

Guidelines for Prevention and Control of Anthrax



**ZOONOSIS DIVISION
NATIONAL INSTITUTE OF COMMUNICABLE DISEASES
(Directorate General of Health Services)
22-SHAM NATH MARG, DELHI - 110 054**

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GUIDELINES FOR PREVENTION AND CONTROL OF ANTHRAX

1. Introduction

Anthrax also called Malignant pustule, Malignant oedema, Woollsorter's disease, or Ragpicker's disease, is an acute infectious disease of animals caused by *Bacillus anthracis*, a gram-positive, spore-forming bacillus. Spores of *B.anthraxis* can persist in the environment for many years in some types of soil and enter the body through skin abrasions, inhalation or ingestion and multiply to produce exotoxins. Anthrax is primarily a disease of herbivorous animals that occasionally affects human.

2. Causative agent

The causative organism *B.anthraxis* is a gram-positive, nonmotile, non-haemolytic, and spore-forming bacillus. The virulence of the organism is determined by the capsule and exotoxins; oedema toxin and lethal toxin.

Anthrax is considered an important biological warfare agent because (i) it is highly fatal and when transmitted through inhalation is almost always fatal, (ii) Anthrax spores can remain viable for several decades and can be easily produced in large quantities at a very low cost, and (iii) it is easy to weaponize and disseminate anthrax as an odourless and invisible aerosol which can affect thousands of people at the same time.

3. Epidemiology

3.1 Geographical distribution

Anthrax is known to occur globally, though it is more often a risk in countries with less standardized and less effective public health programs. Anthrax is most common in dry agricultural zones. These include South and Central America, Southern and Eastern Europe, Asia, Africa, the Caribbean, and the Middle East.

3.2 Situation in India

Anthrax is enzootic in southern and eastern India but is less frequent in the northern Indian states. In the past years the anthrax cases have been reported from Andhra Pradesh, Jammu and Kashmir, Tamil Nadu, Orissa, Karnataka and West Bengal. Outbreaks of Anthrax have been reported from Karnataka (1999 and 2001), Orissa (2003 and 2005), West Bengal (2000).

3.3 Transmission

Animals usually become infected by ingestion of contaminated soil or feeds. Infected animals shed the bacilli during terminal hemorrhage from all the natural orifices, or if the blood of the dead animal is spilled accidentally. On exposure to the air, the vegetative forms sporulate. These spores are markedly resistant to many disinfectants and adverse environmental conditions and remain viable in the contaminated soil for many years.

Cutaneous anthrax is the most common anthrax infection. Transmission occurs after exposure to infected animals and contaminated animal products such as hair, hides, wool, bones, or skin. Inhalation anthrax results from inhalation of spores in particles less than 5 µm in diameter that may reach the terminal alveoli of the lungs. Aerosols of such particles may be created by the agitation of the hair or wool in the industry settings. Intestinal and oropharyngeal anthrax results from ingestion of contaminated meat of sick and dead animal.

Accidental infection may occur among laboratory workers. Direct person to person spread of anthrax is extremely rare. The disease mostly affects adults, especially males. It is due to high exposure rate among these groups.

Anthrax is a seasonal disease. Climate probably acts directly or indirectly 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 and sparse.

Incubation period is from one day to seven days. Incubation period up to 60 days is possible. Most cases occur within 48 hours of exposure.

4. Clinical manifestations

4.1 In animals

Important clinical manifestations in animals

- In ruminants, sudden death, bleeding from natural orifices, subcutaneous haemorrhage, without prior symptoms or following a brief period of fever and disorientation should lead to suspicion of anthrax.
- In pigs, occasionally when carnivores and primates are involved the manifestation would be, local oedema and swelling of face and neck or of lymph nodes, particularly mandibular and pharyngeal and/or mesenteric.

The incubation period in the susceptible herbivore ranges from about 36 to 72 hours. The first signs of an anthrax outbreak are one or more sudden deaths in the affected livestock. Other signs include going off feed, or producing less milk than usual. During the systemic phase, the animals become distressed, appear to have difficult breathing and cease eating and drinking. Swellings in the submandibular fossa may be apparent, and temperature may rise. If the animal fails to respond to the treatment, it lapses into coma followed by death from shock.

4.2 In humans

Anthrax infection occurs in three forms: cutaneous, inhalation, and gastrointestinal depending on the mode of transmission. Symptoms of disease vary depending on how the disease was contracted, but symptoms usually occur within seven days.

Cutaneous anthrax: Most anthrax infections occur when the bacterium enters a cut or abrasion on the skin, such as when handling contaminated wool, hides, leather or hair products of infected animals. The incubation period for cutaneous anthrax is 1-7 days. Skin infection begins as a painless, pruritic papule that resembles an insect bite but within 1-2 days develops into a vesicle (usually 1-3 cm in diameter) and then a painless ulcer with a characteristic black necrotic (dying) area in the center. Systemic symptoms are mild and may include malaise and low-grade fever. There may be regional lymphangitis and lymphadenopathy. Occasionally more severe form of cutaneous anthrax may occur with extensive local oedema, induration and toxemia. The infection can also spread to the

bloodstream with overwhelming septicemia. About 20% of untreated cases of cutaneous anthrax will result in death. Deaths are infrequent with appropriate antimicrobial therapy.

Inhalation anthrax: Initial symptoms may resemble a common cold. After several days, the symptoms may progress to severe breathing problems and shock. Mediastinal widening is seen in the X-Ray chest. Diagnosis is difficult but inhalation anthrax should be suspected if there is a history of exposure to an aerosol that contains *B.anthraxis*. Inhalation anthrax usually results in death in 1-2 days after onset of the acute symptoms. Mortality is 30% in untreated cases and those who survive acute illness will fully recover.

Intestinal anthrax: The intestinal disease form of anthrax may follow the consumption of contaminated meat and is characterized by an acute inflammation of the intestinal tract. There are two clinical forms of intestinal anthrax.

- ***Intestinal anthrax:*** Symptoms include nausea, vomiting, fever, abdominal pain, haematemesis, bloody diarrhoea and massive ascites. Unless treatment starts early toxemia and shock develop resulting in death. Gastro-intestinal anthrax is difficult to diagnose as the symptoms are non-specific. However, history of ingesting meat of a sick animal and cases of cutaneous anthrax in the area may support diagnosis. Intestinal anthrax results in death in 25% to 60% of cases.
- ***Oropharyngeal anthrax:*** Main clinical features are sore throat, dysphagia, fever, lymphadenopathy in the neck and toxemia. Even with treatment mortality is high, about 50%.

Meningitis may complicate any of the three primary forms. It resembles meningitis due to other causes although it is frequently haemorrhagic. Diagnosis is confirmed by demonstration of the organism in the CSF by microscopy or culture or both.

5. Case management

5.1 Chemotherapy

Antibiotics are effective if the disease is recognized early and the full recommended dose and course of the antibiotic is completed. If left untreated or if the antibiotic

treatment is discontinued early, the disease can be fatal. General measures for treatment of shock are also necessary.

Cutaneous anthrax:

1. Ciprofloxacin 500 mg BD orally for 10 days or
Doxycycline 100 mg BD orally for 10 days or
Amoxicillin 500 mg TDS orally for 10 days
2. Oral penicillin V 500 mg 6 hourly or
Procaine penicillin 1 million unit 12 to 24 hourly by IM route
3. Chloramphenicol, Rifampicin, erythromycin, clindamycin or clarithromycin may also be given.

Pulmonary and intestinal anthrax:

Ciprofloxacin 400 mg IV BD till 2 weeks after the clinical response or Penicillin G, 2 million units per day by infusion or by slow intravenous injection should be administered until the temperature returns to normal. After that, treatment should be continued in the form of intramuscular procaine penicillin, 1 million units every 12-24 hours. Streptomycin, 1-2 grams per day intramuscularly, may act synergistically with penicillin.

Supportive care:

- Oxygen inhalation
- Respiratory support
- Treatment of shock if present

5.2 Vaccination

Human vaccine: In China and former USSR live spore vaccines are in use. However, in most other countries the same are not licensed and killed vaccines for humans are used. As it is not known whether the anthrax vaccine can cause foetal harm, pregnant woman should not be vaccinated.

Animal vaccine: Most animal vaccine for anthrax in use around the world utilize the toxigenic, non-capsulating *B.anthraxis* strain 34F₂. The protection by single

dose of strains 34F₂ vaccine is said to last about 1 year, therefore annual boosters are recommended for livestock in endemic areas.

6. Case definition

Recommended case definition in humans

Suspect: A case that is compatible with the clinical description and has an epidemiological link to confirmed or suspected animal cases or contaminated animal products and also if smear from clinical specimen is positive. In enzootic areas, all sudden death in animals with haemorrhage from natural orifices should be regarded as suspect case.

Presumptive: A suspected case where:

- The clinical specimen in culture shows typical characteristic
- In smear short chain of capsulated bacilli are seen when stained with polychrome methylene blue

Confirmed: A suspected case that is laboratory confirmed by one or more of the following:

- Where it shows encapsulated, non-motile, non-haemolytic gram positive bacilli susceptible to penicillin and the isolate is susceptible to gamma phage lysis.
- PCR confirming presence of toxin and capsule genes.

7. Laboratory diagnosis

7.1 Collection, storage & transportation of samples from suspected anthrax cases

- Laboratory diagnosis -for anthrax should be attempted only by laboratory well trained to do so.
- High index of suspicion of the disease is important.
- Collection and transportation should be carried out under strict aseptic condition

Collection of Specimen

(a) Cutaneous Anthrax

- In early stage vesicular exudate from the lesions by sterile swab can be collected.
- In later stage swabs to be taken from underneath of eschar after lifting up of eschar with sterile forceps.

The swab should be put in Carry-Blair transport medium and with another swab smear on microscopic slide may be prepared and heat fixed. Smear should be made wherever feasible.

(b) Intestinal Anthrax

- If patient is not severely ill, a faecal specimen can be collected
- If patient is severely ill ascitic fluid (peritoneal fluid) can be collected.

(c) Pulmonary Anthrax

- If patient is not severely ill, sputum can be collected.
- In severely ill children gastric lavage should be collected.

Storage and Transportation

(I). Storage:

All samples collected should be stored properly at room temperature, if delay in transportation takes place then specimen should be stored at 4 - 8°C.

(II). Transportation

Specimen container

- Should be leak proof, break-resistant plastic or glass container
- Screw cap, containers are preferable.
- After the container is closed and sealed - Wipe with a disinfectant - a chlorite solution (sodium hypochlorite)
- Dry it and send it in a properly labelled packet (3-layer packing).

7.2 Laboratory procedures

Laboratory confirmation for anthrax is made by:

- (i) Direct demonstration *B.anthraxis* from blood, skin lesion or respiratory secretion by polychrome methylene blue staining and Gram staining which shows encapsulated broad rods in short chain 2 – 4 cells. Modified acid fast staining should be used to visualize the spores.
- (ii) Culture – specimens are cultured on sheep blood agar aerobically at 35 – 37°C. After 15 to 24 hours of incubation, colonies are well isolated, non-hemolytic, 2 – 5 mm in diameter, flat or slightly convex, irregularly round with edge that are slightly undulated greyish white and have a ground glass appearance. The edge of the colonies are curled or fringed having a ‘medusa head’ appearance. The gram stain morphology shows broad gram positive rods (1-1.5 x 3-5µ) in long chain with oval, central to sub-terminal spores: 1 – 1.5µ with no significant swelling of cells.
- (iii) Biochemical reaction – *B.anthraxis* is non-motile, ferment glucose, sucrose and maltose with acid only. Gelatin is liquified and starch is hydrolyzed. The confirmation of the isolate is done by gammaphage lysis.
- (iv) Animal pathogenicity – 5 – 10 mice are injected intra-peritoneally with suspension of culture material. Observed for mortality. 100% mortality within 24 hours in case of *Bacillus anthracis*. Material (spleen) from dead mice processed for detection of *Bacillus anthracis*.
- (v) Molecular methods – Direct PCR and genetic study for 846 bp capsule gene, 639 bp S-layer gene and 596 bp PA genes of *B.anthraxis*.

8. Prevention and control

The problem of anthrax continues because of the following:

- The custom of butchering and eating roasted meat from sudden death animals and utilizing their hair, hides, bones etc.
- Lack of cooperation over reporting sudden deaths in animals
- Long delays in diagnosis due to poor communication and inadequate local laboratory facilities
- Failure to implement policies on disposal of carcasses and subsequent disinfection and decontamination

Control measures aim at breaking the cycle of infection. It is primarily around proper disposal of anthrax carcasses, disinfection, decontamination and disposal

of contaminated materials, and vaccination of exposed susceptible animals and humans in at risk occupations

8.1 Guidelines for an effective control programme

- **Surveillance**

All unexplained livestock deaths or suspected human and animals cases must be investigated with laboratory support. In animals, the samples should be collected with the help of veterinarian.

- **Reporting**

- Mandatory reporting of sudden deaths among livestock
- Mandatory reporting of all human cases

- **Disposal**

After confirmation as being a case of anthrax, a carcass should not be opened and should be disposed of by incineration or rendering (Annexure-1). Deep burial after disinfection is a less favoured option. Blood from the dead animal should be collected aseptically for confirmation of diagnosis. Necropsy should not be done, as this has the risk of spread of the infection.

- **Disinfection**

Disinfectants should be available in reasonable quantities at veterinary hospitals (Annexure-2). Veterinary assistants, surgeons and livestock owners should be trained in their use. Decontaminate soil seeded by carcasses.

- **Education**

Educate employees handling potentially contaminated articles about modes of anthrax transmission, care of skin abrasions and personal cleanliness.

- Control dust and properly ventilate all hazardous industries particularly which are handling raw animal materials.
- Do not use/sell hides of animals exposed to anthrax nor use their carcasses as food or feed supplements.
- Treat properly the effluents from hazardous industries handling animals etc.

- **Treatment**

All symptomatic animals should be treated. Immunize after cessation of treatment.

- **Intersectoral cooperation**

Good communication and cooperation including sharing laboratory facilities and knowledge between veterinary, medical and wild life services are essential to control of anthrax.

9. Actions to be taken in the event of an outbreak of Anthrax

Every effort is to be made to investigate the outbreak, to confirm through laboratory diagnosis and epidemiological investigation. Every effort should be made to search for the source.

In the affected areas, the following measures must be applied.

For Animal

- The carcasses of infected cattle are to be either burnt at the site of death and the ashes buried deeply, or wrapped in double thickness plastic bag to prevent spilling of body fluids and removed to a more suitable site where they are burnt and the ashes buried.
- The site where the animal died is to be disinfected with 5% formaldehyde after disposal of the carcass.
- All other animals in the affected herd are to be vaccinated.
- Affected premises are to be quarantined for at least 20 days after the last case.
- All cattle on neighboring premises should also be vaccinated.
- A buffer zone, 20-30 Km wide, is to be established around the infected area within which all cattle and exposed sheep are vaccinated and quarantined.
- Any milk collected from a cow, buffalo or goat showing signs of anthrax within 8 hours of milking is to be destroyed, along with any other milk that may have been mixed with the suspected milk.

For Human

- People entering infected premises are required to wear protective clothing and footwear, which are disinfected before leaving the premises.
- Such persons should avoid any contact with other persons or animals without first changing clothing, washing hands and taking appropriate disinfection measures.
- Where there is a risk of aerosolization of spores, further precautions should be considered such as damping down the material, possibly with 5% formalin, wearing face masks etc.
- ***Chemoprophylaxis*** – asymptomatic exposed individuals are put on a four week course of doxycycline 100 mg twice daily or ciprofloxacin 500 mg twice daily.

GUIDELINES FOR DISPOSAL OF ANTHRAX CARCASSES

Sporulation of *B.anthraxis* requires oxygen and therefore does not occur inside a closed carcass, regulations forbid post mortem examination of animals when anthrax is suspected. "The methods of disposal of an anthrax carcass are incineration, rendering or burial.

A. Incineration

The preferred method of disposal of an anthrax carcass is incineration. Incineration must be done with appropriate care to ensure complete burning from beneath. All the procedures described below take many hours for a large domestic animal, such as cow or buffalo.

(i) Pit Method

For a large animal, a pit about 0.5m deep and exceeding the length and breadth of the carcass by about 0.25m on each side should be dug near the carcass. A trench approximately 0.25m wide by 0.25m deep should be dug along the length of the centre of the pit extending beyond the ends by about 0.75m; this serves the purpose of allowing air for the fire under the carcass. The bottom of the pit and the trench should be covered with straw which is then soaked in kerosene.

Above the kerosene-soaked straw, place a few pieces of heavy timber (or other type of beams which will hold the carcass well above the bottom of the pit) across the pit and then scatter thin pieces of wood over beams and straw. Then add larger pieces of wood and, if available, coal, until the pit is filled upto top ground level. Saturate all the fuel with kerosene.

The carcass can then be drawn onto the pyre, preferably propped up so that it is lying on its back. Further kerosene should be poured over the carcass. The fire

is started at either end of the longitudinal trench. Once the incineration is well underway (probably after about the first hour), the pyre should be covered with corrugated iron or other metal sheeting in such a way as to reduce the heat loss without cutting off the ventilation.

The approximate quantities of fuel that will be needed for a large domestic animal are 20 kg of straw, 10 liters of kerosene, and either 2 tonnes of wood or 0.5 tonnes of wood and 0.5 tonnes of coal. It will be necessary to decontaminate the ground where the carcass lay and from where it was removed to the pit and also the ground, equipment, etc. contaminated during this moving process.

(ii) Raised carcass method

This method may be appropriate when labour is scarce or the ground unsuitable for the construction of a pit.

Place straw over a 2 x 1.5 metre area. Place two wooden beams (approximately 2m lengths of small tree trunks, railway sleepers, etc.) over the straw parallel to each other and about 1.2~m apart and aligned with the direction of prevailing wind. Soak the straw with kerosene and cover with thin and thick pieces of wood and coal if available. Place further stout cross-pieces of wood or other material across the two main beams to support the carcass. The fuel (wood or coal) is banked up either side (but not at the ends where the air must be allowed to enter upon the carcass) of the carcass and fuel and carcass further doused with kerosene.

The fire can then be started and as before, when well underway, it should be covered with metal sheeting to retain heat but without inhibiting ventilation. Further fuel should be added if and when necessary.

More fuel may be required than with the pit method. For a large domestic animal, an estimate is 0.75 tonnes coal + 0.5 tonnes wood or, if coal is unavailable, approximately 3 tonnes of wood, plus 20 kg straw and 20 liters of kerosene.

As with pit method, it will be necessary to decontaminate the site where the carcass lay before incineration and the ground and equipment contaminated in moving it from there to the pyre.

B. Rendering

Rendering is essentially a cooking process which results in sterilization or raw materials of animal origin such that parts of carcass may be utilized safely for subsequent commercial purposes.

There are a number of variations of the rendering process, broadly divided into batch processes and continuous processes. In general, the raw materials are finely chopped and then passed into a steam heated chamber and subjected to temperatures ranging from 100°C to 150°C for 10-60 minutes (this does not include the time taken to bring the material to the peak temperature or the subsequent cooling period time).

C. Burial

Where neither incineration nor rendering is possible, for example due to lack of fuel, burial is the alternative. Deep burial (2m) of carcass covered with lime and soil in 1:3 ratio. Burial should be discouraged in favour of incineration or rendering wherever possible.

Periodic reports of viable anthrax spores at burial sites of animals which died many years back have testified to the unreliability of burial procedures for long term control of the disease. Disturbance of such sites, for example by ploughing, or laying drainage, brings the spores to the surface; even without site disturbance, spores can work their way up to the soil surface. In either case, this may result in new live stock cases.

Further disadvantages to the burial sites are that scavengers may dig down to reach the carcass and in dry, dusty areas, the digging process can spread the contaminated soil extensively.

D. The last resort

The last resort in situations where incineration, rendering or burial may not be feasible is to leave the carcass unmoved and adequately closed off from other animals, particularly scavengers, or people. The carcass should be fenced off and covered using branches of trees, corrugated iron or any other available materials,

and hazard signs should be posted around the site. *B.anthraxis* within the animal carcass does not sporulate and is inactivated by the putrefactive process in a few days. However, environmental contamination due to bloody exudate escaping from the mouth, nose and anus of the death animals may still occur. The resulting environmental contamination could be minimized by scorching the site with fire, after the carcass has effectively putrefied, though this may be many months later. Clearly this is far less satisfactory than incineration, rendering, or burial which should be carried out as the least preferred option.

GUIDELINES FOR DISINFECTION, DECONTAMINATION AND DISPOSAL OF INFECTED/CONTAMINATED MATERIAL

Anthrax is a bacterial disease caused by the spore forming bacteria, *Bacillus anthracis*. When conditions are not conducive to growth and multiplication of the bacilli, they tend to form spores. The spore forms are markedly resistant to extremes of heat, cold, pH, desiccation, chemicals (and thus to disinfection), irradiation and other such adverse conditions. Therefore, the spore forms are the predominant phase in the environment and it is very largely through the uptake of spores that anthrax is contracted.

Within the infected host the spores germinate to produce the vegetative forms which multiply, eventually killing the host. Spores will germinate outside an animal if they fall into appropriate conditions, i.e. a temperature between about 8° and 45°C, a pH between about 5 to 9, a relative humidity greater than 95% and the presence of adequate nutrients. The recorded survival time of spores ranges from 6 months to 71 years under different condition.

To break the local cycle of anthrax infection, disinfection, decontamination and correct disposal of infected/contaminated materials are of considerable importance in preventing transmission of infection.

The details of the disinfectants in respect to the decontamination of various infected materials are as follows:

A. Spore form by using either of following:

- 2% Glutaraldehyde
- 5-10% Formaline
- 5% Lysol

- 5-10% Sodium hydroxide — At least for 2 hours
- 3 % peracetic acid
- 1 in 5000 solution of bichloride of mercury
- 1 % Formic acid
- 2% Hydrochloric Acid
- Ethylene oxide vapour for 10 hours
- Moist heat 121°C for 30 minutes
- Dry heat 120° - 140°C for 3 hours
- Flame gun - on floors and crevices

B. Vegetative form

- If carcass is unopened bacilli lasts for 3 days at 25-30°C, 4 weeks at 5-10°C and for few minutes at 60°C.

C. Manure/Dung/Bedding

- By incineration
- By autoclaving at 121°C for 30 minutes
- Or immersion in 4 % formaldehyde for 12 hours

D. Floor space/shed/vehicle

- Preliminary disinfection using 10% formaldehyde; (1-1.5 It/ sq.m.) or 4% glutaraldehyde for atleast 2 hours
- Cleaning - by washing or scrubbing with hot water
- Final disinfection by one of the following disinfectants applied for atleast 2 hours.
 - 10% formaldehyde
 - 4% glutaraldehyde
 - 3% hydrogen peroxide or
 - 1% peracetic acid

E. Closed rooms/ cabinets

- Fumigation - Boiling of water containing concentrated formalin in an electric kettle and leaving overnight or at least for 4 hours.

F. Spills/Splashes/Accidents

- Floor - by Hypochlorite solution containing 10000ppm available chlorine.
- Clothes - should be autoclaved or fumigated.
- Eyes - should be flushed out with copious quantity of water immediately. Rubbing to be avoided. Medical help to be taken
- Skin - should be bathed in hypochlorite solution containing 5000 ppm available chlorine for one minute and washed with soap and water. In broken skin bleeding to be encouraged, washed with water. Medical help to be taken at the earliest.
- Mouth - Mouth pipetting should not be done in any case. If accidentally done, mouth should be thoroughly washed with hypochlorite solution containing 2000 ppm available chlorine.

G. Liquid Manure

- By treating per m³ of slurry with 37% formaldehyde solution (approx. 50-100 kg. of formalin). The mixture is left for 4 days.

H. Sewage sludge

- By 5% formaldehyde as 8% of dry matter for 10 hours
- Or 3% peracetic acid for 30 minutes

I. Water

- By autoclaving, filtration or by 5-10% formaldehyde for 10 hours.

J. Soil

- By incineration/heat treatment 121°C for 30 minutes.
- 5% formaldehyde as 50 lts/sq.m.
- or covering with concrete/ tarmac

K. Other materials

- By incineration
- Autoclaving

- Overnight soaking in 4% formaldehyde/2% glutaraldehyde
- Or by fumigation (Ethylene oxide/formaldehyde)

L. Wool and Hair

- By duckering process (five stages) i.e.
- Immersion in 0.25-0.3% soda liquor
- Immersion in soap liquor;
- Two immersions in 2% formaldehyde solution; and
- Rinsing in water

M. Hide and Skin

- By formaldehyde or ethylene oxide fumigation

N. Bone, Hoof and Horn

- No statutory regulations exist, but sterilization is essential before making feed ingredients and fertilizers.

