Guidelines for Preparedness, Surveillance and Control of Anthrax in Human and Animals in Bhutan

ROYAL GOVERNMENT OF BHUTAN
MINISTRY OF HEALTH
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Foreword

Anthrax is a notifiable zoonotic disease in Bhutan. Sporadic outbreaks in animals are reported from different parts of the country. However, some cases may have gone unreported both in animals and in humans due to passive reporting system and absence of clear control guidelines. Therefore, a comprehensive guideline for preparedness, surveillance and control of anthrax in humans and animals is felt necessary for the successful prevention and control of anthrax in Bhutan through 'One Health' approach.

This first edition of the anthrax guidelines 2013 is developed by anthrax working group consisting of experts from public health, Ministry of Health (MoH) and animal health of the Ministry of Agriculture and Forest (MoAF). This document is prepared with the objective to provide relevant source of information and guidelines for the management of anthrax in humans and animals and will be updated based on the epidemiology of the disease and emerging needs.

This guideline provides ready reference on all aspects of anthrax - diagnosis, treatment, surveillance, outbreak investigation, surveillance and control program.

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Abbreviation

ANM:	*Assistant Nursery Midwife*
BAFRA:	*Bhutan Agriculture and Food Regulatory Authority*
BHU:	*Basic Health Unit*
CBC:	*complete blood count*
CMO:	*Chief Medical Officer*
CNS:	*Central Nervous System*
CSF:	*Cerebrospinal fluid*
DLO:	*Dzongkhag Livestock Officer*
DH:	*Dzongkhag Hospital*
DT:	*Dzongkhag Tshogdue*
DVO:	*Dzongkhag Veterinary Officer*
DVH:	*Dzongkhag Veterinary Hospital*
DVL:	*Dzongkhag Veterinary Laboratory*
DH:	*Dzongkhag Hospital*
DHO:	*Dzongkhag Health Officer*
EA:	*Extension Agent*
FBC:	*Full Blood Count*
GT:	*Geog Tshogdue*
GPS:	*Global Positioning system*
HA:	*Health Assistant*
JDWNRH:	*Jigme Dorji Wangchuk National Referral Hospital*
LEC:	*Livestock Extension Centre*
LFT:	*Liver function test*
MO:	*Medical Officer*
MoH:	*Ministry of Health*
MoAF:	*Ministry of Agriculture and Forests*
NCAH:	*National Centre for Animal Health*
OIE:	*Office International des epizooties (World Animal Health Organization)*

PHL:	*Public Health Laboratory*
RBP:	*Royal Bhutan Police*
RRT:	*Rapid Response Team*
RLDC:	*Regional Livestock Development Centre*
RN-REC:	*Renewable Natural Resources Extension Centre*
RRH:	*Regional Referral Hospitals*
SVL:	*Satellite Veterinary Laboratory*
SOP:	*Standard Operating procedure*
VBDCP:	*Vector Borne Disease Control Program*
VVT:	*Veterinary Vigilance Team*
VO:	*Veterinary Officer*

Contents

1. Introduction

Anthrax is a bacterial zoonotic disease caused by the spore forming *Bacillus anthracis*. It is primarily a disease of herbivorous animals with clinical features of hyperacute or acute symptoms and usually results in death. It can also cause disease in other warm blooded animals including humans. Humans contact the disease directly or indirectly from animals or animal products. Prior to the development of the vaccine in the 1930s, anthrax was regarded as a disease of major health or economic importance and was the foremost cause of uncontrolled mortality in cattle, sheep, goats, horses and pigs worldwide. The disease is still enzootic worldwide and *B.anthracis* has always been high on the list of potential agents with respect to biological warfare and bioterrorism.

In Bhutan, anthrax is sporadically reported in domestic animals, primarily in cattle and also causes disease in humans. For instance, between 1998 and 2012, 34 anthrax outbreaks in animals have been reported from Samtse, Dagana, Chukha, Zhemgang, Wangdue, Mongar, Trashigang, Tsirang, Trongsa, Sarpang and Haa with 11 outbreaks of anthrax reported in 2012 alone (Fig. 1).

Figure 1: Reported anthrax outbreak in animals in Bhutan at the Dzongkhag level (1998 – 2012). The numerical numbers indicate the number of outbreaks whereas the number within the bracket ( ) indicate the number of cases in domestic animals.

A major anthrax outbreak occurred at Kagtong village under Ngangla geog, Zhemgang Dzongkhag in 2010 and caused deaths of several domestic animals. In addition, nine people had contacted cutaneous form of anthrax through contacts with infected animals or consumption of anthrax infected meat. One person died due to suspected inhalation form of anthrax in the outbreak area. Human deaths have also been reported in Trongsa in 1989 and Wangdue in 1998 following contact and consumption of meat derived from anthrax infected carcasses.

Although anthrax is a notifiable disease in Bhutan, many cases may have gone unreported both in animals and in humans due to passive reporting system and absence of clear control guidelines. Therefore, a comprehensive guideline for preparedness, surveillance and control of anthrax in humans and animals is necessary for the successful prevention and control of anthrax in Bhutan through 'One Health approach'.

This first edition of the anthrax guidelines 2013 was developed by anthrax working group consisting of experts from public health, Ministry of Health (MoH) and animal health of the Ministry of Agriculture and Forest (MoAF). This document is prepared with the objective to provide relevant source of information and guidelines for the management of anthrax in humans and animals and will be updated based on the epidemiology of the disease and emerging needs.

2. Etiology

Anthrax is a bacterial disease caused by the spore forming, gram positive, rod shaped bacterium, *Bacillus anthracis. Bacillus anthracis* occurs in vegetative and spore form. The vegetative form occurs within host and in low oxygen environment of the infected host. Once outside the host, sporulation commences upon exposure to the air. Spores are extremely resistant to heat; cold, pH, desiccation, chemicals, irradiation and other adverse conditions, and can survive for years in soil, wool, and hair of infected animals. Upon entering the host animal through inhalation, ingestion and cuts in the skin, the spores germinate, multiply and cause disease.

Anthrax is a seasonal disease and its occurrence is affected by temperature, rain or drought. Climate probably acts directly by influencing the way in which the animal comes into contact with the spores (for example, grazing closer to the soil in dry periods when grass is short), or indirectly by affecting the general status of health of the host and thereby affecting the level of resistance to infection.

3. Anthrax in animals

3.1. Host range and susceptibility

Anthrax is primarily a disease of herbivores (cattle, sheep, and goats. It can also affect other species such as pigs, horses, dogs, cats and other vertebrates.

3.2. Transmission

The spores shed by an animal dying or dead from anthrax act as the source of infection for other animals (Fig. 2). The routes of anthrax transmission in animals are as follows:

- Ingestion of the spores while grazing or browsing close to the soil is a frequent mode of infection.
- Carnivores and pigs may acquire infection through ingestion of meat of an animal that died of anthrax or food waste which inadvertently included meat and bones from anthrax carcasses.
- The organism can enter the body through cut wounds or lesions in the skin.
- Inhalation of spores during grazing occasionally transmits anthrax.
- Flies (for example, by insect bites) appear to play an important role in explosive outbreaks.
- Human activities in the form of trade in animal products (meat, hides, hair, wool or bones and contaminated feedstuffs) are responsible for the spread of the disease.
- Deliberate release of anthrax spore (bioaggression/bioterrorism)

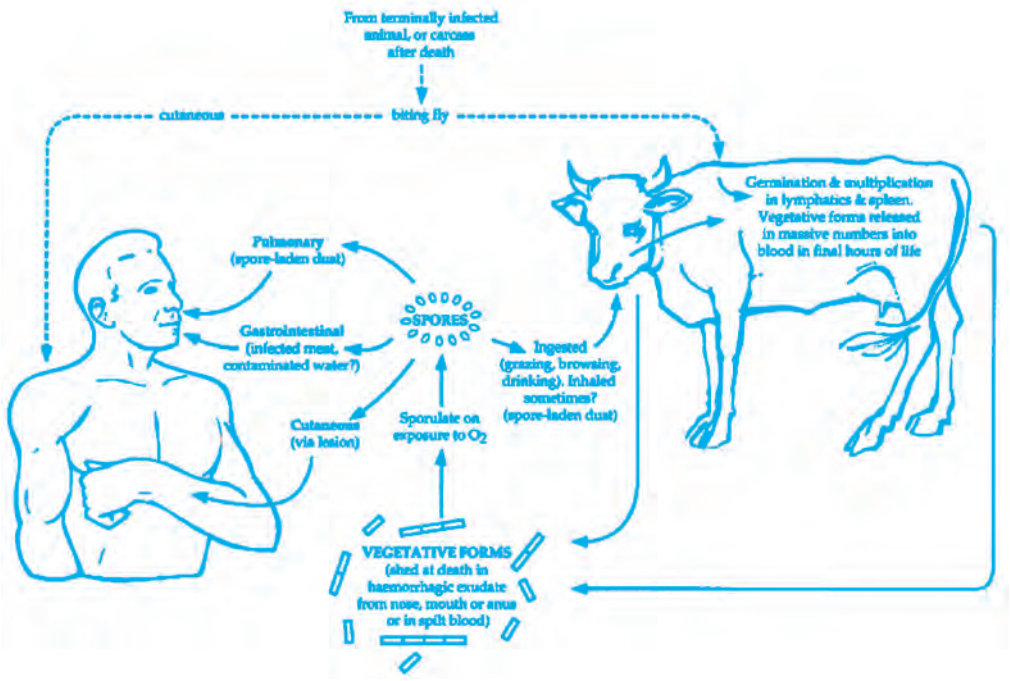


Figure 2: Transmission cycle of anthrax infection in animals and humans (Source: WHO, 2008)

3.3. Pathogenesis

After entering the body, the spores get carried to the lymphatics where they multiply and are released into the blood stream. The toxin produced in the blood stream destroy the endothelial cell lining of the blood vessels resulting in internal bleeding and oozing of unclotted blood from the natural orifices (ears, nostril, mouth, anus, vagina).

3.4. Clinical manifestations and incubation period

The incubation period in naturally infected cattle is about 1–14 days or more. For trade purposes, the OIE incubation period for anthrax is 20 days. The general clinical manifestations of anthrax in animals vary from species to species, presumably reflecting differences in susceptibility. The first signs in the more susceptible livestock species are sudden deaths with bloody discharges from natural orifices, absence of clotting of the blood, bloating and incomplete *rigor mortis*. The other signs in less susceptible animals may include oedematous swellings along the neck, pharyngeal region, flanks or lumbar region, respiratory distress, loss of appetite, rise in temperatures, and terminal coma and death if not treated in time (Fig. 3). (Refer case definition under Surveillance section 5.1.1). Pigs are more resistant to anthrax than cattle, sheep, goats and horses.


Figure 3: Sudden death of animal, bloating of carcass, discharge of unclotted tarry blood from nostril in cattle

The specific clinical signs in different species of animals include:

Species	Clinical signs
Bovine	• Acute febrile disease without obvious localization • Rise in temperature • Dullness, off feed and difficulty in breathing • Sudden death and blood may exude from the rectum and other natural orifices. • In subacute condition, there will be oedematous swellings along the neck, flanks or lumbar region • Death usually occurs in 2–3 days if no treatment is given
Sheep and goats	• Usually occur as a per acute infection (sudden death)
Horses	• Intestinal lesions may result in colic and diarrhoea. • Large oedemas on the breast, abdomen, neck and shoulders. • May manifest as acute symptoms and die in 2–3 days.
Pigs	• Anthrax in pig can occur as pharyngeal and intestinal form. Pharyngeal form occurs due to consumption of meat and bones from infected carcasses, containing both vegetative cells and spores and is characterized by an ulcerative stomatitis, laryngitis, and markedly oedematous swelling of the throat and cervical lymph glands that could interfere mechanically with respiration, feeding and drinking • The intestinal forms occurs after consumption of spore-contaminated mineral supplements which does not contain vegetative forms and is associated with anorexia, vomiting, diarrhoea (sometimes bloody) or constipation, blood in the faeces, ataxia and rise in temperature.
Dogs and cats	• Dogs are considered very resistant to anthrax, but dogs that have scavenged anthrax infected carcasses will show the symptoms such as severe inflammation and oedematous swelling of the throat, stomach, and intestine and of the lip, jowl, tongue and gum.

3.5. Diagnosis

3.5.1. 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 (see section 3.3).

3.5.2. Laboratory diagnosis

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

Examination of blood smear

A blood smear should be prepared by cutting a small tip of ear as it is easily accessible and supplied with extensive capillary network, or by means of a syringe from an accessible vein. The smear should be stained with polychrome methylene blue and observed under microscope for characteristic rod-shaped bacilli with pink capsule (M' Fadyen reaction) (Fig. 4) (Refer Annex 11.1: SOP for collection of specimen from anthrax suspected animal or carcases and annex 11.2: SOP for laboratory diagnosis of anthrax in animals).

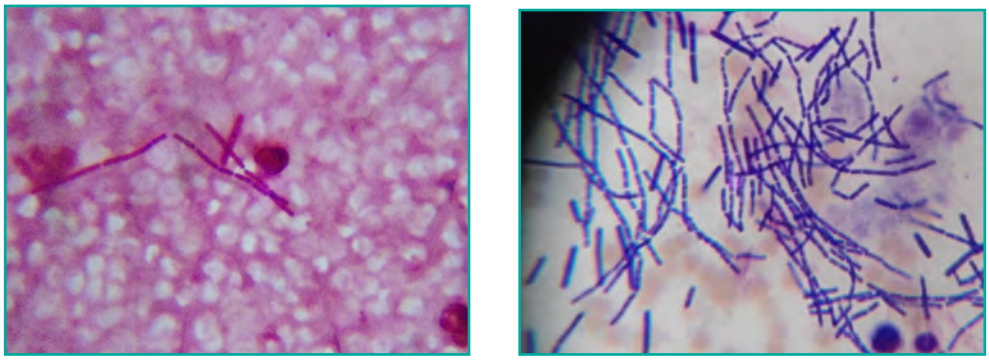


Figure 4: Rod shaped bacilli

However, a Gram's stain may not reveal capsule and could result in false negative diagnosis if the carcass is not fresh (after 24 hours of death) or putrified. In addition, it may not be possible to find bacilli in smears or to isolate *B. anthracis* from animals that were treated with antibiotics before death.

Blood culture

Blood for culture should be obtained by means of a syringe from an accessible vein within 24 hours of death or alternatively, a dry swab inserted into a small incision in a region well supplied with blood vessels. It should then be cultured in the laboratory (recommended to be undertaken in BSL II plus) and examined for presence of bacilli by staining (Refer annexes 10.1 and 10.2).

Serology

Anthrax can also be diagnosed by serological methods.

Antigen detection test

Conventionally Ascoli precipitin test (thermostable antigen test) has been used to diagnose anthrax. Now a rapid antigen detection test using *immunochromatography* will be used in Bhutan. It is simple, rapid and highly sensitive and specific and can be easily conducted in the field without the need of laboratory facilities.

Molecular diagnosis

Molecular diagnosis using PCR can be applied as confirmatory test.

3.5.3. 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).

3.6. Treatment and prophylaxis

Antimicrobial therapy

The anthrax suspected animal should be treated immediately with long acting benzathene penicillin, or procaine pencillin, @ dose range of 11000 – 22000 unit per kg body weight by deep intramuscular injection or strepto-penicillin at dose of 8 - 10mg per kg per body weight in large animals (2ml/50kg body weight after reconstitution with distilled water).

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.

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. 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.

4. Anthrax in humans

4.1. Transmission

Humans can get anthrax infection by handling animal products from infected animals or by inhaling anthrax spores from contaminated animal products. Anthrax can also be spread by eating undercooked meat from infected animals. Direct human to human transmission is unknown.

4.2. Different forms of anthrax infection in humans

Anthrax infection in humans can occur mainly in three forms: cutaneous (skin), inhalation (pulmonary) and gastrointestinal (oral route) based on exposed areas of the body (head, neck, forearm, hands, legs) and route of entry.

4.2.1. Cutaneous anthrax

Most (about 95%) anthrax infections occur when the bacterium (usually as spores) enters through a skin lesions (a cuts, abrasion and insect bites), during handling of contaminated wool, hides, leather or hair products of infected animals. The incubation period ranges from 9 hours to 3 weeks, mostly 2 – 7 days.

Skin infection begins as an initial itching at the affected sites (within 1–2 days) that develops into a papules, vesicle and then to a depressed black eschar (a characteristic black necrotic area in the center) that usually develops within 2–6 days of infection (Fig. 5). The ulcer will be painless and without pus unless there is secondary infection. The exposed areas of the body are the common sites of infection (head, neck, forearm, hands, and legs) (Refer Annex 10.3: Flow chart for clinical diagnosis and management of anthrax in humans).

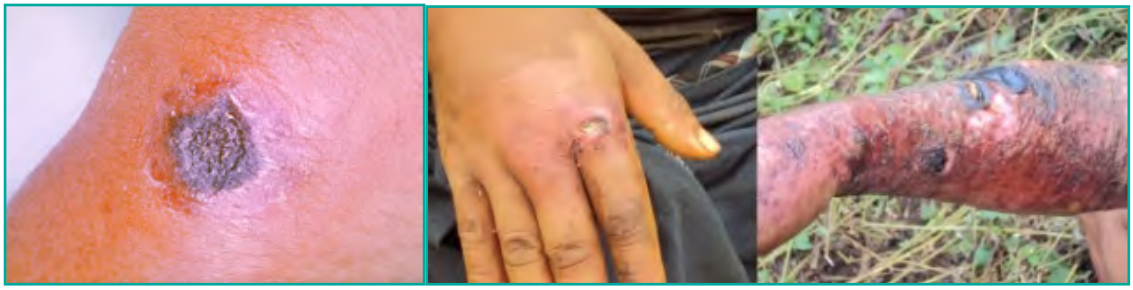


Figure 5: Cutaneous form of anthrax in humans with eschar lesion and extensive skin reaction.

Complications can include obstructive airway with oedema of face and neck and untreated infection spreads to regional lymph nodes and blood stream resulting in septicemia with involvement of meninges. Deaths are rare with appropriate antimicrobial therapy but the case fatality rate ranges between 5 – 20% in untreated cases.

4.2.2. Inhalation (pulmonary) anthrax

Human acquire infection through inhalation of anthrax spores. Person working in wool industries are at higher risk of getting inhalation anthrax. The average incubation period is about 4 days ranging from 1-7 days although incubation period up to 60 days are possible.

Initial symptoms are mild and non specific and may include mild fever, muscle aches and malaise, mild cough, sore throat, and chest pain. The symptoms may progress to severe breathing problems, shock, disorientation with coma, and death. Inhalation anthrax is usually more fatal than other forms of anthrax (Refer Annex 10.4: Flow chart for diagnosis and management of inhalation form of anthrax in humans).

4.2.3. Gastrointestinal anthrax

Gastrointestinal anthrax can occur in two forms: oropharyngeal and intestinal anthrax, occurring after consumption of meat or drinks contaminated with *B. anthracis*

The intestinal form of anthrax is characterized by an acute inflammation of the intestinal tract and the symptoms may include nausea, loss of appetite, vomiting, fever, abdominal pain, vomiting of blood, and severe (bloody) diarrhea and massive ascitis. If not treated early, intestinal anthrax results in death in 25–60% of cases due to toxemia and shock (Refer Annex 10.5: Flow chart for diagnosis and management of gastrointestinal form of anthrax in humans).

The main clinical features of oropharyngeal form of anthrax include sore throat, dysphagia, fever, regional lymphadenophathy in the neck and death may result due to toxaemia.

4.3. Diagnosis of human anthrax

The clinician should be aware and alert the laboratory about the anthrax suspected cases.

Forms of anthrax	Diagnosis	Differential diagnosis
Cutaneous anthrax	-Clinical history and symptoms as per section 4.2.1 -Microscopic examination	Boil (early lesion), spider bites, ulcer (especially tropical); erysipelas, glanders, plague, syphilitic chancre, ulceroglandular tularaemia; clostridial infection; rickettsial diseases (scrub typhus); vaccinia and cowpox, rat-bite fever, or leishmaniasis.
Inhalation anthrax	-Clinical history & symptoms as per section 4.2.2 -Chest X ray -Culture from sputum & blood - Complete blood count and LFT.	Mycoplasmal pneumonia, legionnaires' disease, psittacosis, tularaemia, Q fever, viral pneumonia, histoplasmosis, coccidiomycosis, malignancy
Gastrointestinal anthrax	-Clinical history & symptoms as per section 4.2.3 -Culture from stool & blood -Complete blood count and LFT.	Oropharyngeal anthrax has to be differentiated from diphtheria and complicated tonsillitis, streptococcal pharyngitis, angina, parapharyngeal abscess, and deep-tissue infection of the neck whereas gastrointestinal anthrax has to be differentiated from food poisoning (in the early stages of intestinal anthrax), acute abdomen owing to other reasons, and haemorrhagic gastroenteritis caused by other microorganisms, particularly necrotizing enteritis caused by Clostridium perfringens and dysentery (amoebic or bacterial).

Note: Refer Annex 11.6: SOP for sampling for anthrax in humans.

4.4. Treatment of humans

Mild uncomplicated cases

Adult: procaine penicillin, 500-600 mg (800000-1 million units), every 12 -24 hours or Penicillin V 500mg every 6 hours or amoxicillin 500mg orally every 8 hours for 3-7 days. Other oral antibiotics can also be used (Refer Table 1 for details and options)
Children: penicillin V 25-50mg/kg/day OR procaine penicillin 2500-50000 units/kg/day for 3-7 days.

Severe or potentially life threatening cases

In severely affected patients or when pulmonary or gastrointestinal anthrax is suspected, the patient should be treated as follows:

Adult: penicillin G, 2400 mg (4 million units) every 4-6 hours by IV infusions until the patients temperature returns to normal; at this point treatment should continue in the form of intramuscular procaine penicillin injection as per dose mentioned in above section. In very severe cases penicillin G can be combined with other antibiotics such as doxycycline or ciprofloxacine as per dosage mentioned in Table 1.

In children: Penicillin G 300000-400000 units /kg/day and in very severe cases it can be combined with other suitable antibiotics.

Quinolones and aminoglycosides can be used as second line or if above drugs are contraindicated or as per culture and sensitivity report as per Table 1.

Table 1: Antimicrobial dose to be used in Anthrax cases in humans

Antibiotic	Dosage for adults	Dosage for Children	Remark
Penicillin V	500mg orally 4 times/ day	25-50mg/kg/day orally in 4 divided doses	Do not use if there is history of penicillin allergy.
Procaine Penicillin	0.6-1.2mU every 12-24hr	25000-50000 U/kg/ day IM	
Benzyl Penicillin G	4mU intravenously	300000-400000U/ kg/day in 4-6 divided doses	
Amoxycillin	500mg orally every 8 hrs	40mg/kg orally in 3 divided doses	
Ciprofloxacin	500mg orally every 12 h or 400mg iv every 12 hours	10-15mg/kg twice daily not exceeding 1g/day	Generally not recommended for children or in pregnancy unless benefits outweighs risk
Doxycycline	100mg every 12 hrs	2.2mg/kg twice daily	Generally not recommended for children or in pregnancy unless benefits outweighs risk. Do not have good CNS penetration.
Erythromycin	500mg orally every 6 hrs	30-50mg/kg/day in 4 divided doses	
Chloramphenicol	500 mg orally 6 hours	50mg/kg/day in 4 divided doses	
Cotrimoxazole	160/800 mg every 12 hours	4/20mg per kg every 12 hours	

Supportive care

In addition to antibiotic therapy, analgesics, antipyretics, anti inflammatory may be given as appropriate. In case of cutaneous anthrax, wound care should be given. Severe cases may require assisted respiration, fluid and electrolyte supplement, cerebral oedema management and referral to next higher health centre.

Post exposure prophylactic antibiotics are recommended in specific cases involving direct contact with animal carcasses and contaminated materials. In event of suspicious signs or symptoms, exposed people should be instructed to seek medical care.

5. Anthrax Surveillance System

Surveillance is the collection, collation and analysis of health data that enables the prompt dissemination of the information to take appropriate action. Routine information sharing and notification between the veterinary and human health sectors should be established for prompt decision making.

Anthrax is a notifiable disease under the Livestock Act of Bhutan 2001 and in Public Health notifiable disease guidelines. Therefore, it is mandatory to report any suspected cases to the relevant authorities.

Surveillance during the preventive phase

The objective of the surveillance during the preventive phase is for early case detection and prevention of outbreaks in animals and humans. The process comprises of routine clinical and laboratory surveillance. However, in the high risk areas/groups/population, targeted and ecological surveillance should be undertaken.

Surveillance during the outbreak and post-outbreak phase

The main objectives of the surveillance during the outbreak phase are to assess the success of the control measures being implemented and also to prevent further spill over of the infection into the susceptible population.

5.1. Surveillance of anthrax in animals

The main objectives of a veterinary surveillance program is to evaluate the health status of animals at risk, evaluate the prevention and control activities, monitor the epidemiological pattern of the disease and ensure proper feedback mechanisms (Refer annex 10.7: SOP for anthrax surveillance in animals).

5.1.1. Case definition

The epidemiological unit for anthrax in animals for Bhutan is at the herd-level.

Suspected case

Any case of sudden death of animals accompanied by bleeding (unclotted dark tarry blood) from natural orifices, absence of *rigor mortis,* and rapid bloating of carcasses will be considered as suspected case of anthrax. In pigs, carnivores and primates the main symptoms suspected for anthrax are local edema and swelling of the face and neck. *In endemic areas all sudden death of animals should be regarded as suspected anthrax case unless proven otherwise.*

Confirmed case

A confirmed case of Anthrax requires detection of *Bacillus anthracis* either in smears, rapid test or through bacterial isolation (Refer Annex 10.2: SOP for laboratory diagnosis of anthrax in animals).

5.2. Surveillance of anthrax in human

The main objectives of surveillance in human are to identify the characteristics of the disease in the affected populations, formulate prevention and control program for the human health and veterinary sectors, detect outbreaks and monitor changes in the epidemiological patterns of the disease (Refer Annex 10.8: SOP for anthrax surveillance in humans).

5.2.1. Case definition

The epidemiological unit for anthrax in humans is at the individual level.

Suspected case

Cutaneous anthrax: An acute illness with a painless skin lesion developing over 2 to 6 days from a papular through a vesicular stage into a depressed black eschar with surrounding edema, and has history of exposure to anthrax suspected animals or/and their products (see Fig. 5 and section 4.2.1). Fever, malaise and lymphadenopathy may be seen

Inhalation (pulmonary anthrax): An acute illness, a prodrome resembling a viral respiratory illness, followed by rapid onset of hypoxia, dyspnea or acute respiratory distress with resulting cyanosis and shock. Radiological evidence of mediastinal widening or pleural effusion and has history of exposure to anthrax suspected animals or/and their products

Gastrointestinal anthrax: An acute illness, severe abdominal pain, nausea, vomiting, hematemesis, bloody diarrhea, anorexia, fever, abdominal swelling, septicemia and has history of exposure to anthrax suspected animals or/and their products.

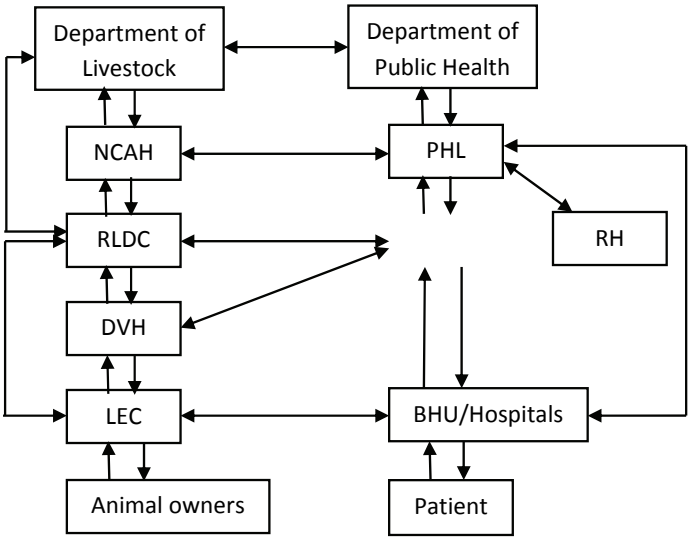
Oropharyngeal anthrax: An acute illness with a painless mucosal lesion in the oral cavity or oropharynx, with cervical adenopathy, edema, pharyngitis, fever, septicemia and has history of exposure to anthrax suspected animals or/and their products.

Meningeal anthrax: An acute illness with fever, convulsions, coma, or meningeal signs with history of exposure to anthrax suspected animals or/and their products. Signs of another form will likely be evident as this syndrome is usually secondary to the above syndromes..

Confirmed case

The detection of *Bacillus anthracis* in smears, culture, detection of four fold rise in antibodies titer by serology and PCR.

6. Reporting and information system



Department of Livestock

At the geog level, the LEC/RNR-EC/DVH) is the first focal point of official contact with the infected herd in the village and have to send a flash report of the suspected cases to DLO/RLDC/SVL/ NCAH.

At the regional level, the RLDCs will be responsible for epidemiological investigation and laboratory confirmation of the disease, analysis of the data, monitoring of prevention and control activities and feedback of information to the Dzongkhag/geog level and reporting to the National Centre for Animal Health (NCAH).

The NCAH will be responsible for the analysis of the data at the national level, to study the epidemiological links, trends and achievement of control targets, formulate national policies and allocate resources and provides advanced laboratory diagnostic support to RLDCs and Dzongkhags.

Department of Public Health

The current reporting system and information flow is from BHU/Hospitals/RH to PHL and to DoPH. The national level disease reporting to the Department and Ministry of Health will also be done as per the existing standard notifiable disease reporting system. The information from the BHU, district and regional level are compiled and analyzed at PHL for further reporting to Department and Ministry including WHO. The reporting of cases from MoH to MoAF at different levels should occur at regular intervals to enhance prevention and control of anthrax.

In general, the reporting of the disease outbreak and the flow of information will be done in line with the existing reporting system of the Department of Livestock and Department of Public Health.

7. Anthrax outbreak investigation and response plan

The animal and human health officials should immediately investigate all suspected cases of anthrax in animals and humans with assistance from other relevant agencies (BAFRA, RBP, local authorities). The plan of action during the outbreak should follow the recommendations outlined in the guidelines for anthrax outbreak (Refer Annex 10.9 and 10.10: SOPs for anthrax outbreak investigation in animals and in humans, respectively).

7.1. Suspected case(s) and infected premises

The field staff must ensure that once the suspected case(s) of anthrax either in animals and humans is reported, preliminary investigation should be done and appropriate action taken as per the standard operating procedure described in annexes 10.9 and 10.10).

7.2. Declaration of provisional outbreak

When anthrax outbreak is suspected in animals, RLDC/DLO/DVH shall inform NCAH and BAFRA officials in the respective areas for the quarantining of the outbreak areas/premises. Accordingly the concerned health authorities will be notified. In case of suspected anthrax case (s) in humans, the concerned health authorities should immediately inform the veterinary authorities for investigation. Necessary pre-emptive control measures should be implemented to contain the outbreaks until confirmed by laboratory.

7.3. Outbreak response

The outbreak response in animals and humans should be done as per the standard operating procedures laid down in annexes 10.9 and 10.10.

7.4. Declaration of Protection Zone

Official declaration of the outbreak should be made by the Dzongkhag administration upon recommendation of the technical units. The Dzongkhag will issue an executive order banning movement of livestock/livestock products with information to all relevant stakeholders. This will then follow proper disposal of carcass, disinfection of the premises and sanitary measures to all the properties which had direct or indirect contact with the infected materials and animal. Strict surveillance and movement control should be done in the protection zone.

7.5. Disposal and decontamination

7.5.1. Disposal of anthrax carcasses

Carcasses should not be opened for postmortem or other purposes as sporulation would take place once exposed to atmospheric oxygen and thereby would acts as a potential source of infection. The carcasses should be properly disposed-off by burial with proper sanitary measures (Refer annex 10.11: SOP for anthrax carcass disposal by burial). Disposal should be completed as soon as possible after mortality/destruction, to prevent the spread of infection**.** If the carcasses has been opened and processed (e.g. dried meat) for human consumption including hides, such items should be collected and burnt (not to be buried to prevent from exhumation and consumption by people).

7.5.2. Disinfection, decontamination and disposal of contaminated materials

The primary objective of disinfection, decontamination and disposal of contaminated materials and wastes is to prevent the spread of infection, which is part of an emergency animal disease control programme.

In the infected premises materials like soil, bedding, unused feed, and manure may get contaminated by exudations from the dying or dead animals and act as potential source of infection. Since the anthrax bacteria spores can survive in soil for many years in adverse conditions, it is important that all contaminated materials are disinfected and decontaminated with appropriate chemicals (Refer annex 10.12: SOP for disinfection and decontaminations of contaminated premises and materials).

7.6. Declaration of the surveillance zone

Surveillance should be undertaken immediately outside the infected herds or premises (Refer annex 10.7).

7.7. Regulatory and movement control

The movement of livestock and their products within the protection zone should be strictly regulated by BAFRA as per the Livestock Rules and Regulation of Bhutan 2008.

7.8. Withdrawal of the declaration of the outbreak

Ban on the movement of livestock and products should be lifted three weeks after the last reported case in animal by the Dzongkhag authorities based on the recommendation by the technical personnel of the Department of Livestock..

7.9. Continuation of the surveillance in post-outbreak phase

The respective livestock and health authorities should continue surveillance of disease for at least one to two weeks post withdrawal of the declaration of the outbreak to find out any residual infection in the surveillance zone.

8. Communication strategies

Communication effort should be made to educate the farmers and general public on various aspects of anthrax using an appropriate methodology with an aim to prevent and control the disease.

Strategy	Phase	Targeted audience	Materials	Contents
Sensitization on anthrax to local government members through GT/DT by animal and human health officials	Preventive	Gewog officials	Power points, posters, leaflets in Dzongkha/ English	Brief background on anthrax, public health importance, signs and symptoms, reporting mechanism, prevention and control program, national policy guidelines.
Awareness to the general public/farmers by animal and human health officials	Prevention and Control	General public, farmers	Posters, Banners, leaflets, media, meeting	About disease, signs and symptoms, Dos and Don'ts in handling anthrax cases, reporting system

The concerned officials (animal and human health) should provide correct and relevant information to the general public through media (if required) to avoid miscommunication and misinformation.

9. References

WHO, 1998. Guidelines for the Surveillance and Control of Anthrax in Human and Animals. 3rd edition. WHO/EMC/ZDI/98.6

WHO, 2008. Anthrax in humans and animals. Fourth edition.

OIE - Terrestrial Animal Health Code, Volume II. Twentieth edition, 2011. World Organisation for Animal Health (OIE) 2011.

Baldock, C; Cameron, A; Black, Principle of disease investigation and surveillance in livestock system, Animal Health in South East Asia, 33-55.

10. Annexes

Annex 10.1: Standard Operating Procedure for sample collection from anthrax suspected animals or carcasses

Purpose:

To have a standardized and uniform sample collection procedure

Scope:

The document describes collection of samples from animals or carcasses for laboratory diagnosis.

User:

Veterinarians, para-veterinarians and laboratory personnel

Manpower:

Veterinary Officer / Laboratory technician, para-veterinarians

Materials/Equipment:

Gloves, Syringes 2ml/5ml, Needles – 18 gauge, Test tubes/screw capped bottles, Sterile swabs and specimen transportation kit, Glass slides with slide box, Scalpel blade, Scissors, Cotton/tissue paper, 70% ethanol, Self sealing plastic bags, Lab marker/sample labels, Sample data sheet, Ice packs, Cool box, Packing tape, PPE.

Procedure for sampling from carcasses

- Appropriate PPE should be used and follow good laboratory practice before sampling
- Follow the below table for appropriate sample collection
- Label sample identification number corresponding to that in the sample submission data sheet
- Pack properly in the plastic bags and store in cool box before transporting to the laboratory
- If the sample is intended for culture, it should be stored and transport to the laboratory at room temperature.Transporting in ice box may result in negative culture since organism is sensitive to cold.

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

Circumstances	Specimen	Container	Test
Anthrax suspected sick animal	Collect blood from vein (0.1 ml), nasal swabs	Sterile vial, or leave in syringe.	Use for smear and culture. Test with antigen detection device (immunochromatography assay) if available.
Fresh carcass	Collect blood from vein (0.1 ml) or, if opened by scavengers or unknowingy, collect blood and/orfluid from body cavity or piece of highly vascularized tissue (usually ear clipping).	Sterile vial, or leave in syringe.	1. Use for smear and culture[a]. The smear should be prepared on the spot 2. Test with antigen detection device (immunochromatography assay) if available.
Putrefied carcass	Collect piece of highly vascularized tissue and swabs from vascularized regions (nostrils, eye socket, any bloody material). Blood stained soil from under head or tail.	Swab tubes. For soils, collect in the screw capped container	For culture on blood agar (preferably with polymyxin) and selective agar. Culture soil on selective agar.
Very old carcass. ,	Collect hides, bones, soil around/ under carcass, swabs of nostrils, eye sockets.	Swab tubes. For soils, collect in the screw capped container	Culture on selective agar.

[a] smear and culture should be done within hours of collecting blood. Vegetative cells disintegrate in blood held for much more than a day. 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. This will encourage sporulation of the B. anthracis on the swab, which is then reliable for culture for long periods.

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
- Food 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.

Annex 10.2: Standard Operating Procedure for laboratory diagnosis of anthrax in animals

Purpose:

To diagnose anthrax by standard methods available in the country.

Scope:

To process sample, perform test/s and provide interpretation.

Users:

Veterinarians and laboratory staff of DVL, SVL, RLDC & NCAH

Manpower

Veterinary Officer/ Laboratory technician

Materials/Equipment:

- Polychrome methylene blue stain, microscope, glass slides and cover slip, biological safety cabinet,incubator, centrifuge, water bath
- Culture media - (blood agar, nutrient agar, PLET agar (and/or other selective agar) ingredients other stains – spore stain.
- Antibiotic disc
- PPE, 95-100% alcohol, disinfectants (hypochlorite solution), biohazard bags

I. Procedure for demonstration of B. anthracis instained smears from dead animals using Gram's stain

1. Make two thin smears of clinical material from swabs or from a small drop of blood
2. Air dry the smear
3. Fix gently by passing over a flame
4. Cover the smear with Gention violet and leave it for one minute
5. Rinse the slide gently with tap or distilled water into container containing hypochlorite solution
6. Cover the slide with Gram's iodine and leave it for one minute
7. Rinse the slide gently with tap or distilled water into container containing hypochlorite solution
8. Cover the smear with acetone for 30 seconds
9. Rinse the slide gently with tap or distilled water into container containing hypochlorite solution
10. Cover the smear with Safranine and leave it for one minute
11. Rinse the slide gently with tap or distilled water into container containing hypochlorite solution
12. Air dry the slide and examine under 100X objective
13. Examine for any Gram positive *anthrax bacilli*

II. Procedure for demonstration of *B. anthracis* using Polychrome methylene blue (M' Fadyean reaction)

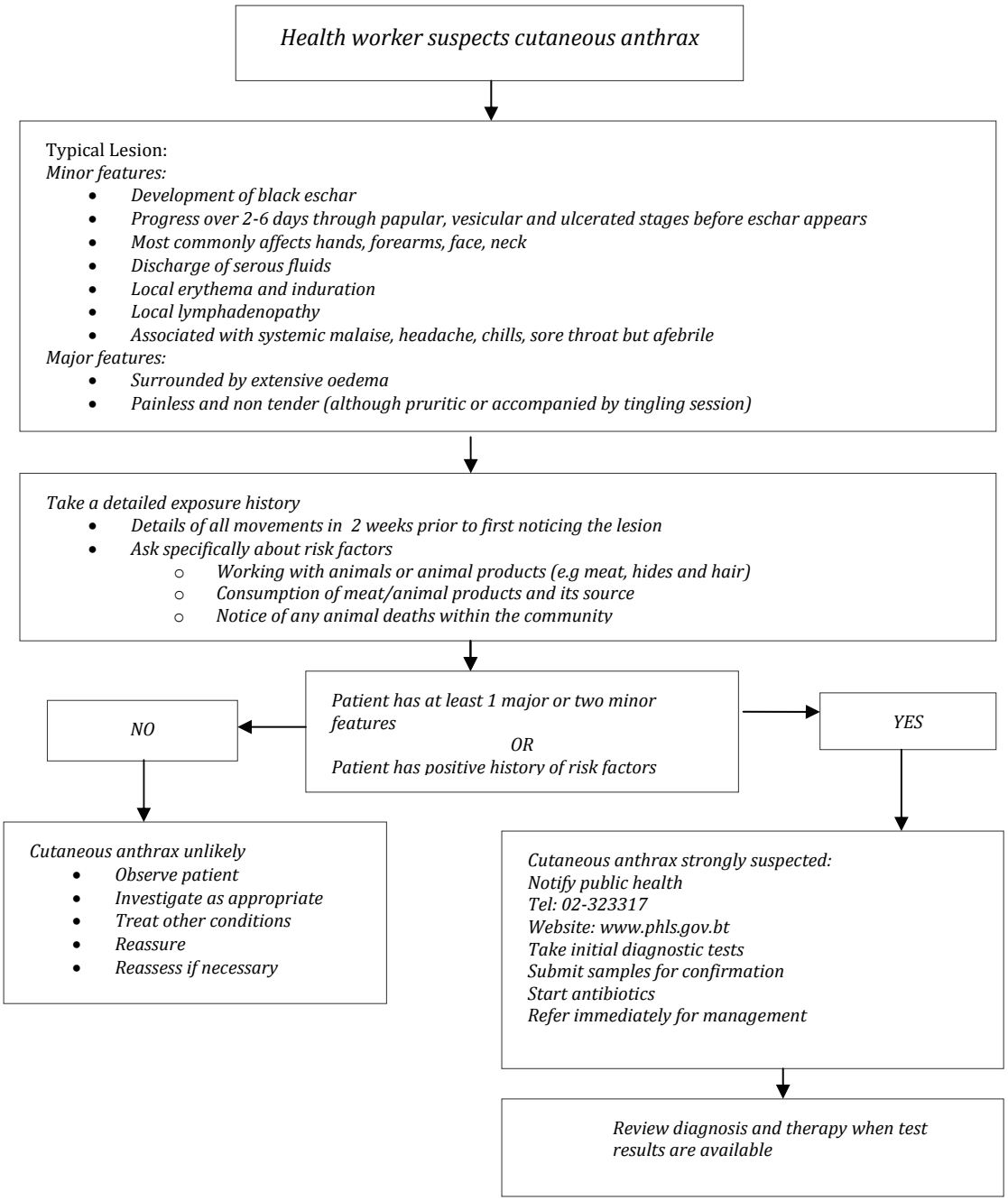
1. Make two thin smears from small drop of blood or swabs or tissue fluids
2. Air dry the smear
3. Fix the smear by dipping in absolute alcohol (95 -100%) or methanol for 30 to 60 seconds
4. Air dry the smear slide
1. Cover the smear with Polychrome methylene blue and leave it for one minute
2. Rinse the smear gently with tap or distilled water into container containing hypochlorite solution
3. Air dry the slide and examine under 100X objective (oil immersion)
4. Examine for blue black, usually square ended bacilli surrounded by pink capsule

A positive (wild-type isolate, Pasteur (pXO1-/2+) control should be included with every test.

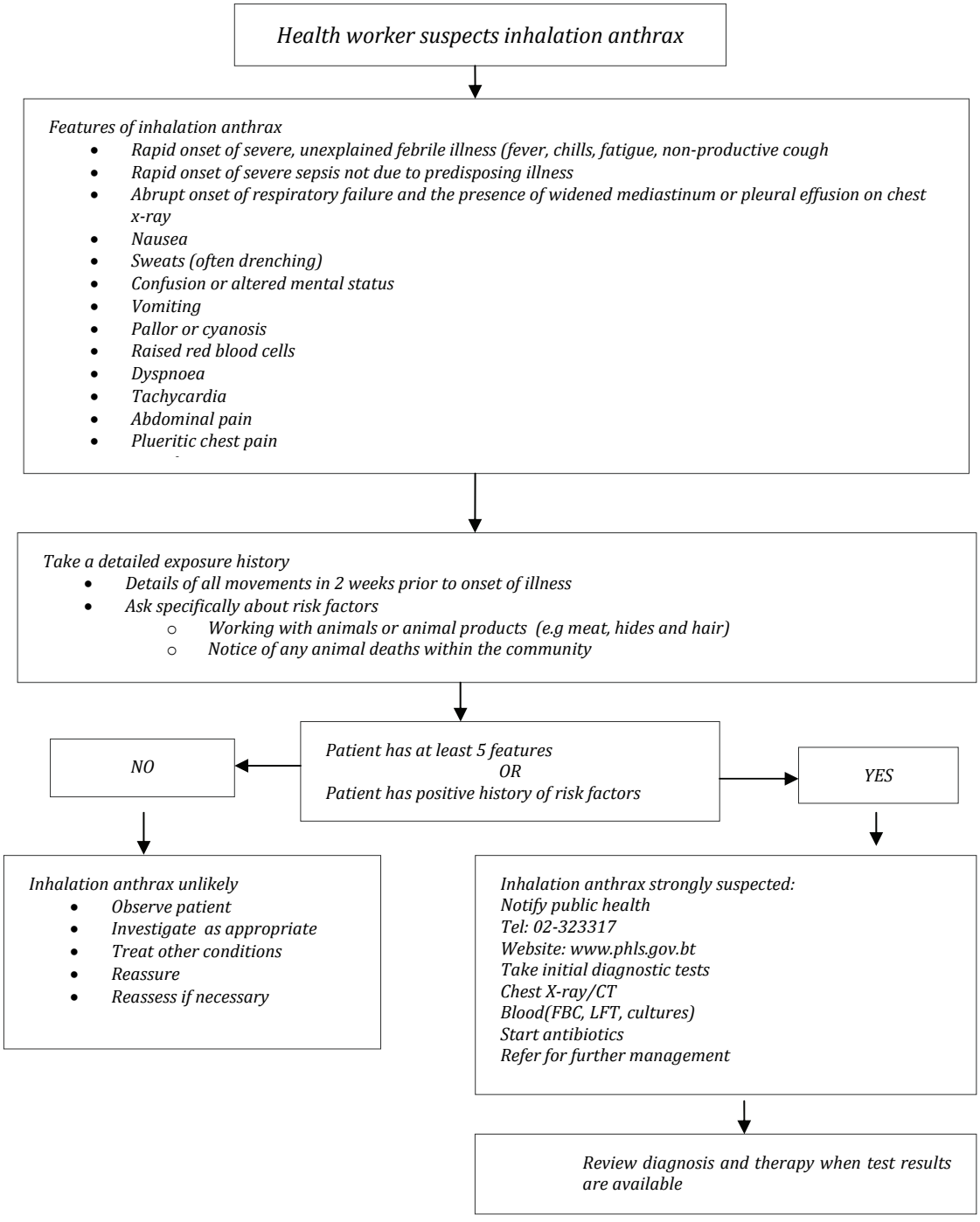
III. Antigen detection test

A rapid test kit (e.g. immunochromatographic techniques) should be used as per the manufacturer instructions given as insert with the kits.

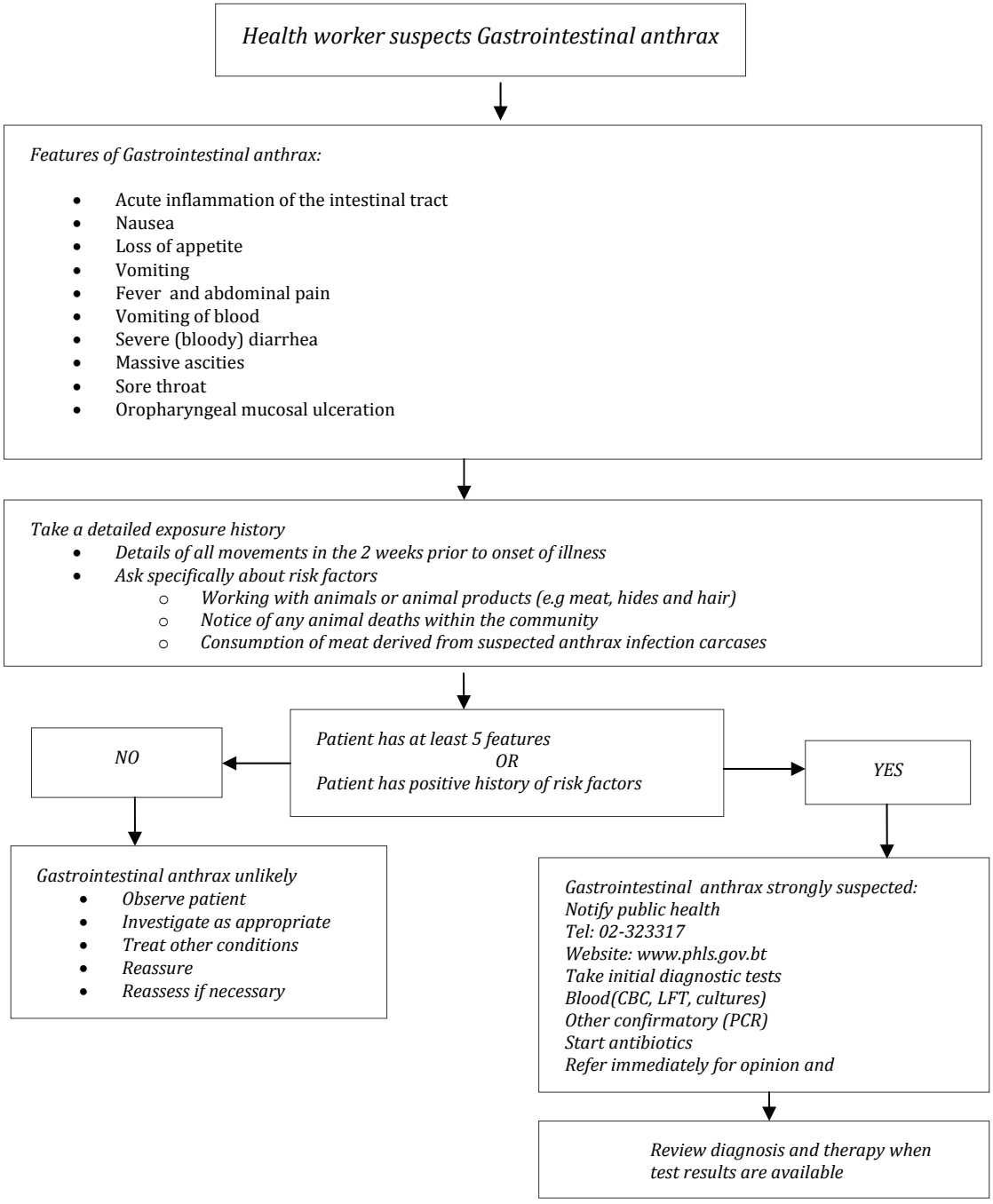
Annex 10.3. Flow chart for clinical diagnosis and management of cutaneous form of anthrax in humans



Annex 10.4. Flow chart for diagnosis and management of inhalation form of anthrax in humans



Annex 10.5. Flow chart for diagnosis and management of gastrointestinal form of anthrax in humans



Annex 10.6: Standard Operating Procedure for sample collection from human anthrax suspected/cases

Purpose: To have a standardized and uniform sample collection procedure in humans

Scope: The document describes collection of samples from humans for laboratory diagnosis.

User: Pathologists/Medical Technologists, laboratory technician

Materials/Equipment:

Disposable gloves, Syringes 2ml/5ml, Needles – 18 gauge, test tubes/screw capped bottles, Sterile swabs and specimen transportation kit, Glass slides with slide box; Scalpel blade, Scissors, Cotton/tissue paper, 70% ethanol, zip locked plastic bags, Lab marker/sample labels, Sample data sheet, Ice packs, Cool box, Packing tape, PPE

Procedure:

- Appropriate PPE should be used and follow good laboratory practice before sampling
- Follow the below table for appropriate sample collection
- Label sample identification number corresponding to that in the sample submission data sheet
- Pack properly in the plastic bags and store in cool box before transporting to the laboratory
- If the sample is intended for culture, it should be stored and transport to the laboratory at room temperature. Transporting in ice box may result in negative culture since organism is sensitive to cold.

Clinical picture	Specimen	Quantity	Container	Tests
Cutaneous anthrax	Vesicular fluid, wound scrapping or wound swab (scrapping and swabs should be taken from underneath the eschar)	3 ml for vesicle fluid	Sterile container, sterile swabs	Smear, culture, rapid antigen detection test if available
Inhalational or Pulmonary anthrax	Blood	10ml	Blood collection tubes (anticoagulant not needed)	
	Sputum	3-5 ml	Sterile screw capped container	
	Nasal swabs	2 swabs	Sterile swabs	Smear, culture
Gastrointestinal anthrax	Blood	5-10 ml	Blood culture bottle	Smear, culture, rapid antigen detection test if available
	Ascitic fluid	2 ml	Sterile screw capped container	
Anthrax meningitis	CSF	0.5 ml	Sterile screw capped container	Smear and culture
	Blood	5 to 10 ml	Blood culture bottle	Smear, culture, rapid antigen detection test if available

Annex 10.7: Standard Operating Procedure for anthrax surveillance in animals

Purpose:
To have standardized surveillance procedure for early detection of anthrax cases in the animal population

Scope: This SOP covers the surveillance guidelines during preventive, outbreak and post outbreak phase

User/Target:
Veterinarians, Laboratory technician and field para-veterinarians

Surveillance Team composition:
Veterinary epidemiologist/Veterinary Officer from NCAH, RLDCs, DVLs,Laboratory technicians and para-veterinarians

Materials and Equipment:
Questionnaires/survey forms, Note pad and pen, mobility, Communication facilities (mobile), Sampling kits (swabs, needle, syringes, permanent marker pen, sample submission forms), eppendorf tubes, fecal vials, transport media, cotton, antiseptics, face mask, gloves, soap, apron, soil sampling kits, diagnostic kits – Gram's stain, Polychrome methylene blue stain, glass slides, GPS,Laptop, extension gears (tent, sleeping gears, rain coat/ umbrella, cap, torch, walking boots.

Types and steps for surveillance:

I. Surveillance during the prevention phase

Surveillance during preventive phase comprises of clinical and laboratory surveillance in the Dzongkhags with intensive programs in high risk areas/Dzongkhags. Based on the epidemiology of anthrax, the high risk Dzongkhags may include Trongsa, Samtse, Dagana, Chukha, Zhemgang, Wangdue, Mongar, Trashigang, Tsirang, Punakha, Sarpang and Haa.

i. Clinical disease surveillance

Clinical surveillance is aimed at detection of clinical cases of anthrax at the herd level.
The following trigger points may provide guidance in suspecting an anthrax infection in animals (also refer section 3.4.1 on clinical signs for anthrax).

1. Sudden death of animals with oozing of un-clotted blood from the natural orifices in herbivores (cattle, sheep, goats, yaks)
2. Rapid bloating of carcasses and absence of *rigor mortis*
3. Presence of facial swelling and subcutaneous edema in pigs.

The field staff should report the findings of clinical surveillances to the DLO/RLDC/NCAH. The village focal persons in each village should also report any suspected cases to the livestock field staff and act as contact point for reporting and dissemination of information to the farmers in the villages. Detailed investigation should be followed based suspected cases of anthrax.

ii. Laboratory surveillance

Although clinical surveillance should be a daily farm routine, purposive sampling and laboratory testing should be be carried out in the high risk areas.
Differential diagnosis should be made against the following diseases: Black quarter, lightning, acute poisoning, Haemorrhagic Septicaemia, snake bite(Refer section 3.4.2 and 3.4.3).

iii. Targeted surveillance

Targeted surveillance should be carried out in the high-risk areas (areas where incidences of Anthrax has been reported) and in farming system where the risk of disease occurrence is high such as in farms where bone meal/commercial feed is fed and also where free-grazing is practiced. Surveillance should also be undertaken in the feed plants where bone meal is being used as an ingredient in the animal feed.

iv. Ecological surveillance

As soil can harbor the bacterial spores for many years, it is essential to have a surveillance system whereby soil mapping should be done in terms of the texture and pH so that the high risk areas can be monitored more intensively for anthrax. It will be also useful if soil samples from outbreak areas could be screened for the presence of anthrax bacilli so that vaccination can be recommended in the high risk areas. A indicator/sentinel surveillance should be conducted in domestic dogs and cats against anthrax infection. Dogs and cats are resistant to anthrax and they develop antibodies after ingestion of anthrax contaminated meat. Sera sample from dogs and cats should be screened for anthrax using ELISA test which will provide an indication of anthrax bacterial contamination in the area.

II. 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.

Sample Collection and testing Procedures

Sampling and laboratory diagnostic procedures during surveillance should be undertaken as per the SOPs.

Annex 10.8: Standard operating procedures for anthrax surveillance in human

Purpose

To have standardized surveillance procedure for early detection of anthrax cases in human

Scope: This SOP covers the surveillance procedures during preventive, outbreak and post outbreak phase

User/Target: Clinicians, Health Workers and Laboratory staff

Surveillance Team composition:

Epidemiologist, MO, relevant specialists, health workers and laboratory staffs of district health sectors, hospitals and PHL.

Materials and Equipment:

Case reporting form, case investigation form, line listing form.

Sampling kits – swabs, needle, syringes, permanent marker pen, sample collection form, leak proof containers, transport media, cotton, antiseptics, and PPE.

Diagnostic kits – rapid diagnostic kits.

Types and steps for surveillance

I. Surveillance during the prevention phase

Surveillance during preventive phase comprises of clinical and laboratory surveillance in the Dzongkhags with intensive programs in high risk areas/Dzongkhags (Samtse, Chukha, Zhemgang, Trongsa, Wangdue, Mongar, Trashigang,Tsirang, Punakha, Haa, Sarpang & Dagana) and when there is imminent threat of anthrax incursion.
The reporting should be done as per the notifiable diseases surveillance reporting system given in the notifiable disease surveillance operational manual.

i. Clinical disease surveillance

Clinical surveillance is aimed at detection of anthrax cases based on clinical signs and symptoms of anthrax at the individual/household level and health centers. The anthrax should be suspected when any patients is found or visit health centers with cutaneous lesion on hand, legs, face and neck, abdominal distress characterized by nausea, vomiting, and anorexia, respiratory distress syndrome and acute encephalitis syndrome with history of exposure to anthrax suspected animals or/and their products.

ii. Laboratory Surveillance

Laboratory surveillance is not applicable for anthrax in human however whenever there is suspected case, appropriate samples should be collected and sent to the laboratory for confirmation.

iii. Targeted surveillance

Targeted surveillance in human is recommended only in the high-risk areas or households where incidences of animal anthrax has been reported in the past and in those people who handle meat and meat products (e.g. butchers/slaughter house workers).

iv. Surveillance plan for Anthrax in humans following an outbreak and post-outbreak phase

Surveillance must be carried out in human based on epidemiological risk assessment and on the basis history of contact, consumption and trade of animal products suspected to have died of anthrax.

Annex 10.9: Standard Operating Procedures for investigation of anthrax outbreak in animals

Purpose:

To have standardized outbreak investigation procedure following suspected anthrax outbreaks in animals.

Scope:

This SOP outline the general principles and steps for investigation of anthrax in animals

Users or targets

Veterinary Officers, Laboratory staff and para-veterinarians

Manpower/Team composition

Veterinary Epidemiologist, Veterinary Officer; Laboratory staff and field para veterinarians

Materials and equipment

PPE sets, investigation and laboratory submission forms, sampling kits, GPS and extension gears.

Steps for Investigation

i. Pre-investigation preparation

- Form investigation team and planthe investigation procedure among team members
- Discuss each person's roles and responsibilities
- Arrange materials and logistics *(refer materials and equipment requirement)*
- Gather preliminary information: Following information needs to be collected by the team prior to their departure
- Farmers name and phone number (if available),
- Name of village, Geog, Dzongkhag
- Type of enterprise and number of animals (commercial, semi commercial, backyard, (specify),
- Date and time of report of outbreak from farmer to LEC/ DVH.
- Date and time of report from LEC/ DVH to RVL/NCAH.
- Date and time of visit by veterinarian or field staff,
- Name of contact field staff, address and phone number.
- Provide information about the team visit to outbreak area.
- Date and time of visit

ii. Field investigation

a. Background information to collect

- Farm and village background information
- Different animal categories and numbers (herd size,)
- Farm type and husbandry practices
- Whether any inter-mixing of animals from other premises
- General information regarding source of animals
- General information buying and selling of animals and their products
- General information about the affected village/ farm (no. of households; household rearing livestock, average herd size, farming system etc.)
- Geographical information such as location (X Y coordinates altitude, road network, Government offices, frequency of movement of people in an out of the outbreak area

b. Baseline mortality and clinical signs

- Determine baseline mortality for period (week or month) before the outbreak and in previous year, both generally, and more specifically for the same seasonal time period as the present outbreak in the previous year;
- General information of the present disease outbreak such as number of households affected, population at risk, animals population in the surrounding villages etc.
- Record of the daily morbidity, mortality and case fatality figures in the farm/ village
- Record of the detail clinical signs during these periods.

c. Bio-security arrangements

- Describe bio-security arrangement of the farm. Mixing of different animals from different locations.

d. Feed source

- Describe feed sources/s and assess the possibility of using bone, hide and other animal meals.

e. Water source

- Describe soil type/texture and collect soil sample
- Assess water source for possibility of any contamination.

f. Wild animals

- Determine the presence of any wild animals in the area
- Assess contact with wild animals through sharing of common water bodies (ponds, lakes, wetlands) or through water source/s.

g. Weather situation

- Any occurrence of rainfall/flood/ or drought in the area

h. Veterinary interventions

- Record vaccination programs, drug use and other veterinary interventions

i. Laboratory investigation

Laboratory investigation in the field (refer specific SOP for sampling, packaging and transportation to the laboratory and rapid field test)

- Put on minimum PPE items
- Collect relevant clinical samples from live and dead animals as per the standard list of samples from different parts of the animal and transport to the laboratory.
- Collect environmental samples (water, feeds, soil etc) and submit to the laboratory.

j. Laboratory diagnosis (refer specific SOPs for laboratory diagnosis)

Following laboratory tests will be done at different levels:

At the field

- Carry out rapid antigen detection test using immunochromatographic assay device (if available).

At DVL//SVL/RLDC:

- Blood smears (blood from natural orifices of dead animals and from ear tips from sick/ dead animals) should be stained using with Polychrome Methylene Blue stain methods animals should be stained using and examined for presence of capsulated rod shaped bacilli organism.

At NCAH for confirmation:

- Reconfirmation of unstained and stained blood smears and exudates smears from the natural orifices stained with Polychrome Mehtylene Blue stain. Further confirmation with bacterial isolation and identification and PCR test.

k. Characterize the outbreak

- Establish or verify the outbreak
- Provisional diagnosis made on clinical signs and epidemiological pattern followed by field test at DVL.
- Interim immediate emergency disease control response should be in place before the confirmatory laboratory diagnosis is made from the reference samples from NCAH, Serbithang

l. Establish the case definition for Anthrax

- Suspect: sudden death of animals with unclotted tarry coloured blood oozes from the natural orifices with rapid bloating and absence of rigor mortis in herbivore animal and presence of oedema and swelling of face, neck in pigs.
- Confirmed: A combination of symptoms consistent for Anthrax with the identifcation of bacillus anthracis organism in blood smears at DVL/SVL/RLDC with further confirmation from NCAH Serbithang through similar tests or and PCR test positives.
- Differential diagnosis has to be made against Lightning, acute poisoning, Black Quarter and Bloat (refer setion 3.4.2 and 3.4.3).

m. Describe outbreak in terms of time, animal and place.

Time (draw epidemic curve by plotting cases against the time from available data-preferably time series)

- When was the index case?
- What is the exact period of outbreak?
- Given the diagnosis what is probable period of exposure?
- Is the outbreak most likely to be point source or propagated or both?

Animal (attack rates, risks etc)

- Any differences in the attack rates among different sizes of herds.
- Which groups has the highest and which have the lowest attack rate?
- Any difference in the attack rate among different age group of animals?

Place (plot the location of outbreak on a map with physical characteristics such as road, water bodies, mountains, infrastructures etc)

- What are the geographical distributions of the cases?
- What is the pattern of the cases among different poultry house or management system?
- Whether case farm is close to water bodies or other spatial risk factors?

Develop hypothesis based on the pattern of disease (animal, time and place).

- Source of disease outbreak-forward and back ward contact tracing
- Mode of transmission.
- Whether the outbreak is a common source or propagating
- If a common source, whether it is point or multiple exposure
- What are the risk factors associated with problem?

Control and Prevention (Refer specific SOPS for RRT; surveillance; disposal; decontamination etc)

- Interim immediate emergency disease control response should be in place before the confirmatory laboratory diagnosis is made (refer specific SOPs for disease outbreak response)

Declaration of protection zone

When Anthrax is suspected, Veterinary vigilance team (VVT) should inform the NCAH and immediately quarantine the suspect infected place (farm premises or a village) and the surrounding area a radius of about 500 meters around the infected foci) as a protection zone. This zone will include infected premises; suspected premises and dangerous contact premises and should be demarcated after due consideration of the epidemiologic risk and natural geographical settings.

All places with animals within this Zone shall be considered at-risk/ suspect and should be visited to establish their infection status.
Quarantine and movement control on animal and animal products, farm workers; vehicles etc should be imposed (refer SOP fro quarantine and movement control).
Strict surveillance and movement control should be maintained on all other properties within this Zone.

Declaration of protection zone:

Once the outbreak is confirmed Rapid Response Team (RRT) should be immediately activated.
RRT should carry out (a) disposal of carcass and infected materials; (b) disinfection and (c) sanitary measures, on all the properties within this zone (refer specific SOPs).

Declaration of Surveillance Zone (Refer SOP for surveillance)

Disposal of carcass and infected animals (Refer SOP for disposal)

Disinfection of infected premises (Refer SOP for decontamination)

Reporting

- Document the findings (Background; investigation procedures, epidemiological and laboratory findings; economic impact etc.
- Provide recommendations to all the relevant stakeholders (farmers/ producers; DoL, MOH, BAFRA and other agencies)
- Submit the final report.

Risk communication

- Surveillance and monitoring (will be done by the surveillance team).
- Is the control programme effective?
- Is the frequency of the disease remaining constant; increasing or decreasing
- Does the disease have any impact on productivity

Form-1: Disease Investigation Form

Reference No.:		Date:
Owner details		
Name of the farm:		Name of farm owner:
Contact telephone number:		
Address: Village:	Geog:	Dzongkhag:
Geo coordinates	Longitude (E)	Latitude (N)
Information about the farm		
Type of farm: Commercial [ ]; Semi-commercial [ ]; Backyard [ ];		
Livestock population (mention in detail including sex, breed, age and categories)		

Detail history of outbreak

Actual location and area (Geographical identification)

Date and time of report of outbreak from farmer to LEC/ DVH/SVL:

Date and time of report from LEC/ DVH/SVL to RLDC/ NCAH:

Date and time of onset of clinical signs:

Date and time of onset of mortality:

Any outbreak in the past years (mention the details)

Any meat or milk consumed from the herd or the sick/dead animals (if yes mention the details)

Animals affected

Sl. No. (n)	Species (a)	Age & sex group affected (b)	Number of villages affected (c)	Numbers at risk (d)	Number of		Source of Infection (g)
					Cases (e)	Deaths (f)	

Vaccination history of the last vaccination

(If there is control strategy with vaccination for the disease suspected or confirmed)

Name of the Vaccine	b) Vaccination frequency	Target population		No. vaccinated		h) Vaccine type (batch no./ expiry date)	Time of last vaccination
		d)Geog	e)Dzongkhag	f)Geog	g) Dzongkhag		

Clinical observation

Detail clinical signs (including duration of illness)

How the vaccine is transported to the centre and how is the cold chain maintained

Treatments instituted/advised

Necropsy findings (if any)

Comments on epidemiology of the disease (*origin of disease, mode of entry, how the disease spread in the population, any human infection, etc)*

Laboratory findings

Differential Diagnosis

Disease suspected/confirmed

Control measures recommended

Sample collected and referred							
Sample Id.	Category of livestock	Specimen type	No. of specimens	Laboratory referred to	Date of shipment	Test requested for	
Name & Designation of Investigation Team:					Signature		

6.B. FLASH REPORT ON NOTIFIABLE DISEASE OUTBREAK

1. Disease	
2. Date of report	
3. Date of previous report	
4. Number of separate outbreaks identified	
5. Geographical location of the outbreak	
6. Species affected	
7. No. of cases	
8. No. of deaths	
9. Probable source of infection	
10. Control measures taken	
11. Reported by	

Annex 10.10. Standard Operating Procedure for investigation of Anthrax outbreak in human

Purpose:

To have standardized outbreak investigation procedure following suspected anthrax outbreaks in human

Scope: This SOP outline the general principles and steps for investigation of anthrax outbreak in human

User/target:
Doctors, Health Workers and Laboratory staff

Manpower/Team composition: Epidemiologist/ Public Health experts/Clinicians/Laboratory statf and other relevant health staff

Materials/Equipments:
PPE sets, investigation and sample submission forms, sampling kits, sample shipment container.

Investigation steps

Step 1: Establish the existence of outbreak

Before proceeding in the field for outbreak investigation, the health authority of the concerned district should take up the responsible for the response. Establish existence of an outbreak based on outbreak notification and preliminary information sent by health centers.
Contact the health workers who have reported the outbreak and ask for additional information if required.

Step 2: Verify the diagnosis of anthrax

After the establishing an outbreak, verify the diagnosis of anthrax case reported by health worker by reviewing both clinical findings and laboratory findings if smear testing is performed.
Instruct to send sample to nearest hospital laboratory if health center does not have facility to perform smear test.
Also, contact the concerned livestock center and find out anthrax case in animal. If there is confirmed anthrax in animal and verified the diagnosis in human, ask health worker to institute the control measures immediately.
The concerned district health authority should immediately alert the rapid response team (RRT) of the district instituted, if not form outbreak control team with relevant people (refer notifiable disease surveillance guideline). Identify the team leader and dispatch the team in the outbreak place/field.

Notify PHL/DoPH about the outbreak or seek technical guidance if require.

Step 3: Define and identify the anthrax cases

In the field, the team should establish a working case definition after review cases record/data to find out more cases. The working case definition should be defined by place, person and time

Find out more cases by using working case definition in the outbreak places and record in the line listing form

Group the cases based on case classification definition (refer surveillance SOP)

Implement control measures

Step 4: Perform Descriptive epidemiological analysis

Organize the data from line listing and enter into the computer
Describe the case by person, place and time using epi-curve, map, frequency of signs, symptoms and age of cases and find out the attack rate.

Step 5: Find out who are at risk of anthrax infection

Based on exposure history to anthrax suspected/confirmed animals or/and their products, list out the people in the locality/place.
Follow up those listed case for development of any signs and symptoms or/and ask to report to health center if any of them develop any signs and symptoms.
Implement control measures

Step 6: Develop hypothesis for an anthrax outbreak

Anthrax being zoonosis, the source and mode of infection is usually known. However, if there is no suspected/confirm animals case in the place or nearby locality, hypothesis should be developed to trace the source of infection.

Step 7: Evaluate hypothesis for an anthrax outbreak

Conduct cohort study
Use two-by-two table to evaluate hypothesis

Step 8: Refine hypothesis for an anthrax outbreak

Refine hypothesis based on evaluation if not answered

Step 9: Implement control measures

- Treat and manage all anthrax cases.
- Carry out advocacy among public in the place.
- Help livestock to implement their control measures.
- Keep active surveillance including follow up of the cases who are identified as at risk

Step 10: Communicate findings

The team should initially submit an interim report to concerned district health authority, PHL and DoPH during the investigation time giving details of the investigation, diagnosis and also the control measures initiated.
The team should write final report in the standard format (see natfiable disease surveillance guideline) and submit final report to concerned district health authority, PHL and DoPH.

Recommendation must be mentioned explicitly based on findings to correct gaps and constraints to avoid future outbreaks

The district health authority (DHO) should continue monitoring the place through active surveillance till outbreak is declare over (when there is no new cases reported for a period of two consecutive incubation period of an anthrax bacteria).

Annex 10.11: Standard Operation procedure for disposal of anthrax carcasses by burial

Purpose:

To have standard procedure for safe disposal of anthrax infected carcasses

Scope:

This section describes procedures for site selection and burial of anthrax carcasses in a safe manner

Users:

Veterinary Officer/Para veterinarians

Manpower:

Veterinary Officer, para-veterinarians, animal owners

Materials/ Equipments required:

Hand gloves; Face masks; Apron (disposable); Eye goggle; Gum boot; Disinfectant-Lime/ Virkon
Digging tools: spades, crowbars, peak-axe

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 carcasses with a minimum depth of 2 meters considering the size of the carcasses.
- Wear apron, face masks, goggle and gloves before handling the carcasses.
- Drop the carcasses into the pit.
- Cover the carcasses with soil, 400 mm is suggested, and add an unbroken layer of lime (calcium carbonate)
- Do not put lime directly on to the carcasses (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.

Annex 10.12: Standard Operating Procedure for disinfection and decontamination of contaminated premises and materials.

Purpose:

To have standard procedure for effective disinfection and decontamination of contaminated premises and materials

Scope:

The document describes procedures for disinfection and decontamination of contaminated materials and premises.

Users:

Veterinary Officers/Para-veterinarians

Manpower:

Veterinary Officer/para-veterinarians; animal owners

Materials/ Equipments required:

Gloves; Apron; Gum boots; Buckets; Mugs/jugs; Water; bleaching powder; hypochlorite

Procedure:

- Prepare 1% hypochlorite solution in a bucket.
- Utensils: Spray and wash barn utensils, tools and equipments with the above solution thoroughly.
- Dry them for reusing.
- Burry 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