African Journal of Basic & Applied Sciences 12 (3): 75-81, 2020
ISSN 2079-2034

DOI: 10.5829/idosi.ajbas.2020.75.81

Management and Disposal Strategies of Died Animals Due to Anthrax

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Abstract: Anthrax is an infectious disease caused by the bacteria *Bacillus anthracic*. The disease can affect both humans and animals, although it is more common among livestock and wild animals. The ecological factors that might be responsible for the survival of anthrax spore in soil are soil type, calcium content and organic carbon content and soil pH. Effective carcass disposal and disinfection of site prevent contaminating the ground with the anthrax spores or organisms. After an outbreak, quarantine the healthy livestock, vaccinate and move them away to clean pastures reduces anthrax epidemics. Appropriate and safe disposal of dead animals, bedding and other contaminated materials and subsequent disinfection and decontamination of all possible surfaces that can harbor anthrax spores are key steps in limiting the spread of anthrax and contamination of the environment. The ideal method of disposal of an anthrax carcass is incineration. Where this method is not possible, deep burial is the alternative. Unlike burial, burning has the advantage of destroying anthrax spores and reducing the number of spores available in the environment and, therefore, reducing the chance of spores resurfacing years later. Anthrax spores can be destroyed by applying dry heat (140°C) for three hours, autoclaving at 120°C for ten minutes or exposure to 10% bleach for two hours. Surfaces contaminated with spores can also be disinfected with 10% formaldehyde, 2% glutaraldehyde, 3% hydrogen peroxide or 0.3% peracetic acid.

Key words: Anthrax · *Bacillus anthracic* · Disposal · Incineration · Carcass

INTRODUCTION

Anthrax is a contagious and highly fatal zoonotic bacterial disease affecting primarily herbivores. Mortality can be very high, especially in herbivores. The disease has world-wide distribution and is a zoonosis. The etiological agent is the endospore-forming, Gram-positive, non-motile, rod-shaped *Bacillus anthracic* [1]. And has an almost worldwide distribution. Once introduced into an area, anthrax is maintained in the environment by resistant spores that may remain dormant in the soil for many years. Spores are more likely to persist in areas with ideal soil conditions (alkaline, calcium rich) [2]. Anthrax is a re-emerging infection and consequently, endemic areas may provide additional sources of alternative strains of *B. anthracic* for bioterrorism placing global security at renewed risk. All mammals appear to be susceptible to anthrax to some degree, but ruminants such as cattle, sheep and goats are the most susceptible and commonly affected, followed by horses and then swine. There are three forms of anthrax, these being cutaneous, gastrointestinal and inhalational [3]. Inhalational anthrax resulting from exposure to aerosolized *B. anthracic* is the most deadly form of the disease in humans with a mortality rate approaching100% even with appropriate treatment especially if initiated after clinical symptoms have already progressed. It has been used widely in biological warfare including bioterrorism for decades [4]. The resilience of the *B. anthracic* endospore combined with the potentially lethal nature of the disease and the efficiency with which it infects via an aerosol route make it an ideal biological weapon and consequently issues about anthrax have drawn considerable attention in recent years [5]. The incidences of the anthrax-laced letters that

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were sent in the wake of the 11 September 2001 terrorist attacks on the World Trade Center and the Pentagon that caused 5 American deaths have shown renewed potential use of this bacterium as a biological weapon [6].

In an emergency animal disease (EAD) response, there are a number of methods for disposing of carcasses and other items. The most common disposal methods for carcasses and other materials are burial (either at an existing licensed landfill site or in a specially designed and excavated pit), cremation (either on a pyre or in an incinerator or pit burner), rendering, composting and disposal by alkaline hydrolysis. The methods used to dispose of animals, animal products and associated wastes during an EAD outbreak must be science based. Much time can be saved by prior consultation with appropriate authorities, such as environment protection agencies, to locate appropriate potential sites and to determine the basic minimum requirements for the various options available. The 2013 National Capacity Profile for Carcass Disposal project, overseen by the National Biosecurity Committee, showed that all jurisdictions are actively engaged at a whole-of-government level in disposal preparedness. A number of predisposal processing treatments may need to be considered before the disposal method is used. These include freezing and storage, grinding, sterilization (using disinfectants, heat, barriers) and carcass breakdown [5, 6].

Etiology and Ecology of Anthrax: Anthrax is a bacterial disease caused by the spore forming Bacillus anthracic, a Gram-positive, rod shaped bacterium and the only obligate pathogen in the large genus Bacillus [7].

Epidemiology of Anthrax

Biology of *Bacillus anthracic*: *Bacillus anthracic* is anaerobic, gram-positive, non-motile rod. The bacterium measures 1-1.5mm by 3-10mm. Spore formation occurs centrally or pericentrally and causes no bacterial. Spore formation occurs when nutrients are depleted as happens after host death and exposure to air [7]. *B. anthracic* spores are highly resistant to various environmental changes and can survive indefinitely in soil, air, water and vegetation despite extreme heat or cold, desiccation, chemical treatment or ultraviolet exposure. The highly resistant nature of the spore aids in the persistence of the disease in an area. The bacteria grow readily on all conventional microbiology media at 37°C including sheep blood agar and produce non-hemolytic colonies. Colony appearance on agar is typically 4-5mm rough, white colonies with a characteristic comma shape or tail often referred to as "curly-hair" or “medusa head” colonies [8]. *B. anthracic* occurs singly or impairs in tissue and forms long chains in culture giving a classic "boxcar" appearance [7]. *B. anthracic* is part of the *B. cereus* group of bacilli which includes *B. cereus*, *B. thuringiensis* and *B. mycoides.* Anthrax can be differentiated from other members of the group by several methods. Members of the *B. cereus* groups, except *B. anthracic*, are resistant to penicillin due to the presence of a chromosomal encoded betalactamase enzyme. Other characteristics, which differentiate *B. anthracic* from other *Bacillus species*, are the absence of hemolysis, lack of motility and the presence of an anti-phagocytic capsule consisting of D-glutamic acid. It is theorized that spore germination may be triggered inside the macrophage by host-specific signals such as elevated temperature (> 37°C) and CO_2 concentrations (> 5%) and presence of serum components [8].

Factors Associated with Anthrax Epidemics

Soil Type: The moisture content of soil mainly depends on its type, which may influence in the long time persistency of anthrax spore. Loamy type soil increases anthrax outbreak, soil with moisture range between 6.31-28.37% might be favorable for the viability of *B. anthracic* spore in the soil. The soil pH controls the availability of many nutrients in soil. Alkaline soil containing high nitrogen, calcium (Ca^{++}) and organic matter gives favorable condition to the spore for growing in soil. The soil that is rich in organic matter and calcium promotes the survival of resilient B. anthracic. In addition to adequate Ca^{++}, nitrogen and organic matter in the soil, anthrax outbreak requires favorable seasonal changes such as warm weather followed by heavy rain. The bacteria are thought to undergo a vegetative cycle when the above conditions are fulfilled. By this process, anthrax spores could be concentrated in top soil to cause disease in grazing animals, occurring outbreak separated by disease-free intervals [9].

Nature of the Spore: The bacterial spore is a resting form of the organism. the essential parts of the vegetative cell (the vegetative cell genome precursors in a dehydrated state, small acid-soluble proteins (SASPs) that bind to and protect the DNA as well as acting as amino acid sources during germination and specific organic acids which act as energy sources for germination) lie in a “core” surrounded by a thick protective cortex, spore coats and a protein aceousexosporium. The inner layer of the cortex is the precursor of the vegetative cell wall and the receptor for germinants lies in the interface between the cortex and spore coats. The pro-enzyme of a germination-specifi cortex-lytic enzyme (GSLe) is activated when the germinant attaches to the receptor. The active GSLe allows uptake of water by the cortex for initiation of germination. The cortex also plays a role in the resistance of the spore to heat. The spore coats, which represent approximately 50% of the volume of the spore, supply the first line of resistance to chemicals and physical disruption. The function of the loose-fitting exosporium is not known but may have a role in adhesion to surfaces. Spores are markedly resistant to biological extremes of heat, cold, pH, desiccation, chemicals (and thus to disinfection), irradiation and other such adverse conditions. The organism can persist in the spore state for long periods of time waiting the moment when conditions favor germination and multiplication. The ability of anthrax spores to persist in the soil and other environments for decades is legendary [10].

Anthrax Toxin: The toxigenic properties of *B. anthracic* were not recognized until 1954. Prior to that time, because of the tremendous number of anthrax bacilli observed in the blood of animals dying of the disease (109 bacteria per ml), it was assumed that death was due to blockage of the capillaries, popularly known as the "log-jam" theory. But experimentally it was shown that only about 3 x 106 cells per ml is necessary to cause death of the animal. Furthermore, the cell-free plasma of animals dying of anthrax infection contained a toxin which causes symptoms of anthrax when injected into normal guinea pigs. These observations left little doubt that a diffusible exotoxin plays a major role in the pathogenesis of anthrax [7].

One component of the anthrax toxin has a lethal mode of the action that is not entirely understood at this time. Death is apparently due to oxygen depletion, secondary shock, increased vascular permeability, respiratory failure and cardiac failure. Death from anthrax in humans or animals frequently occurs suddenly and unexpectedly. The level of the lethal toxin in the circulation increases rapidly quite late in the disease and it closely parallels the concentration of organisms in the blood. The bacilli secrete three proteins, protective antigen (PA), lethal factor (LF) and edema factor (EF) and these proteins combine to form the lethal (PA plus LF) and edema (PA plus EF) toxins. PA is the common cell binding component and is required for toxin activity [11].

Factors to Be Considered During Choice of Disposal Methods: A variety of factors will affect the decision-making process and the disposal method(s) recommended. The relative importance of each factor will depend on the local situation. The epidemiology of the disease may mean that some disposal methods are not appropriate. Most importantly, the disposal methods chosen must prevent the dissemination of infection. They must also gain international acceptance from a disease control perspective; be generally acceptable to the local and broader community; meet legislative requirements and industry standards; and take into account community and operator safety, the local environment and transport availability [12]. Cost-effectiveness and speed of implementation are also fundamental to the choice of disposal method. Long-term factors, such as the maintenance, monitoring and eventual rehabilitation of disposal sites, must be considered. Emergency animal disease (EAD) outbreaks may necessitate the creation of large mono-fill waste disposal facilities that are far harder to manage in the medium and long term than mixed general wastes in terms of odour, gas and leachate generation. The statutory requirements of local, state/territory and national authorities must be met. The industry involved and those associated with it need to be reassured that the disposal process is secure. The public needs to know that food, drinking water and the environment remain safe from contamination. Timely availability of resources, such as information, materials, expertise and equipment, must also be considered [12].

Effective Carcass Disposal: Avoid performing an autopsy when anthrax is being suspected. Ensure that all body openings anus, mouth, ears and nose are plugged with an absorbent material like non-perforated paper towel and cloths to prevent leakage of exudates. To prevent scavenging and spreading of spores by insects, birds, or mammals, once all body openings are plugged and the head securely covered, cover the carcass with a tarp, heavy plastic, or other appropriate material. Weigh down the edges of the covering to prevent removal by wind or predators. Under specific environmental conditions for instance, prolonged rain; carcass inaccessibility (e.g. standing water, heavy bush); or logistical problems (e.g. lack of proper equipment, manpower etc.) the prompt disposal of infected carcasses may be impossible. In these circumstances, to prevent or minimize anthrax environmental contamination, assess the situation

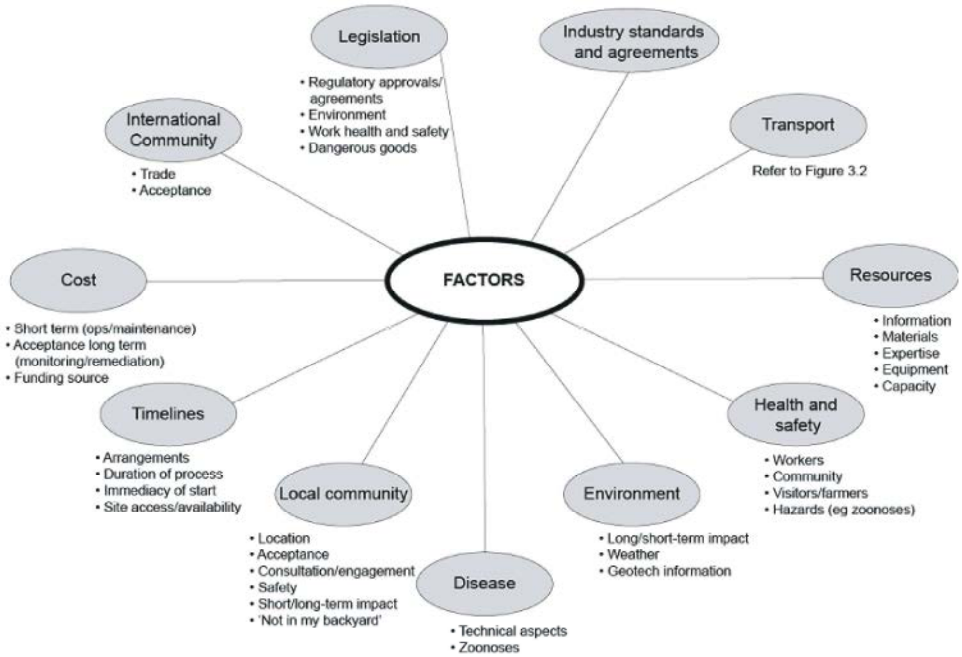

Fig. 1: Summary of factors affecting disposal methods [12]

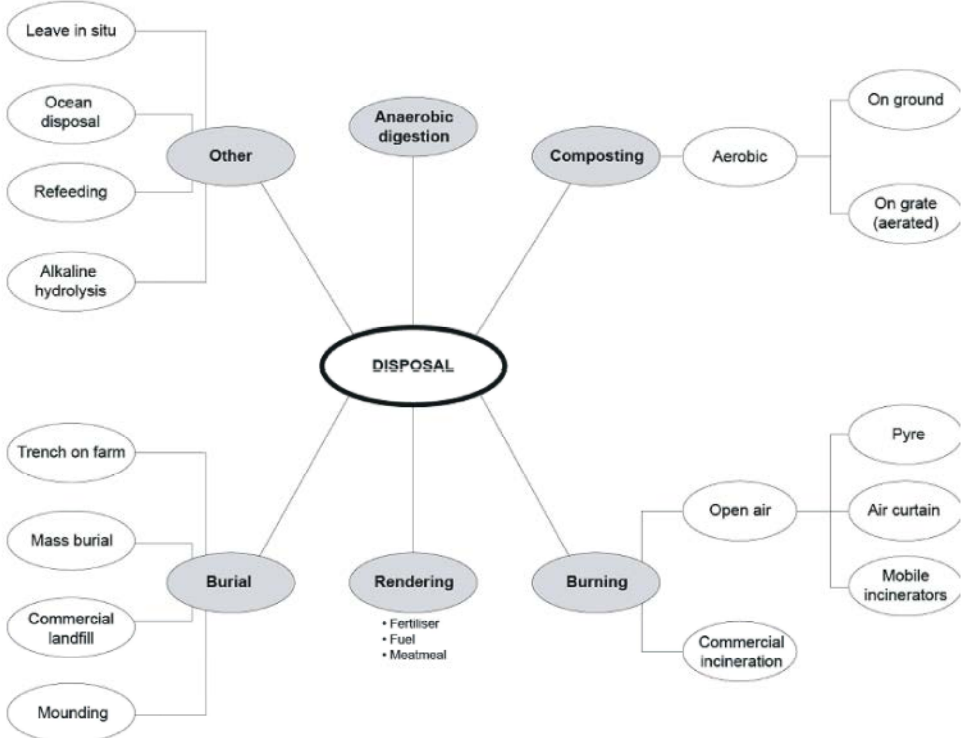

Fig. 2: Disposal methods [13]

to decide on an appropriate course of action within a realistic timeline for disposal. Cover the carcass and the surrounding area with disinfectants, such as 10% formalin or 5% solution of sodium hydroxide [13]. The goal is to destroy as many spores as possible, thereby decreasing environmental anthrax contamination. A complete burn should be achieved. The carcass should be completely reduced to ash. An effective burn primarily leaves ash and bits of bone, with minimal fly attraction to the site. If incineration is not feasible or cannot take place immediately, deep burial may be a viable option. The location of burial sites, using GPS or other mapping methods, should be recorded by those involved and kept indefinitely. Be aware that burial permits may be required by municipal or provincial governing authorities and it should be away from water bodies. Ensure that the pit is 6–8 feet (2 m) deep – the bottom of which should be well above the water table (minimum 3 feet (0.9 m). Clay soil is preferable, whereas, sand or gravel should be avoided. Have a minimum of 3.2 feet (1m) of clay at the base of the pit and cover the carcass with a minimum of 3.2 feet (1m) of clay and topsoil to prevent access by scavengers. Use 10% formalin or 5% lye solution (sodium hydroxide) to decontaminate the carcass and the soil [13].

Disposing Methods of Died Animal Due to Anthrax Diseased

Burial: Periodic reports of viable anthrax spores at burial sites of animals that died many years previously and incidents and outbreaks in animals associated with such sites, 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, presumably 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 livestock cases as shown by [14]. Further disadvantages of 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. In Wood Buffalo national Park, Canada, where burials of bison that had died of anthrax were carried out in the 1960s, the raised burial mounds became excavation sites for foxes and wolves building their dens and nesting sites for ants. Africa as an example, it is hard to stop dogs from digging up buried anthrax (cattle) carcasses the origins of the common recommendation to bury with lime appear to be lost to history. In theory heat would be generated on contact with body fluids and that would thereby be expected to hasten the decomposition of the carcass. It may have been applied originally to deny corpses (human or animal) to relatives/owners after death where that was off cially needed [15]. it is now uncertain just what lime does to buried carcasses, whether it accelerates their disintegration or actually preserves them believed that at least as far as lime treatment of tannery effluent is concerned, this probably followed the use of lime as a cheap chemical for disinfecting sewage sludge however, they showed that at 20 kg/m^3, lime failed to inactivate anthrax spores in sewage sludge. in raising the pH of the soil, addition of lime when burying anthrax carcasses may actually be counterproductive to minimizing long-term spore contamination in summary, burial should be discourage in favor of incineration [5].

Incineration (Cremation): Cremation refers to the burning of carcasses. Sufficient air flow is required to achieve the hottest fire and efficient combustion. Large amounts of fuel are required to fully burn a carcass which may be an issue as can fire restrictions in summer. Before cremating carcasses on-site, your local fire brigade should be contacted in regards to local weather conditions, required permits and possible fire bans in your area. Guidelines on incineration procedures are given in ideally, the soil surrounding and under the carcass, particularly around the nasal and anal regions, should be decontaminated and then incinerated with the carcass. Incineration must be carried out with appropriate care to ensure complete burning from beneath. Usually this involves raising the carcass off the ground before the process is started. Mobile commercial incinerators designed to ensure this are available it must be appreciated that spores that have soaked into the soil may survive the incineration process, although isolation of B. anthracic from incineration sites is rare. The an alternative incineration procedure that ensures severe scorching of the soil to several centimeters of depth Comments are occasionally encountered opposing incineration on the basis that anthrax spores may survive the fire and become aerosolized in the updraft [16]. In general, circumstantial evidence does not support the contention that incineration of anthrax carcasses results in the transmission of anthrax in this manner and the rapid dilution effect on any spores that may become airborne in viable state reduce the chances of these causing an infection to next to nil. the generally high infectious doses for anthrax by routes other than through a lesion even in the more susceptible species supports this contention nevertheless, suspicion that airborne transmission from carcass incineration occurs has arisen. Global effects of virulence gene regulators in a *B. anthracic* strain with both virulence plasmids. Infection and Immunity, the outbreak developed by spreading from the initial focus in a south-westerly direction over a distance of 270 km (40–50 km wide) they considered the distances involved too great for the spread to be accounted for in terms of insect transmission and believed it to result from windborne spores emanating from incineration sites in support of this possibility, they demonstrated that *B. anthracic* could be isolated in low numbers from cotton wool held in the smoke above three carcasses and from the face mask of one of the veterinary officers assisting in the incinerations. Blenkharn and Oakland [17] were able to isolate Gram-positive bacteria, predominantly Bacillus species, from the base of the exhaust stack of a hospital waste incinerator with design-specified operating temperatures of 800°C and 1000°C in the primary and secondary chambers respectively, thereby demonstrating that there is no room for complacency. However, numbers were very low, averaging 56 cfu per cubic meter (range 0–400 cfu per cubic meter, i.e. < 1 cfu per liter) and would be subject to rapid further dilution on leaving the chimney. A badly constructed pyre producing smoke with little or no ?ame might result in a higher survival rate of organisms collected by the updraft. An additional consideration is that anthrax organisms in an unopened carcass are in the vegetative form and are readily susceptible to heat and other adverse conditions [16]. The spores will be confined to where the blood has been shed through the body orifices and will mostly be in the soil beneath these points. Relatively few spore forms, therefore, will enter the fire and updraft; vegetative forms will almost certainly not survive. If concern persists, consideration might be given to pretreating the carcass and associated contaminated soil with 10% formalin a few hours before incineration to minimize the number of viable spores present [18].

Rendering: Rendering is essentially a cooking process that results in sterilization of raw materials of animal origin such that parts of carcasses 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) the rendering procedure involves correct performance at each of three stages: collection, transport and treatment of the carcass [19]. These should be supervised by veterinary authorities the carcass should be bagged and the bag, collection machinery, materials and tools and the carcass site itself appropriately decontaminated and disinfected The rendering plant must be properly divided into "dirty" and "clean" areas, which must not be connected via a common drain to avoid possible cross-contamination by back-flow the dirty side must be suitably equipped for disinfection of the transport vehicles and other equipment involved waste water from the dirty side must be collected and treated by heat or chemicals (preferably heat) to destroy the spores. Before the heat treatment, carcasses should be broken down into pieces not larger than 10 cm^3. In the case of anthrax carcasses, this should be done with very careful attention to hygiene during the process, with the necessary disinfection and decontamination of the rendering premises, tools, clothing, waste run-off, etc. Controlled heat treatment is then carried out with temperature, pressure and time of sterilization recorded [20].

Handling the Premises: The site where the animal died should be disinfected with 5% formaldehyde or peracetic acid to minimize anthrax environmental contamination. Affected premises are to be quarantined for at least 20 days after the disposal of the carcass and disinfection [13]. The list of recommended disinfectants includes: 10% formaldehyde, 4% glutaraldehyde, 3% hydrogen peroxide and 1% peracetic acid. Hydrogen peroxide and peracetic acid will not work in the presence of blood. Soil from areas of anthrax contamination should be removed for incineration or soaked with 5% formaldehyde. Contaminated materials should be incinerated and non-disposable items should be soaked with 4% formaldehyde or 2% glutaraldehyde [21].

CONCLUSION

Although the true worldwide incidence of anthrax is unknown, official reports show that the disease is enzootic in many countries and that sporadic outbreaks are common. Experience shows that countries with inadequate veterinary and public health facilities and areas where it is difficult to implement control programs are the most affected. The repeated occurrence of anthrax in livestock with spillover to humans suggests that improved prevention and control measures are needed to protect both animal and human health. These include: a preventive strategy involving annual vaccination of susceptible livestock animals (usually cattle, sheep and goats) in areas prone to the disease using quality-assured vaccines; an effective surveillance system both in the public health and the veterinary sectors to ensure earliest reporting and investigation of sudden death in livestock and wildlife; prompt disposal of dead animals, bedding and contaminated materials and control of scavengers; increased public awareness and observation of principles of general hygiene, including use of personal protective measures by people who may have contact with diseased or dead animals; enforcement of regulations pertaining to anthrax control including quarantine.

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