

## Methods of Organ Condemnation Infected by Anthrax

<sup>1</sup>Mebrate Getabalew and <sup>2</sup>Derbie Zewdie

<sup>1</sup>Holland Dairy Private Limited Company, Milk Collection Point Quality Coordinator, Debre Zeite, Ethiopia

<sup>2</sup>Ankober Woreda Agricultural and Developmental Office,  
North Showa Zone, Amhara Regional State, Ethiopia

**Abstract:** Anthrax is a seasonal disease typically; an outbreak in an enzootic area follows a prolonged hot dry spell, which in turn was preceded by heavy rains or flooding, or with rain ending a period of drought. However, because conditions and circumstances that predispose to outbreaks vary from location to location, seasonality shows corresponding divergences from this pattern in different locations in the event of a case or outbreak occurring in livestock, control measures consist of correct disposal of the carcass, decontamination of the site and of items used to test and dispose of the carcass and initiation of treatment and/or vaccination of other animals as appropriate. The best disposal method is incineration. Livestock vaccines are available in most countries. Vaccines for humans, in contrast, are not widely available. It is the responsibility of the owner of the premises where the carcass is located to insure that all provincial and municipal regulations that apply to the burial or burning of carcasses are complied with. Wherever possible, contaminated materials should be incinerated or autoclaved at 121°C for 60 minutes. Use of disposable items facilitates this. In the case of contaminated non-disposable items such as clothing, boots, tools, etc., excess dirt should be scraped off and incinerated and the items themselves should be soaked overnight in 10% formalin. Formalin is sold as 37% solution of formaldehyde in water. Always follow manufacturer's label directions to get proper dilution rates. Bleach is a possible alternative if discoloration or corrosion is not of consequence and there is little organic material left on the items after scraping and contaminated materials such as bedding, feedstuffs and manure should be incinerated. Anthrax contaminated feed can safely be fed once two weeks have passed since animals have been vaccinated; however, care should be taken to make sure no dust is produced i.e. do not use a bale shredder in some developing country situations where burial, incineration or rendering is not feasible, the last resort may be to leave the carcass unmoved in situ and ensure that it is inaccessible to other animals, particularly scavengers, or people. So the objective of this review paper is to access methods of organ condemnation infected with the disease Anthrax.

**Key words:** Anthrax • Condemnation • Carcass • Prevention • Burial • Rendering • Incineration

### INTRODUCTION

Anthrax (*Bacillus anthracis*) is a reportable disease found globally. Any case, whether human or animal, domestic or wild, is supposed to be reported to the appropriate veterinary and human health officials to initiate the necessary control measures. Disease control strategy is well established [1] as vaccination is sufficient to stop an outbreak when combined with animal movement and carcass control. However, due to the complexity of the life cycle and the unknowns regarding the bacteria's life in the soil, it is difficult to predict when and where outbreaks will occur. Further, disease control can be unpopular in agricultural areas, as the standard control methods have serious economic implications.

In most countries, the preferred method of disposal of an anthrax carcass is incineration. Controlled heat treatment or "rendering" has been proposed in at least one country of the European Union, but no records were found of this having been done, or of relevant legislative documentation. Where neither of these approaches is possible, for example owing to lack of fuel, burial is the remaining less satisfactory alternative. As discussed in section 3.2, history has many examples of new outbreaks following disturbance of old burial sites. Consideration might be given to treating anthrax carcasses with 10% formalin, leaving them in situ for some days before disposal while natural putrefaction processes within the carcass kill the vegetative anthrax organisms. The formalin would have the action of killing anthrax organisms shed

by the dead animal, preserving the skin so that it retains the anaerobic environment within the putrefying carcass. It may also deter scavengers that would otherwise open up the carcass and thereby increase the contamination and flies that might spread the disease. However, it was the experience in the major outbreak in wildlife in Zimbabwe [2], that formalin treatment of carcasses per se did not deter scavengers or flies, personal communication [3]. The disease is an ancient and virulent zoonotic disease with a complex natural ecology. The bacteria are a close relative to several species of common soil bacteria [4] and Anthracis bacillus also spends much of its life cycle in the ground [5]. This is the part of the life cycle we know least about. Historical research indicates that certain soils are more hospitable to the bacterial spores [6]. There was another outbreak in 2010, which killed a smaller number of wildlife. The most recent outbreak in Uganda was not in the park and it temporarily shut down local beef sales and claimed the lives of both humans (two) and bovines (five) in Sheema district in November [7]. A non-profit that tries to mitigate harmful zoonoses and cross species disease effects near conservation areas [8]. It is the responsibility of the owner of the premises where the carcass is located to insure that all provincial and municipal regulations that apply to the burial or burning of carcasses are complied with.

#### **Anthrax Disease**

**Etiology:** Anthrax is a bacterial disease caused by the spore-forming *Bacillus anthracis*, a Gram-positive, rod shaped bacterium the only obligate pathogen in the large genus *Bacillus* [9].

#### **Transmission as a Result of Trade in Animal Products:**

In economic and public health terms, the importance of the disease lies in its ability to affect large numbers of livestock at one time and to be spread from these to others great distances away. Anthrax carcasses pose a hazard to humans and other animals both in the vicinity and at a distance through their meat, hides, hair, wool or bones hides, hair, wool and bones may be transported long distances for use in industries, feedstuffs or handicrafts [10]. Livestock may acquire the disease continents away from the original infection source through contaminated feedstuffs, or from spores that have reached fields in industrial effluent in countries with advanced agriculture, feedstuffs with contaminated ingredients are traditionally the primary source of infection, especially for dairy cows these ingredients can either be improperly-treated locally-produced meat and bone meals salvaged from moribund stock or, more likely today, infected bones or contaminated meat and bone meal imported from enzootic regions [10]. The spores begin to form 4 to 10 hours after death and the process

of spore formation is complete within 24 to 48 hours. These spores are very hardy and they contaminate the soil, where they can survive for many years. Vegetative cells within an intact carcass will be out competed by other bacteria and die within 2 to 3 days. Ecological factors can influence when anthrax outbreaks occur [7].

#### **Ecological Factors**

**Precipitation:** Rain concentrates spore-containing soil into low-lying areas and surface waters.

The spores are hydrophobic and float from groundwater to surface of soil and they cling to vegetation or roots. Outbreaks occur primarily in hot, dry months following spring rains, which promote pasture growth and grazing. In addition, prolonged spring rains promote large tabanid (biting) fly populations, which may serve as mechanical vectors [11].

**Seasonality:** Anthrax is a hot season disease, as the outbreaks most often occur during hottest summer months or at the end of drought. Animals may gather in low-lying, high-anthrax risk areas to graze on remaining vegetation as spring growth dries up in the heat. Additionally, animals are drawn to new vegetation that occurs following rains at the end of a drought. In both instances, animals may graze down to surface soil or roots. This dry vegetation is abrasive and may cause mucosal trauma to the animals, thus increasing the chances of infection Also, the animals may consume anthrax spores along with the surface soil and roots. Finally, drought conditions may reduce the animal's resistance to infection by increasing stress and causing nutritional changes [12].

**Soil Factors:** Soil alkalinity, high calcium content, moisture and high organic matter content favor spore survival. Calcium preserves spore latency. Spores will disappear from soils with pH less than 6.1 Soil disruption by human activity (loosening and excavating soil from prior anthrax graves or contaminated sites) or natural events causes spores to surface and promotes increased growth of vegetation, which may be contaminated and attract more animals, resulting in new infections [13].

**Host Susceptibility:** Susceptibility varies by animal species Cattle and sheep are highly susceptible and can have hyper-acute to acute disease, including sudden death without prior signs of illness. Horses have hyper-acute to acute disease with signs for 2 to 3 days before death. Pigs and carnivores are more resistant and may have sub-clinical disease. Scavengers are relatively resistant. The incubation period in livestock is typically 3 to 7 days (range 1 to 14 days). The OIE international trade regulation incubation period is 20 days [14].

**Human Infection:** People become incidental hosts through contact with infected animals or contaminated animal products. They can become ill if they: Get spores in a cut or scrape of the skin, Eat food or drink water that is contaminated with spores, Breathe in spores, Inject spore-contaminated illicit drugs [15]. The type anthrax infection (Cutaneous, gastrointestinal, inhalation or injection) depends on how anthrax enters the body while some types of infection are more severe than others, all forms can cause systemic illness and result in death [16].

**Cutaneous Anthrax:** This form causes a clump of small blisters or lumps that may itch, swell and eventually develop into an ulcer with a black center; the lesion may be painless and is often located on the face, neck and arm, or hands, Incubation period: up to 17 days, but most disease occurs in 1 to 7 days [16]. Exposure butchering an animal that died of anthrax or contact with contaminated animal products such as meat, hide, blood, or hair, scratches or cuts on skin may increase risk; hand washing may decrease risk [17].

**Inhalation Anthrax:** Inhalation of the spores causes fever, chills and fatigue may be accompanied by cough; slightly later, chest discomfort, headache and nausea/vomiting occur finally, shortness of breath and confusion, Incubation period: up to 60 days, but most disease occurs in 1 to 7 days, Exposure: working with spore-contaminated hides in an industrial setting or bioterrorism [18].

**Gastrointestinal Anthrax:** In the GIT, fever/chills, fatigue, nausea/vomiting and abdominal pain are common diarrhea, headache and confusion occur in about a quarter of patients; swelling of neck, sore throat, or painful swallowing suggest pharyngeal disease, Incubation period: up to 16 days, but most disease occurs in 1 to 7 days, Exposure: eating meat from an animal that died of anthrax [19]. Injection anthrax- similar to cutaneous anthrax, but injection anthrax has the potential to spread more rapidly throughout the body, Incubation period: up to 20 days, but most disease occurs in 1 to 7 days, Exposure: injecting spore-contaminated illicit drugs (e.g. heroin) if untreated. Incubation period in people ranges from 1 day to more than 2 months [20].

**Control and Prevention:** The primary actions for animal outbreak management include: Rapid identification and treatment of infected animals, enhanced case surveillance, prophylaxis, vaccination and quarantine, restrict access to suspected sources (feed or pastures), appropriate carcass disposal and disinfection of affected premises and materials [21].

**Carcass Control:** In cases where anthrax is highly suspicious, practitioners will play a role in educating the

client in proper carcass management until laboratory diagnosis is confirmed. In the event the carcass is positive, the Ministry of Agriculture will confirm the carcass have been disposed of in an appropriate manner. However, carcass management should begin prior to the lab results being received. It is expected that when veterinary practitioners are called to examine the carcass of an animal that has died suddenly on pasture, they may have opened the carcass to make a tentative diagnosis. In highly suspicious cases for anthrax, after samples are collected, the producer should be advised how to properly dispose of the carcass to prevent further environmental contamination with spores. Please refer to the document "Information for Producers" for information on disposal [22]. When anthrax is possible but lower on your differential list and a full post-mortem exam is required, conduct the examination in a manner that minimizes environmental contamination and be prepared to control or dispose of the carcass. Place a large piece of heavy duty plastic or 6 mil polyethylene sheeting in front of the carcass and lay out any removed organs, etc. on it. When finished with the exam, roll up the plastic and parts and place inside carcass. Cover the carcass with 6 mil polyethylene sheeting and stake down. Heating and putrefaction under the plastic cover will destroy the vegetative form of bacteria within 48 to 72 hours in warm weather conditions or burn or bury carcass immediately [23]. It is the responsibility of the owner of the premises where the carcass is located to insure that all provincial and municipal regulations that apply to the burial or burning of carcasses are complied with carcasses are first covered with lime, then a tarpaulin and left for 9 months before the bones are collected this is said to stop scavenging and is cheaper than burying [23].

**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 Cote *et al.* [24]. 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 in Africa [25] 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 decomposition of the carcass. It may have been applied originally to deny corpses (human or animal) to relatives/owners after death where that was officially needed [26]. 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<sup>3</sup>, 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 discouraged in favor of incineration [27].

**Incineration:** 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. anthracis* 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 [28]. 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. It described an outbreak of anthrax in the Kimberley district of South Africa affecting goats, cattle, sheep, horses, roan antelope, gemsbok, Laldu and Springbok [29].

Global effects of virulence gene regulators in a *Bacillus anthracis* strain with both virulence plasmids. 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. anthracis* 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 [30] 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 flame 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 [31]. 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 pre-treating the carcass and associated contaminated soil with 10% formalin a few hours before incineration to minimize the number of viable spores present [32].

**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 [33]. 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 Wastewater 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<sup>3</sup>. 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. As well as careful hygiene control on the dirty side of the rendering plant, the level of hygiene being maintained on the clean side should also be monitored at least twice yearly by the veterinary authorities [34].

### CONCLUSIONS

The bacteria that cause anthrax can be found naturally in soil and commonly affect domestic and wild animals around the world. The animals can become infected when they ingest spores from contaminated soil, plants, or water. Spores form when bacilli in blood or body fluids from carcasses are exposed to air, or when carcasses are opened by scavengers thanks to successful national programs, there has been a progressive global reduction in livestock anthrax cases over the past three decades. This has had many beneficial aspects but has also created some problems the latter follow from lack of experience of the disease on the part of younger veterinarians, who may fail to recognize or are slow to diagnose cases. the result of this is that single cases become multiple, sometimes with human exposure, whereas previously it would have been limited to the initial deaths the other problem is public ignorance of the disease, with the consequent sale and slaughter of affected livestock this is a particular problem with sheep, where help is requested only when several too many are dead. Because of the resulting environmental contamination, this turns a trivial problem will have long-lasting repercussions. There is also the perennial problem of owners failing to vaccinate livestock in the years immediately following an outbreak while the environmental risk is still present. It is the responsibility of the owner of the premises where the carcass is located to insure that all provincial and municipal regulations that apply to the burial or burning of carcasses are complied with. Formalin is sold as 37% solution of formaldehyde in water. Always follow manufacturer's label directions to get proper dilution rates. When soil is saturated with water or it is a heavy type soil, complete penetration of formalin may not be possible, it is recommended in these

situations to check effectiveness with additional swabs and cultures. Where it is not feasible to remove and incinerate or chemically decontaminate soil, the alternate is to close or seal off the site, covering with concrete or tarmac or planting with thorny bushes and secured fencing.

### REFERENCES

1. Turnbull, P., 2012. Guidelines for the surveillance and control of anthrax in human and animals. (No. WHO/EMC/ZDI/98.6). World Health Organization. Accessed May 2012.
2. Clegg, S., 2006. Preparedness for anthrax epizootics in wildlife areas. Emerging Infectious Diseases, July 2006 (<http://www.cdc.gov/ncidod/eid/vol12no07/06-0458.htm>).
3. OIE, 2012. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2016, chapter 2.1.1: Anthrax.[http://www.oie.int/fleadmin/Home/eng/Healthstandards/tahm/2.01.01\\_ANTHRAX.pdf](http://www.oie.int/fleadmin/Home/eng/Healthstandards/tahm/2.01.01_ANTHRAX.pdf).
4. Saile, E. and T. Koehler, 2006. Bacillus anthracis multiplication, persistence and genetic exchange in the rhizosphere of grass plants. Applied and Environmental Microbiology, 72(5): 3168-3174.doi: 10.1128/AEM.72.5.3168-3174.2006.
5. Schuch, R. and V. Fischet, 2009. The secret life of the anthrax agent bacillus anthracis: Bacteriophage-mediated ecological adaptations. Plos One, 4(8): e6532.doi: 10.1371/journal.pone.0006532.
6. Dey, R., P. Hoffman and I. Glomski, 2012. Germination and Amplification of Anthrax Spores by Soil-Dwelling Amoebas. Appl. Environ. Microbiol, 78(22): 8075.
7. OIE, B., 2012. Terrestrial animal health code - anthrax.(No. Article 8.1.1).
8. Kalema-Zikusoka, G., 2011. Integrating wildlife health and community public health to promote conservation and sustainable livelihoods. British Veterinary Zoological Society Proceedings.
9. Chabot, D., 2004. Anthrax capsule vaccine protects against experimental infection. Vaccine, 23: 43-47.
10. Meaney-Delman, D., S. Rasmussen, R. Beigi, M. Zotti, Y. Hutchings, W. Bower, T. Treadwell and D. Jamieson, 2013. Prophylaxis and treatment of anthrax in pregnant women.Obstet Gynecol., 2013 Oct, 122(4): 885-900. <http://www.ncbi.nlm.nih.gov/pubmed/24084549>.
11. Dragon, D., 2005. Natural dissemination of Bacillus anthracis spores in northern Canada. Applied and Environmental Microbiology, 71: 1610-1615.
12. Braack, L.E. and V. De Vos, 1990. Feeding habits and flight range of blow-flies (*Chrysomya* spp.) in

- relation to anthrax transmission in the Kruger national Park, South Africa. *Onderstepoort Journal of Veterinary Research*, 57: 141-142
13. Beyer, W., C. Bartling and H. Neubauer, 2003. Status of methods for detecting *Bacillus anthracis* in clinical and environmental samples]. *Tierärztliche Umschau*, 58: 653–662. (in German).
  14. Bradley, J., G. Peacock, S. Krug, W. Bower, A. Cohn, D. Meaney-Delman and A. Pavia, 2014. AAP Committee on Infectious Diseases and Disaster Preparedness Advisory Council. Pediatric anthrax clinical management. *Pediatrics*. May, 133(5): e1411-36.
  15. Baillie, L., 2004. Characterization of the human immune response to anthrax vaccine. Abstracts of the 2004 ASM Biodefense research Meeting, Baltimore, M.d., uSA, 7–10 March 2004, abstract 77.
  16. Kaufmann, A. and A. Dannenberg, 2002. Age as a risk factor for cutaneous human anthrax: evidence from haiti, 1973-1974. *Emerging Infectious Diseases*, 8: 874-875.
  17. Pillai, S., E. Huang, J. Guarnizo, J. Hoyle, S. Katharios Lanwermyer, T. Turski, W. Bower, K. Hendricks and D. Meaney-Delman, 2015. Antimicrobial Treatment for Systemic Anthrax: Analysis of Cases from 1945 to 2014 Identified Through a Systematic Literature Review. *Health Secur.* 2015 Nov Dec; 13(6): 355-64. <http://www.ncbi.nlm.nih.gov/pubmed/26623698>.
  18. Griffith, J., D. Blaney, S. Shadomy, M. Lehman, N. Pesik, S. Tostenson, L. Delaney, R. Tiller, A. De Vries, T. Gomez, M. Sullivan, C. Blackmore, D. Stanek and R. Lynfeld, 2014. Anthrax Investigation Team. Investigation of inhalation anthrax case, United States. *Emerg Infect Dis.* 2014 Feb, 20(2): 280-3. [http://wwwnc.cdc.gov/eid/article/20/2/13-0021\\_article](http://wwwnc.cdc.gov/eid/article/20/2/13-0021_article).
  19. Kanafani, Z., 2003. Endemic gastrointestinal anthrax in 1960s Lebanon: clinical manifestations and surgical findings. *Emerging Infectious Diseases*, 9: 520-524.
  20. Tapa, N., Tenzin, K. Wangdi, T. Dorji, Migma, J. Dorjee, C. Marston and A. Hoffmaster, 2010. Investigation and control of anthrax outbreak at the human animal interface, Bhutan, *Emerg Infect Dis.* 2014 Sep; 20(9): 1524-6. [http://wwwnc.cdc.gov/eid/article/20/9/14-0181\\_article](http://wwwnc.cdc.gov/eid/article/20/9/14-0181_article)
  21. Binkley, C., 2002. *Bacillus anthracis* as an agent of bioterrorism – a review emphasizing surgical treatment. *Annals of Surgery*, 236: 9-16.
  22. Drysdale, M., 2004. Anthrax controls *Bacillus anthracis* capsule synthesis via *acpA* and a newly discovered regulator, *acpB*. *Journal of Bacteriology*, 186: 307-315.
  23. Turnbull, P., A. Frawley and L. Bull, 2006. Heat activation/shock temperatures for *Bacillus anthracis* spores and the issue of spore plate counts versus true numbers of spores. *Journal of Microbiological Methods*, 68: 353-357.
  24. Cote, C., N. Van Rooijen and S. Welkos, 2006. Roles of macrophages and neutrophils in the early host responses to *Bacillus anthracis* spores in a mouse model of infection. *Infection and Immunity*, 74: 469-480
  25. Dull, P., 2002. *Bacillus anthracis* aerosolization associated with a contaminated mail sorting machine. *Emerging Infectious Diseases*, 8: 1044-1047.
  26. Hugh-Jones, M. and V. De Vos, 2004. Anthrax and wildlife. *Revue Scientifique et Technique de l'Office International des Epizooties*, 21: 359-383.
  27. Inglesby, V., 2002. Anthrax as a biological weapon, 2002. Updated recommendations for management. *Journal of the American Medical Association*, 287: 2236-2252.
  28. Bowen, J., 1999. Factors affecting conventional and molecular detection of *Bacillus anthracis* in the environment and the stability of *B. anthracis* identification plasmids pX01 and pX02 *in vitro* [Phd thesis]. Milton Keynes, open university.
  29. Graber, M., A. Michael and D. Solomon, 2005. Helminthes and Helminthiasis of Domestic and Wild Animal in Ethiopia. *Revue Elev. Med. Vet. Pays. Trop.*, 1: 13-95.
  30. Blenkarn, J. and D. Oakland, 1989. Emission of viable bacteria in the exhaust flue gases from a hospital incinerator. *Journal of Hospital Infection*, 14: 73-78.
  31. Bowen, J., 1996. Inactivation of *Bacillus anthracis* vegetative cells and spores by gamma irradiation. *Salisbury Medical Bulletin*, 87S: 70-72.
  32. Keim, P. and K. Smith, 2002. *Bacillus anthracis* evolution and epidemiology. in: Koehler T.M., ed. *Anthrax. Current Topics in Microbiology and Immunology*, 271: 23-32.
  33. Anon, W., 2004. Anthrax spores can germinate, grow and reproduce in soil. university of Michigan health System, press release, 16 February 2004 (<http://www.med.umich.edu/opm/newspage/2004/anthrax.htm>). *And in Infection control today* (<http://www.infectioncontroltoday.com/hotnews/42h1682536.html>).
  34. Bergman, N., 2005. Murine macrophage transcriptional responses to *Bacillus anthracis* infection and intoxication. *Infection and Immunity*, 73: 1069-1080.