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Review Article

Historical evolution of human anthrax from occupational disease to potentially global threat as bioweapon

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ABSTRACT

Purpose: Anthrax is caused by *Bacillus anthracis*, which can naturally infect livestock, wildlife and occupationally exposed humans. However, for its resistance due to spore formation, ease of dissemination, persistence in the environment and high virulence, *B. anthracis* has been considered the most serious bioterrorism agent for a long time. During the last century anthrax evolved from limited natural disease to potentially global threat if used as bioweapon. Several factors may mitigate the consequences of an anthrax attack, including 1. the capability to promptly recognize and manage the illness and its public health consequences; 2. the limitation of secondary contamination risk through an appropriate decontamination; and 3. the evolution of genotyping methods (for microbes characterization at high resolution level) that can influence the course and/or focus of investigations, impacting the response of the government to an attack.
Methods: A PubMed search has been done using the key words "bioterrorism anthrax".
Results: Over one thousand papers have been screened and the most significant examined to present a comprehensive literature review in order to discuss the current knowledge and strategies in preparedness for a possible deliberate release of *B. anthracis* spores and to indicate the most current and complete documents in which to deepen.
Conclusions: The comprehensive analysis of the two most relevant unnatural anthrax release events, Sverdlovsk in the former Soviet Union (1979) and the contaminated letters in the USA (2001), shows that inhalational anthrax may easily and cheaply be spread resulting in serious consequences. The damage caused by an anthrax attack can be limited if public health organization, first responders, researchers and investigators will be able to promptly manage anthrax cases and use new technologies for decontamination methods and in forensic microbiology.

Contents

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1. Introduction

Anthrax is a potentially lethal zoonosis, caused by *Bacillus anthracis*, a Gram-positive, rod-shaped, spore-forming and toxigenic bacterium. Upon adverse conditions like heat, cold, desiccation and starvation, free oxygen presence, vegetative cells of *B. anthracis* have the ability to form spores characterized by a complex, multilayered structure. The spores are easily spread and resistant to environmental stress for prolonged periods. In nature, when environmental conditions are permissive and nutrients become available, the spores are triggered into a germination process, which results in the synthesis of vegetative cells. *B. anthracis* ecology is complex and not completely understood. In the established transmission pathway, cattle and other ungulates, in addition to ingest, may even inhale spores from the soil while grazing, leading to germination and subsequent killing of the host (von Terzi et al., 2014; WHO, 2008).

Humans can be exposed to spores from the environment or contaminated animal products, such as meat, wool or skin.

The genome of *B. anthracis* is characterized by a chromosome and two plasmids, pXO1 and pXO2, encoding for the major virulence factors. The lack of one or both of these extra-chromosomal DNA may cause a significant virulence attenuation in certain animal models (Levy et al., 2014; Liu et al., 2012; McGillivray et al., 2009; Wahab et al., 2005). However, this does not explain the variation in virulence observed in studies comparing fully virulent isolates, the different response in diverse hosts or the attenuation of some strains containing both plasmids (Chand et al., 2009; Coker et al., 2003; Harrington et al., 2013).

In terms of genetic evolution, *B. anthracis* is considered a relatively new organism emerged some thousands of years ago from its close relative *Bacillus cereus* group (Pilo and Frey, 2011). The chromosome of *B. anthracis* is essentially identical to that of *B. cereus*, while the pathogen acquired additional plasmid-borne virulence factors (Read et al., 2003). However, some *B. cereus* strains carrying pXO1 and a second, capsule-encoding plasmid, not pXO2, were isolated in humans with an anthrax-like disease. This fact indicates the ability for some horizontal transfer to occur in nature among members of *B. cereus* group (Hoffmaster et al., 2004; Oh et al., 2011). The reduction of genomic horizontal transfer, a slow rate of accumulation of genetic variations, and the paucity of large rearrangements within the genome of the bacterium contribute to its highly clonal nature, indicating that isolates across the world derive from a common ancestor. Because of genomic characteristic, only molecular techniques with high discrimination power have proved effective in differentiating strains (Beyer et al., 2012; Ciammaruconi et al., 2008; Derzelle and Thierry, 2013; Hoffmaster et al., 2002; Keim et al., 2000; Keim et al., 2004, 2009; Le Flèche et al., 2001; Lista et al., 2006). In particular, it is possible to differentiate *B. anthracis* strains into distinct lineages (A, B, C, D) and sub-lineages (Aß or E as defined by Lista et al., 2006, A1a, A1b, A2, A3a, A3b, A4, B1, B2) (Pilo and Frey, 2011).

Due to its resistance, easy transportability, and high virulence *B. anthracis* has been considered ideal as biological weapon for a long time (Christopher et al., 1997; Cole, 2005; Turnbull and Shadomy, 2011). During the last century, the anthrax-associated danger evolved, therefore, from limited occupational disease to potentially global threat of bioaggression with high consequence in terms of significant morbidity, mortality and socio-economic disruption. After the 2001 episode of the anthrax-contaminated letters, a deliberate attack with an aerosolized biological agent should no more be considered impossible. The consequences of this event are dependent on the organization and management of biothreat response protocols, involving specialized personnel (including clinicians, laboratory scientists, emergency responders, etc.) that may take advantage of periodical updating on the topic (CDC, 2000; Khan, 2011).

A search in PubMed has been done on the basis of the key words "bioterrorism anthrax" in order to summarize evolution, pathogenesis, diagnosis and management of natural and intentionally released anthrax. Nearly twelve hundred papers have been examined to prepare the current review.

2. The history of anthrax between natural disease and deliberate release scenarios

2.1. The history of anthrax as natural disease

Anthrax has a long history that it is thought to have originated in Egypt and Mesopotamia. It could be possible that in Moses time anthrax had caused the fifth of the 10 plagues affecting horses, cattle, sheep, camels and oxen. Ancient Greece and Rome were also well acquainted with anthrax, as referred by many scholars, whereas in America (Louisiana) anthrax was introduced in the early 18th century during the French settlement (Turnbull and Shadomy, 2011). The first clinical descriptions of cutaneous anthrax were given by Maret (1752) and Fournier (1769) (cited in Turnbull and Shadomy, 2011 or alternatively Schwartz, 2009). During the 1800s, doctors saw cases of anthrax and noticed that a link between the disease and the animal hair industry could be established, but *B. anthracis* had not yet been discovered. The pathology became known as "woolsorter's disease" and, by the middle of the century, was associated with the presence of rod-shaped bodies that were seen in the blood of infected animals. These bodies were later identified as bacteria and given the name of *B. anthracis*. In 1877, Robert Koch isolated and grew the organism in pure culture and injected it to animals to study its life cycle. In 1881, Louis Pasteur prepared the first vaccine (live, heat-attenuated, non-toxigenic, encapsulated) for anthrax (Pasteur et al., 1881). The Pasteur's vaccine was used for the immunization of domestic animals for approximately 50 years (Baillie, 2009) and, in the thirties of the last century, was largely replaced by the more effective and stable Sterne vaccine, successfully developed by Max Sterne (in thirties) (Turnbull and Shadomy, 2011). This vaccine, based on living anthrax spores of a non-encapsulated, toxigenic, avirulent strain derived from a case of bovine anthrax, has been, and is still, successfully and extensively used in animals (Artenstein and Opal, 2012; Baillie, 2009; Sterne, 1939). In Russia and China, instead, living spore vaccines (STI-1 [Sanitary Technical Institute] and A16R, respectively), obtained from attenuated strains similar to Sterne, are even used in humans (Grabenstein, 2008). Atypical veterinary vaccine strains (Pasteur n. 1, n. 2-H and n. 2-17JB; strains A and B used in Argentina) containing both plasmids, were described by Uchida et al. (1985) and Cataldi et al. (2000). In Italy, the attenuated live spore Carbosap vaccine, toxigenic and encapsulated, was used to immunize cattle and sheep until 2006, when the Italian government established the mandatory use of Sterne vaccine. Carbosap, although equipped with both plasmids, is less virulent than the wild-type strains (Adone et al., 2002; Chiocco and Sobrero, 1985; Fasanella et al., 2001; La Rosa et al., 2006). The reason of its attenuation is still unknown but whole-sequencing showed large chromosomal deletions (Harrington et al., 2013; Gentile et al., unpublished data).

The availability of veterinary vaccines resulted in the effective anthrax control in many countries with considerable reduction, sometimes complete elimination, of the disease in animals and humans, who generally acquire it from livestock (Turnbull, 1991). Natural animal anthrax remains common in agricultural regions in North and Latin America, sub-Saharan Africa, Central and South Asia, and Southern and Eastern Europe (Pile et al., 1998; WHO, 2008; Hicks et al., 2012). The human cases are strictly linked to animal epidemiology: approximately, 1 human cutaneous case per 10 livestock carcasses butchered in Northern Europe (and countries with similar epidemiological situation) and 10 cutaneous/enteric cases per 1 single carcass in Africa, India and the Southern Russian Federation (WHO, 2008). The cases are often concentrated within specific areas associated with agriculture and in close proximity to urban centers, where infected livestock or meat are imported and usually sold at informal meat markets (Kracalik et al., 2014). Molecular and epidemiological data show that the *B. anthracis* A lineage is globally distributed and forms the basis for