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The Secret Life of *Bacillus anthracis*

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Bacillus anthracis hemliga liv

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SUMMARY

Bacillus anthracis is the causative agent of anthrax, a serious and globally distributed zoonosis affecting a wide range of wild and domestic animals, invariably also humans. However, although known to humans since biblical times, much remains to be elucidated concerning the ecology and transmission of this bacterium. Of particular interest is the *Bacillus anthracis* spore, the uptake of which is the predominant way to contract anthrax and which is legendary for its resilience in the environment and thus crucial for persistence and spread of the disease. Hence, the aim of this study is to review the natural transmission of *Bacillus anthracis* and investigate potential means by which soil persisting *Bacillus anthracis* spores reach concentrations sufficient to infect susceptible hosts. When reviewing the literature, three different theories can be distinguished. Firstly, "the incubator area" hypothesis suggests that favourable soil factors, possibly in association with amoebas, may constitute an environment supporting repeated spore-bacterium-spore cycling, thus increasing the local amount of spores. Secondly, water runoff from heavy rains or flooding has been proposed to collect spores and dispose them in closely restricted sites, thus creating "concentrator areas" with locally high amounts of spores. Lastly, the outermost layer of the spore, the exosporium, is proposed to tie spores to the environment where they were first shed and hence maintain infectious spore concentrations at a particular site. Considering that these theories all have their agreements and disagreements with the ecology and epidemiology of anthrax, it is reasonable to assume that all three exert an impact on spore concentrations, possibly at different degrees at various sites and regions. Howsoever, the ecology of *Bacillus anthracis* requires further research to fully understand the mechanism responsible for transmission and spread of anthrax. Only then can efficient methods for eradication of the disease from contaminated fields, and hence reducing the risk of future epidemics, be developed.

SAMMANFATTNING

Bacillus anthracis är en zoonotisk bakterie som orsakar den allvarliga och globalt förekommande sjukdomen anthrax, eller mjältbrand. Trots att både agens och sjukdom är välbekanta sedan antikens dagar och har varit i fokus för gedigen forskning i över 150 år, kvarstår häpnadsväckande många frågetecken. Av särskilt intresse är *Bacillus anthracis* alternerande livscykel mellan vegetativ, replikerande cell och metaboliskt vilande, men fortfarande infektiös, spor. Då sporerna har en närmast legendarisk motståndskraft mot yttre omgivningsfaktorer kan de kvarstå i miljön under mycket lång tid, och således upprätthålla ett infektiöst fokus av *Bacillus anthracis* i jordmånen, från vilket nya mottagliga individer kan infekteras. Då anthrax är en sjukdom som huvudsakligen smittar via sporer och har en hög infektionsdos är syftet med denna litteraturöversikt följaktligen att utröna hur infektiösa nivåer av sporer bildas och bibehållas i omgivningen, samt undersöka möjliga spridningsvägar för nämnda sporer. I litteraturen framträder tre huvudsakliga teorier angående hur sporer koncentreras: För det första, i den så kallade ”incubator area”-hypotesen föreslås *Bacillus anthracis* sporer uppnå höga antal genom att självständigt, eller möjligtvis via amöbor, germinera, replikera och re-sporulera i miljön, dvs. utanför ett värddjur, under förutsättning att yttre faktorer är gynnsamma. Ett ytterligare alternativ förs fram i ”concentrator area”-teorin där *Bacillus anthracis* sporer tros följa med avrinningsvatten efter skyfall eller översvämningar för att ackumuleras i låglänta områden där höga lokala sporkoncentrationer således upprättas. Slutligen framförs möjligheten att *Bacillus anthracis* sporernas yttersta hölje, exosporium, binder sporerna till jordpartiklar och följaktligen kvarhålls de på det ställe där de först hamnar efter bildning. Under beaktande att alla tre teorier uppvisar både enigheter och motstridigheter med *Bacillus anthracis* epidemiologi och ekologi, är det rimligt att anta att de alla, var och en på sitt sätt, bidrar till att etablera och bibehålla infektiösa spormängder, troligtvis av varierande grad i olika miljöer och regioner. Dock kvarstår mycket forskning för att fullt kunna kartlägga *Bacillus anthracis* komplexa ekologi och därmed möjliggöra utvecklingen av effektiva metoder för sanering av sporkontaminerade områden och således förebygga framtida anthrax epidemier.

INTRODUCTION

Being the archetype zoonosis, anthrax affects a wide range of species, invariably also humans, although herbivores is the most susceptible group. This serious and often fatal disease is caused by *Bacillus anthracis*, a bacterium globally distributed and endemic in many parts of the world. Although a subject of study for some 150 years, anthrax still confounds researchers and many questions remain unanswered. Notably, the lack of a definable source of infection for the index case during an outbreak evokes queries regarding the fate of *Bacillus anthracis* in the environment and transmission of the disease.

The aim of this review is to study the microbiology and ecology of the infectious agent *Bacillus anthracis*, and its different modes of transmission, focusing on how spores persist in the environment to reach a number sufficient for causing infection in a susceptible host.

MATERIALS AND METHODS

Literature search for this review was performed scanning the database Web of Knowledge and PubMed using the search terms (anthrax OR *Bacillus anthracis*) AND (soil OR water OR ecology) AND (transmission OR spread) AND (sporulation OR spore). Furthermore, lists of references in review articles were used as a supplement to find further articles of relevance.

LITERATURE OVERVIEW

The infectious agent *Bacillus anthracis*

Historical overview

Anthrax is a disease described long since with the first reports appearing already in the Bible where it is featured as the fifth and sixth plagues ravaging the livestock in Egypt. During antiquity anthrax was well known to man and inquisitively investigated, resulting in anthrax being named by the founder of western medicine, Hippocrates, for the black skin lesions and darkly coloured blood observed in infected animals (anthrax being Greek for "coal") (Guichard *et al.*, 2011). The continued study of anthrax yielded several eventful episodes of experimental medicine with the discovery of *Bacillus anthracis* as the causative agent of anthrax holding a much prominent place in medical history. For the first time could an infections disease be ascribed a specific pathogen, as demonstrated by Robert Koch, and later reinforced by Luis Pasteur, in his 1876 paper. Koch showed the presence of rod-like microorganisms in blood and tissue of diseased animals; the formation of spores during starvation; the return of these spores to viable bacilli in a favourable environment; and, last but not least, the ability of spores to be cultured and infective to further animals. From his work on anthrax Koch formulated his three famous postulates for the identification of a specific agent responsible for an infectious disease. In align with Koch´s observation that *Bacillus anthracis* yields spores, Pasteur demonstrated the presence of such spores in fields "cursed with anthrax", i.e. pastures in which grazing stock invariably contracted the disease. (Schwarts, 2009). Albeit great advances in understanding the ecology of *Bacillus anthracis*, anthrax continued to cause substantial losses of European livestock and human life throughout the 19th and 20th century (Dragon & Rennie, 1995). Not until the introduction of

Sterne´s livestock vaccine, in combination with antibiotics and different control programmes, was the disease substantially reduced between 1940-1960 (Beyer & Turnbull, 2009). However, although outbreaks are more or less infrequently occurring today, especially in developed countries, anthrax is still of relevance and in order to control outbreaks it is of major importance to gain further understanding of the ecology of *Bacillus anthracis* (Dragon & Rennie, 1995).

Microbiology of the bacterium and the spore

Bacillus anthracis, the causative agent of anthrax, is a large, Gram-positive, aerobic, rod-shaped, zoonotic bacterium and the only obligate pathogen belonging to the family *Bacilliacea*. Further characteristics include being capsulated and, in similarity with other members of the *Bacillus* genus, capable of spore-formation (Fasanella *et al.*, 2010). Metabolically inactive endospores are formed within vegetative *Bacillus* cells in response to nutrient deprivation. Each bacterial cell yields one endospore and after completion of sporogenesis the cell undergoes lysis releasing the emergent spore. Hence, the bacterial spore is a dormant form of the vegetative bacteria and, as such, the *Bacillus* remains protected from disadvantageous environments until conditions are favourable for growth. Spores of *Bacillus anthracis* are renowned for being notably resistant to extremes in temperature, pH, irradiation, chemicals and desiccation. Consequently, it can persist infectious in the environment for decades. As an example of its legendary resilience, in the Kruger National Park, South Africa, *Bacilllus anthracis* was found viable in bones estimated to be around 200 years old (Dragon & Rennie, 1995).

Being both resting and resistant, the spore nevertheless interacts with its environment. Most communication with the surroundings is made through the outermost layers of the spore, namely the exosporium and the spore coat. These layers provide the spore with surface hydrophobicity and protect the inside of the spore, which consists of a thick cortex, a membrane and, innermost, a core holding the bacterial genome. Furthermore, the spore has low water content, a feature yielding it a high buoyant density as well as resistance towards heat and UV-radiation, which would otherwise risk damaging the essential core components (Gould, 1977). Due to the spore not being static in its environment, prolonged exposure to environmental factors may deplete the spore of molecules essential for resistance, dormancy and germination, rendering the metabolically inactive spore incapable of remaining resilient. Consequently, the environment in which the spore rests is of great importance for how long the spore may be able remain infectious (Dragon & Rennie, 1995).

The uptake of infectious *Bacillus anthracis* spores from the environment is the predominant way of contracting anthrax. Depending on the portal of entry, anthrax develops as one of three possible forms: cutaneous anthrax occurs when a skin breaches is contaminated with spores, intestinal anthrax develops upon ingestion of spores, whereas, less frequently seen, pulmonary anthrax (also known as Woolsorters´ disease in man) occurs after inhalation of spores. When gaining access to a new host, spores germinate into vegetative bacilli that rapidly propagate and enter the bloodstream, resulting in dissemination to various organs and septicaemia. During replication the bacterium form a tripartite toxin, the action of which is responsible for the outcome of the disease. The severity of anthrax depends also on other factors, including the susceptibility of the host, the infectious dose and the portal of entry (Fasanella *et al.*, 2010).

Epidemiology of *Bacillus anthracis*

Geographical distribution

Over centuries, anthrax has caused uncontrolled mortality in livestock globally, and although successful countermeasures have brought by a reduction in incidence, anthrax is still considered enzootic in most countries of Africa and Asia, several European countries as in a number of areas of North and South America. Additionally, in many other countries it still occurs sporadically and anthrax is thus seen as a global health issue. In Europe and parts of North America sporadic outbreaks are triggered first and foremost from disruption of soil, such as ditching and road works, in areas harbouring forgotten graves of cattle carcasses from past outbreaks (Turnbull, 2008). This has been the case also in Sweden, most recently in Örebro 2011 where about twenty cattle succumbed upon having encountered anthrax spores from a nearby ditch work, resulting in a major geographical mapping of historical anthrax-graves (Elvander & Persson, 2011). In enzootic areas, anthrax shows seasonality in its occurrence, with epidemics being observed most typically after extended periods of hot dry weather following spells of heavy rain or flooding. In agreement with anthrax being transmitted mainly by spores and the ability of *Bacillus anthracis* to sporulate outside the host body only in temperatures ranging from 14°C to 42°C, anthrax enzootic areas are generally located to warmer climates (Fasanella *et al.*, 2010). However, there are regions nearby the Arctic Circle in which the disease has existed or still exists, e.g. infected bison in the Wood Buffalo National Park and the MacKenzie Bison Sanctuary, Canada. A common feature for these areas is their long temperate summers, adequate for sporulation to occur (Turnbull, 2008).

Routes of transmission

Although many discrepancies and questions remain regarding the transmission of *Bacillus anthracis*, there are several long-held beliefs considering routes of infection. Since *Bacillus anthracis* is a soil-dwelling bacterium, it is a well-recognized assumption that animals take up anthrax spores while grazing or browsing; hence it is predominantly a disease of herbivores. Bovines are regarded as particularly susceptible and thought to possess highest risk of infection partly due to their absence of upper incisors, rendering them to use their tongue when ripping up grass and thus facilitating the ingestion of loose, spore-infected, soil (Fasanella *et al.*, 2010). Also, as mentioned above, anthrax spores have a high buoyant density and may hence contaminate water and infect animals drinking from such a water source, as was the case in Örebro 2011 (Larsson, 2011). Additionally, but less frequent, animals may contract anthrax from inhalation of spore-contaminated dust. It is reasonable to believe that such transmission could be seen in animals grazing over dry, dusty contaminated fields where they could well be exposed to aerosolized spores. Although carriage states or latent infection have not been proven for anthrax, experimental data suggest that inhaled spores may remain ungerminated in the lungs of host animals for several months, constituting a type of chronic carriage with a prolonged incubation period (Turnbull, 2008). Considering that anthrax spores can travel anywhere dust travels, long distance transmission might be taken into consideration. However, the significance of this kind of transmission is arguable with few spores being detected downwind of anthrax sites, suggesting a dilution effect of wind that minimizes the risk of spread over large areas (Turnbull, 1998). Inhalation of aerosolized spores has been suggested responsible for epidemics in bison in northern Canada. Stamping and wallowing behaviour, especially among adult rutting bulls, create large dust clouds during hot and dry weather, hence increasing the risk of exposure to aerosolized spores (Dragon & Rennie, 1995).

Furthermore, since anthrax lacks the ability of direct animal-to-animal transmission it needs to kill its host in order to propagate itself. However, when the host dies its inner body environment becomes anaerobic, causing the bacillus to cease its activities. Meantime, in the putrefactive carcass anaerobic bacteria grow rapidly, thus antagonistically outcompete *Bacillus anthracis*, which is destroyed within 48-72 hours (Fasanella *et al.*, 2010). Rarely are carrions left undisturbed in nature for such a long time though, and the opening of the dead animal encourages aerosolization of body fluids and tissues, introducing *Bacillus anthracis* to unfriendly conditions and thus promoting sporulation. Hence, the activity of scavenging animals aid in the spreading of spores. In addition, since carnivores are relatively resistant to infection with *Bacillus anthracis*, they act as carriers dispersing spores in their faeces and on their fur over considerable distances, a feature especially true for avian scavengers such as vultures (Dragon & Rennie, 1995). Many more vectors are suggested to spread spores during anthrax epidemics; most notably flies are believed to play a lead role during explosive outbreaks. When in contact with body fluids of dead animals, flies are likely to pick up spores of *Bacillus anthracis* on their bodies, or ingest them when feeding. Next, they contaminate nearby vegetation by depositing faeces or vomit, thus transmitting bacteria onto the plant to be eaten by grazers (Blackburn, 2010) Moreover, biting flies may act as mechanical vectors spreading anthrax directly from animal to animal during blood meals (Blackburn *et al.*, 2010).

Regardless of transmission route, the age and condition of the animal influences whether or not it contracts anthrax (Turnbull, 2008). In humans, anthrax is primarily seen as the result of exposure to infected animals or products from such. Agriculturally, this occurs following direct contact with anthrax-infected animals or through the handling of carcasses of deceased animals, rendering farmers, veterinarians, slaughterhouse workers and butchers especially at risk (Shadomy & Smith, 2008). Industrially, anthrax is acquired through the exposure of spores from processes involving animal products, such as wool, hides or bones. For this reason, tanneries have long since been associated with the disease (Turnbull, 2008).

To sum up, when an anthrax outbreak is established there are a number of possible ways for the spores of *Bacillus anthracis* to be disseminated and presented to new susceptible hosts (Turnbull, 2008).

What happens in soil stays in soil?

An animal deceased from anthrax sheds *Bacillus anthracis* spores at high concentrations, and hence an occasional case of anthrax constitutes a focus of infection for other animals. However, taken into consideration that anthrax is normally a "high dose" infection requiring a relatively large amount of spores to cause infection (Beyer & Turnbull, 2009), the question arises: How does *Bacillus anthracis*, in soil environment, reach a number sufficient for the index animal to succumb?

Van Ness suggested in his much quoted study that anthrax has the ability to multiply outside an affected host and hence, in suitable soils, is able to uphold a bacterium-spore-bacterium cycle for a long period of time without infecting further animals. Such a suitable soil is found in areas where anthrax outbreaks are frequent and, accordingly, it encompasses land rich in organic matter and calcium, with a pH above 6.0 and an ambient temperature higher than 15.5 °C. Areas corresponding to these characteristic features are thought to allow environmental cycling of *Bacillus anthracis* and Van Ness described them as "incubator areas". Specifically, an "incubator area" commonly arises in depressions in which collect water has devitalized the vegetation and calcium and salts have accumulated from hillside runoff, composing a medium suitable for germination and growth of bacteria. The resulting overall increase in spores will be lethal to a susceptible host and thus sufficient to fuel new outbreaks (Van Ness, 1971). In close proximity to this hypothesis, and supporting the idea of an environmental cycling, is the recent finding that *Bacillus anthracis* is capable of germination and propagation within amoeba phagosomes. In a laboratory setting resembling moist soil vicinities, inoculation of *Acanthamoeba castellanii* with *Bacillus anthracis* spores resulted in bacterial germination, growth and re-sporulation with an outcome of 50-fold more spores at temperatures ranging from 30-37°C. (Dey *et al.*, 2012).

Alternatively, it has been proposed that spores attain high concentrations by means of water run-off to so called "concentrator areas" in lowlands. As mentioned above, anthrax spores show high hydrophobicity and consequently spores can be carried in coalition with clumps of organic matter by rainwater to collect in pools. Considering the high buoyant density of anthrax spores, evaporation of standing water will further increase the amount of spores yielding hot spots with very high concentrations. Indeed, over time, as more and more spores collect to the "concentrator area" a lethal amount will finally be acquired (de Vos, 1990, as reviewed by Turnbull, 2008; Dragon & Rennie, 1995).

Additionally, another possible explanation is that of the exosporium holding spores to their immediate surrounding. The exosporium displays a net negative charge, and hence the spore is able to adhere to humus and limestone particles, which under alkaline soil conditions, displays a net positive charge. In similarity with the above "concentrator" theory, the high buoyant density is of importance, as it will cause the spores to rise to the surface upon local flooding. Next, water evaporation increases the number of spores at a specific area and, consequently, high concentrations of anthrax spore are locally established and accessible to susceptible grazing animals (Hugh-Jones & Blackburn, 2009).

DISCUSSION

Many questions await further elucidation concerning the ecology of *Bacillus anthracis* and its life cycle in the environment. Of particular interest is the issue of a potential germination and replication of spores in a non-animal host surrounding. Thus, the objective of this overview was to compile available literature regarding whether or not such an environmental cycling is possible and, if not, by what other means *Bacillus anthracis* spores may reach a number sufficient to cause lethal infection.

When Van Ness postulated that spores of *Bacillus anthracis* would germinate to vegetative bacteria, multiply and then re-sporulate in soil, he based this controversial and debatable "incubator area" theory on thorough investigations of regions in southern United States that had suffered frequent anthrax outbreaks among livestock. True for all of these areas were their calcareous soils developed from limestone (Van Ness, 1971), a correlation seen also by other researchers when studying epidemics in England and Wales (Dragon & Rennie, 1995). Indeed, in laboratory settings, spores of *Bacillus anthracis* have been made to sporulate under moist, alkaline conditions using sterilized soil. However, this experiment was not replicable with unsterilized soil (Minett and Dhanda, 1941).

Nonetheless, it is tempting to argue that the highly specific local geological conditions seen in the defined "incubator areas" would not so much influence the static spore as it would the more vulnerable vegetative bacilli. A stating from which it might be concluded that the spores, at some point, transform into viable cells, sensitive to and affected by its vicinity (Van Ness, 1971). Subsequent experimental evidence, though, suggest the vegetative bacterium so fragile and specific in nutrient and physiological demands, that it is incapable of surviving outside a host or an artificial laboratory medium. Its requirements include frequent replenishment of nutrients, desirably animal blood or viscera, a preference hard to maintain in nature. Should natural surroundings, at some point, fulfil all prerequisites necessary for germination and growth of spores, *Bacillus anthracis* would anyhow be antagonistically outcompeted by other microflora present in soil. Hence, unless replenished by the occurrence of new anthrax cases, it will ultimately be eliminated (Dragon & Rennie, 1995). The absence of vegetative *Bacillus anthracis* in soil, is further explained by experiments proving the bacterium most unwilling to germinate and replicate in soil but instead effectively sporulating (Hugh-Jones & Blackburn, 2009). Furthermore, it has been reported that when *Bacillus anthracis* is grown outside of a host, the bacterium rapidly becomes avirulent, rendering it incapable of infecting an index animal. In fact, this is a knowing historically used to manufacture several successful anthrax vaccines, such as Sterne´s livestock vaccine.
So, apart from laboratory set-ups, animal hosts must be regarded the most suitable medium for successful propagation of *Bacillus anthracis* (Dragon & Rennie, 1995). Also, it can be argued that the concept of environmental cycling coined by Van Ness, might be contradicted by the fact that his theory rests mainly upon observations from the 1957 epidemic in Oklahoma and Kansas, a period of regular outbreaks caused by anthrax contaminated bone meals. In addition, several of these outbreaks relayed on index cases occurring on higher grounds with non-alkaline soils, i.e. inconsistent with a typical "incubator area". Also, speaking for itself against the incubator theory, these parts of Oklahoma and Kansas have

been free of outbreaks since the 1957 epidemic (Van Ness, 1970; Hugh-Jones & Blackburn, 2009).

Still, not necessarily in agreement with "incubator areas", but supportive of a potential bacterial environmental cycling, is the finding that *Bacillus anthracis* can use *Acanthamoeba castellanii* as a cicerone for soil germination and amplification. In contexture with anthrax outbreaks being anticipated most frequently in warmer climates, it seems reasonable to believe that soil amoebas, thriving in warm weather, might serve as natural hosts facilitating spore multiplication and persistence. If so, the use of amoebas as reservoirs is a feature shared with many other human pathogens, such as *Legionella pneumophila*. Supposing such interactions occur in the real world also for *Bacillus anthracis*, it is possible that soil-dwelling amoebas might shed new light on the "incubator area" hypothesis. By providing a host and hence a possibility for *Bacillus anthracis* to persist and replicate in soil without being outcompeted, restrained by its specific environmental requirements or losing its virulence, amoebas might represent the missing link in the Van Ness theory (Dey *et al.*, 2012). If so, the "incubator areas" should be viewed not as vicinities in which *Bacillus anthracis* freely propagates in soil, but as areas in which amoebas thrive and thus provide shelter and replication possibilities for the bacterium.

Neglecting a potential bacterial cycle in the environment for a while, another possible way of attaining substantial amounts of spores in nature is that of water collecting and transporting spores to "concentrator areas", as put forth by de Vos, 1990 (reviewed by Turnbull, 2008) and Dragon & Rennie, 1995. From a historical epidemiological perspective, this is in alliance with the fact that many enzootic areas in the United Kingdom and the Netherlands have been situated downstream from tanneries, indicating streams of water translocating contaminated tannery spills to ditches and other water sources. Such contaminations still cause sporadic outbreaks as a result of land disturbances forcing spores to rise to the surface of the soil, from which they are then carried by water and further concentrated at infectious sites (Turnbull, 2008). In support of the "concentrator theory", the weather patterns characteristically preceding anthrax epidemics and the humidity observed in storage areas implies water being of major importance for the ecology and dissemination of anthrax spores (Dragon & Rennie, 1995). It is dubious, however, whether these weather cycles of drought following heavy rains are more relevant by means of rendering animals susceptible to infection rather than generating the water-flows required for concentration of spores (Turnbull, 2008). It is generally held that the resistance of animals to anthrax is negatively affected by extreme changes in climate, bringing forth alterations in behaviour, food search and general health status. Consequently, infectious and lethal doses are unmistakably reduced, furthering the manifestation of the index case needed to initiate an outbreak. Also, even though waters have been shown to carry spores associated to humus particles, it is just as probable that these spores will be dispersed over extended areas instead of converging to form "concentrator areas" (Hugh-Jones & Blackburn, 2009).

In contrast to the theory of "concentrator areas", the exosporium theory claims a limited physical movement of spores. Indeed, anthrax contaminated carcass locations in the Etosha National Park, Namibia, have in several cases been observed to remain defined and with spores persisting at high concentrations in very local sites albeit these dry and dusty soils are exposed to both strong winds and hard rains (Turnbull, 1998). The exosporium of *Bacillus anthracis* has been demonstrated crucial for hydrophobicity, which in turn is necessary for adherence to various surfaces. Thus, this suggests that the exosporium is of major importance for soil attachment and hence responsible for spore persistence and accumulation at restricted soil sites (Williams *et al.*, 2012).

Concluding remarks and future directions

To sum it up, all these theories have their agreements and disagreements regarding the ecology and epidemiology observed for *Bacillus anthracis*. Hence, it is difficult, if not to say impossible, to decide upon which one is responsible for the environmental accumulation of infectious spore doses. It seems reasonable to believe that all of them may well have an impact on spore concentration, perhaps at different degrees at various sites and regions. Possibly, diversities in soil structure and composition, as well as various weather factors, such as temperature, rain, drought and wind, determine if, and by which means, spores are concentrated. It is also necessary to take into consideration that these environmental factors act not only on spore quantities, but also on animals. Thus, the risk of contracting anthrax does not rely entirely on high amounts of spores but also on the susceptibility of the potential host animal.

Howsoever, as the index case has succumbed, a chain of events is put into practise yielding *Bacillus anthracis* opportunities to sporulate, spread and infect new hosts, thus initiating a full outbreak. Once an epidemic is established, spores can be disseminated in multiple ways, many of which rely on vectors and/or environmental factors. The observed seasonality of anthrax outbreaks, in combination with future climate changes, leads to expectations of great changes ahead regarding persistence and geographical distribution of the disease.

All in all, little agreement has been reached concerning what role all the above mentioned factors might play in the epidemiology of the disease, and hence it is a topic in need of further research. A better apprehension of how *Bacillus anthracis* persists in soil environments and how it is affected by different natural events, will substantially contribute to the understanding of its epidemiology and microbiology, and potentially aid in the development of methods for eradication of the disease from contaminated farmlands, hence greatly reducing the risk of future anthrax epidemics.

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