

## CASE STUDIES OF ANTHRAX OUTBREAKS

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### Contents

1. Etiology
  2. Distribution
  3. Disease in Animals
  4. Disease in Humans
    - 4.1. Cutaneous Anthrax
    - 4.2. Intestinal Anthrax
    - 4.3. Pulmonary (inhalation) Anthrax
    - 4.4. Other Forms of Anthrax
  5. Vaccines and Treatment
  6. The cases of large occurrence
    - 6.1. An Unusual Outbreak of Anthrax in Australia
    - 6.2. Human Anthrax in Zimbabwe
    - 6.3. The Sverdlovsk Anthrax Outbreak of 1979
    - 6.4. Outbreaks of Anthrax Zoonosis Occurred in Two Regions of France in 1997
  7. Conclusion
- Bibliography

### Summary

Anthrax is a disease primarily affecting herbivorous animals, although all mammals, including humans, are vulnerable. This disease has been known from antiquity and has inflicted great losses in agricultural economies and caused significant disease in humans. From the earliest historical records, up to the advent of antibiotics and the mid-twentieth century development of an effective veterinary vaccine, the disease has represented a major scourge of animals and humans and is one of the foremost worldwide causes of uncontrolled mortality in cattle, sheep, goats, horses, and pigs. The mortality rate in animals affected by anthrax can be very high, especially in herbivores. Anthrax can also occur in humans, albeit infrequently, subsequent to exposure to infected animals or their products. Today, parts of Africa, Asia, southern Europe, and South America have suffered repeated outbreaks, and this disease is still seen sporadically in many countries.

### 1. Etiology

Anthrax is a bacterial disease caused by *Bacillus anthracis*, which was the first bacterium shown to be the cause of a disease. In 1877, Robert Koch grew it in a pure culture, demonstrated its ability to form spores, and produced experimental anthrax by injecting it into animals. *B. anthracis*, is a Gram-positive aerobic or facultatively anaerobic endospore-forming rod-shaped bacterium approximately 4 by 1 μm, although

under the microscope, it frequently appears in chains from two to countless cells in length. In the presence of oxygen and towards the end of its exponential phase of growth, one ellipsoidal spore is formed per cell, generally situated centrally. In a vegetative condition the bacteria is killed by 1 hour of moist heat at a temperature of 55 °C. The spores are resistant to heat, cold, and chemical disinfectants, and they can survive many years in contaminated soil, hide, hair, wool and bristles, bone meal, and dried blood. In the terminal stages of the disease, large numbers of bacilli are excreted in all natural excretions, as well as pathological exudation, and these organisms sporulate and perpetuate infections. In temperate and tropical climates, alkaline and calcareous soils subject to periodic flooding which allow for the formation of small pools containing decaying plant matter provide suitable conditions for growth of the organism and its maintenance in the environment.

Two kinds of virulence factors of *B. anthracis* are known. One is the capsule which consists of a gamma-linked poly-D-glutamic acid, and the other is exotoxins. The capsule appears to protect bacteria from phagocytosis, and therefore plays an essential role during the establishment of an infection. *Bacillus anthracis* produces three distinct antigenic components that are termed anthrax toxin components, edema factor (EF), protective antigen (PA), and lethal factor (LF). Each of the components is a protein with a molecular weight of approximately 83 to 90 kDa. EF is necessary for the edema producing activity of the toxin which is known to be an inherent adenylate cyclase. LF is essential for the lethal effects of the anthrax toxin. None of the three factors exhibits significant biological activity in animals.

However, two or three of the toxin components in combination yield toxic activity. EF + PA produce edema in animals, and have been shown to elevate cyclic AMP to extraordinary levels in susceptible cells. Changes in intracellular cAMP are known to affect changes in membrane permeability and may account for edema. LF and PA combined produces lethal activity. The lethal factor is a Zn<sup>++</sup> dependent protease that induces cytokine production in macrophages and lymphocytes. Recently, its activity has been shown to be due to a protease that cleaves the amino terminus of mitogen-activated protein kinase kinases 1 and 2 (MAPKK1 and MAPKK2), and that this cleavage inactivates MAPKK1 and inhibits the MAPK signal transduction pathway. PA plays the role of introducing EF and LF to the cells. Eighty-three kilodalton PA first binds to a specific cell receptor and is cleaved by a cellular protease, liberating a 20 kDa fragment and leaving the 63-kDa (PA63). LF and EF bind the PA63, and the resulting PA-63-LF or PA63-EF complex is then internalized and its toxic activity is expressed in the cytosol. PA is the principal and essential protective antigen against anthrax.

*Bacillus anthracis* coordinates the expression of its virulence factors in response to a specific environmental signal. Anthrax toxin proteins and the antiphagocytic capsule are produced in response to growth in increased atmospheric CO<sub>2</sub>. This CO<sub>2</sub> signal is thought to be of physiological significance for a pathogen which invades mammalian host tissues. Virulent strains of *Bacillus anthracis* contain two large plasmids: a 96.5 kb and a 184 kb plasmid. Virulence requires the presence of both plasmids, as is evident from the comparison of strains which have lost either of the two plasmids. Examination of the variants obtained by curing proved that in the case of 184 kb plasmid, pXO1 was needed for the production of toxin, and for the 96.5 kb, pXO2 was needed for capsule

formation. The genes coding for PA, LF, and EF are contained on pXO1 and designated as *pag*, *lef*, and *cya*, respectively. The genes required for capsule synthesis, *capB*, *capC*, *capA*, and *dep*, which constitute an operon, are on pXO2. The *B. anthracis* plasmid can be selectively cured. The toxin plasmid is cured by repeated passage at 42-43 °C, and the capsule plasmid is cured by growth in novobiocin. Almost 100 years ago, Louis Pasteur successfully developed an anthrax vaccine. Pasteur attenuated *B. anthracis* by passage of bacteria at 42-43 °C in liquid media, and used this bacterial suspension for the vaccination of sheep.

Therefore, in the present day, Pasteur's attenuation of *B. anthracis* is considered to have been made by curing the toxin plasmid. However, it is now well established that *B. anthracis* strains must produce toxin in order to induce protective immunity. Elimination of the toxin plasmid therefore yields an avirulent strain, but such a strain does not induce immunity. In retrospect, it is now evident that Louis Pasteur's attenuation of the virulence was due to the partial curing of the toxin plasmid. The cultures he used to successfully immunize sheep probably contained a small number of virulent bacteria containing both toxin and capsule plasmid, with a large number of avirulent bacteria.

Since there is no evidence that the capsule or surface antigens play a role in protective immunity, it appears that the efficacy of Pasteur's vaccine depended on a small number of virulent bacteria, which would induce antibodies to PA. Currently, the vaccine commonly used to immunize livestock against anthrax consists of a non-capsulated strain that has been cured of the capsule plasmid, pXO2, but still carries the toxin plasmid, pXO1. This type of strain was demonstrated by Sterne to be very effective in immunizing animals.

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