened, the total content of the vial should be used on the same day and any quantity of remaining vaccine should be discarded by boiling in water or by autoclaving.

Surveillance plan for Anthrax in animals following an outbreak and post outbreak phase

Following an outbreak of anthrax in an area, a protection zone should be designated within 500 meters radius around the infected foci or the zone should be demarcated based on the epidemiological risk assessment and geographical terrain. All susceptible animals residing within this zone should be treated with antibiotics and intensive surveillance should be carried out to prevent spill over of infection into the nearby areas. Similarly, a surveillance zone should be declared up to 1 km beyond the infected foci based on epidemiological risk assessment and geographical settings. The main activities to be undertaken in the surveillance zone include vaccination, surveillance (clinical and laboratory), and awareness activities.

Intensive surveillance should be carried out until 3 weeks of the last clinical case (incubation period is 20 days) and satisfactory completion of sanitary measures. In addition routine surveillance should be carried out for at least 2 weeks after the declaration of end of outbreak in these zones.

Diagnosis: (As per World Organisation for Animal Health)

Clinical signs

Sudden death in apparently healthy animals which may be accompanied by bloody discharges from natural orifices, rapid bloating of the carcass, incomplete *rigor mortis* and the absence of clotting of the blood are the common characteristics of anthrax in herbivores.

Laboratory diagnosis

Of the many laboratory diagnostic methods available, Gram's staining, MacFadyean's reaction and rapid antigen detection test are used to diagnose anthrax.

Examination of blood smear

Anthrax is diagnosed by examining blood (or other tissues) for the presence of the bacteria. Samples must be collected carefully to avoid contamination of the environment and to prevent human exposure to the bacteria. Blood samples from relatively fresh carcasses will contain large numbers of *B. anthracis*, which when performed a simple polychrome methylene blue straining can be seen under a microscope as rod shaped square ended, short chains Gram positive bacteria.

Isolation and Cultural examination:

Isolation and cultural examination of *B. anthracis* should be attempted only in designated high bio-security laboratories.

Molecular diagnosis

Molecular diagnosis using PCR can be applied as confirmatory test.

Differential diagnosis

Other causes of sudden death of animals to be differentiated include: botulism, black quarter, peracute babesiosis, chemical poisoning (heavy metal and other poisoning), plant poisoning, snake bite, lightening strike, metabolic disorders (lactic acidosis), magnesium deficiency and bloat.

Inform the Controlling officers immediately if any suspected death of animals due to Anthrax so that information can be relayed to the Office of the Assistant Director (Disease Investigation).

Annexure-I

Guidelines on appropriate sample collection from animals suspected of having died from anthrax

Circumstances	Specimen	Container
Anthrax suspected sick animal	Collect blood from vein (0.1 ml), nasal swabs	Sterile vial, or leave in syringe.
Fresh carcass	Collect blood from vein (0.1 ml) or, if opened unknowingly or by scavengers, collect blood and/or fluid from body cavity or piece of highly vascularized tissue (usually ear clipping). *(Source: Animal Health Diagnostic Center, Cornell University, College of Veterinary Medicine. Anthrax Sample Collection and Shipping Guidelines. [February 22; 2021].)*	Sterile vial, or leave in syringe, swabs in sterile tubes
Putrefied carcass	Collect piece of highly vascularized tissue and swabs from vascularized regions (nostrils, eye socket, any bloody material). *(Source: Animal Health Diagnostic Center, Cornell University, College of Veterinary Medicine. Anthrax Sample Collection and Shipping Guidelines. [February 22; 2021].)* Blood stained soil from under head or tail.	Swab in sterile tubes. For soils, collect in the screw capped container
Very old carcass. ,	Collect hides, bones, soil around/ under carcass, swabs of nostrils, eye sockets	Swab in sterile tubes. For soils, collect in the screw capped container

Annexure-II

Data Sheet for Sample collection

Name of Sender:

Address:

Sl. No.	Date of collection	Owners name and Address	Species	Breed	Age/ Sex	Animal ID	Brief History

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