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The danger of lime use in agricultural anthrax disinfection procedures: The potential role of calcium in the preservation of anthrax spores

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Abstract — Previously, lime (calcium oxide) was recommended by the Canadian Food Inspection Agency (CFIA) as an anthrax disinfectant. However, a recent scientific review of the subject has found evidence to suggest that exposure of anthrax spores to calcium may aid in their survival and viability. For this reason, the CFIA no longer recommends the use of lime for agricultural anthrax disinfection.

Résumé — Risque relié à l'utilisation de la chaux dans les procédures de désinfection en cas d'anthrax en milieu agricole : rôle potentiel du calcium dans la préservation des spores d'anthrax. Auparavant, la chaux (oxyde de calcium) était recommandée par l'agence canadienne d'inspection des aliments (ACIA) comme désinfectant en cas d'anthrax. Cependant, une récente revue de la littérature scientifique a trouvé des indices laissant croire qu'une exposition au calcium des spores de l'anthrax pourrait aider à leur survie et à leur viabilité. Pour cette raison l'ACIA ne recommande plus l'utilisation de la chaux pour la désinfection lors d'anthrax en milieu agricole.

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Lime (calcium oxide) has been used for centuries in agricultural settings for the purposes of disinfection (1). Its properties as an alkali allow it to destroy most bacteria and some viruses through saponification of the lipid components of biological membranes, which results in structural disruption of the microorganisms (2,3). Additionally, if the concentration of an alkali disinfectant is sufficient to raise the local pH to at least 10.0, it may have the additional microbiocidal effect of disrupting the structure of bacterial peptidoglycans (cell wall components) and causing hydrolysis of viral genome nucleotides (2). Although lime may be an appropriate disinfectant for use in many circumstances, it is thought to have limited activity against bacterial spores (3), and no evidence has been forthcoming to prove its efficacy against these spores in agricultural situations. There is, however, a body of scientific evidence supporting the fact that calcium plays a role in the preservation of bacterial spores, including those of the species *Bacillus*. This suggests that, in agricultural situations, lime, a calcium-based substance, may not only be ineffective against bacterial spores, including those of anthrax *(Bacillus anthracis)*, but may actually facilitate the preservation of these spores. Therefore, the use of lime as an agricultural disinfectant when dealing with anthrax may be contraindicated.

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Anthrax is caused by the aerobic, spore-forming bacterium *B. anthracis* (4). Like many *Bacillus* spp., it has 2 forms: the vegetative form, which survives poorly outside the host, and the spore form, which can be formed rapidly under the appropriate conditions and has the ability to persist in the environment for long periods despite adverse conditions, including extremes in temperature and pH, desiccation, nutrient depletion, and the presence of chemical disinfectants (5). Exposure of the spores to a suitable animal host, through ingestion, inhalation, or percutaneous contact, results in germination and the creation of the vegetative form of the bacterium, which goes on to replicate and produce toxins, causing the disease known as anthrax. The formation and longevity of the spores is especially important to the epidemiology of anthrax, as it is not traditionally contagious (the vegetative form of the bacterium cannot be directly transmitted between animals) and can only be contracted from exposure to spores in the environment. These spores may not encounter an appropriate host for many years after their formation; therefore, their ability to survive and remain viable is essential to the propagation of the disease.

Anthrax spores are formed as a reaction to an oxygen-rich, nutrient-depleted microenvironment (5–7). This is usually a result of exposure of the vegetative bacterium to air; for example, from postmortem leakage of body fluids or scavenging of carcasses (6,8). Each vegetative bacterium produces 1 spore before lysing and releasing that spore into the environment (7). The spores are compact, metabolically dormant versions of their vegetative parents. They consist of several layers, including an inner core, containing essential material preserved from the parent bacterium (DNA, ribosomes, etc.), and outer cortical and integument layers, responsible for spore protection and future germination (5,7). Although the structure of the spore protects

it from a variety of environmental insults, it is not an inert object. In fact, the environment in which a spore is formed, or subsequently lies dormant, can have a profound effect on the biochemical make-up of that spore.

Although there is a paucity of direct experimentation on certain aspects of the biochemistry of *B. anthracis* spores, the fact that spore characteristics are well conserved among *Bacillus* spp. makes it reasonable to extrapolate information from other members of this genus. Therefore, what is to follow will primarily refer to *Bacillus* spp. in general.

Unlike their parent vegetative cells, *Bacillus* spp. spores are enriched with metallic ions that appear to play an essential role in their preservation and viability (5,9). Although spores can be enriched with a number of different ions, the metal that appears to be the most advantageous to the spore is calcium (7,9–12).

During the process of spore formation, calcium may be taken up from the sporulation environment and incorporated into the various layers of the spore (7,9). Most of the calcium, approximately 95%, is located in the core region (7,11). Here, calcium, in combination with dipicolinic acid, forms a salt lattice that stabilizes the DNA and enzymes in the core, which is essential in the maintenance of dormancy and the thermoresistance properties of the spore (5,7,9,11). In general, populations of *B. megaterium* and *B. cereus* spores that are enriched with calcium maintain a higher percentage of viable individuals than do populations enriched with strontium or barium, an effect that is magnified at high temperatures (9).

Approximately 5% of the calcium content of a spore is incorporated into the spore integument, where it appears to play an important role in the resistance, viability, and future germinative capabilities of the spore (7). For example, experimentally, *Bacillus* spp. spores enriched with calcium have been shown to germinate more quickly and completely than those enriched with strontium or barium (9). In addition, calcium enrichment has been shown to alleviate the requirement for exogenous electrolyte supplementation during germination, suggesting that calcium may allow spores to germinate in a wider variety of environments (10).

The spore integument is also important in its ability to exchange ions with its environment (10). This means that spores formed in a calcium-poor environment, if subsequently moved to a calcium-rich environment, are able to exchange integumental ions with their surroundings, accumulating calcium and thus increasing their germinative ability (11).

During spore formation, spores do not preferentially take up one type of metal ion over another; therefore, the metal content of a spore is a consequence of the relative concentrations of metals in the sporulation environment (9). Once formed, however, the spore integument has a definite affinity for certain types of ions. Experimentally, spores rich in sodium and magnesium will exchange these ions for calcium in a calcium-rich environment (11). This calcium is then bound very tightly to the dormant spores, and only extreme chelation, acidic pH, or both can release enough bound calcium from the integument to have an appreciable effect on germination (11,12). Calcium adsorption has been shown to be maximal at higher temperatures and more basic pH conditions (10).

This evidence suggests that calcium increases the resilience of *Bacillus* spp. spores; therefore, the use of lime (calcium oxide) in agricultural anthrax disinfection, rather then destroying the spores, may actually aid in their long-term preservation. As well, lime has the added disadvantage of providing spores with the basic (high pH) environment that is optimal for calcium enrichment (10). The addition of lime to a putrefying, and thus heat producing, carcass may be especially problematic, since high temperature is another catalyst for calcium adsorption (10). It should also be noted that Dragon and Rennie (7), in speculating about conditions that may encourage spore degradation and inactivation, suggest that, over time, a calcium-poor environment can result in leaching of calcium from all layers of the spore, decreasing spore viability (7). This may explain why, historically, anthrax outbreaks are most commonly associated with alkaline, calciferous soils (13).

There are a number of studies that have examined alternatives to the use of lime for disinfection of areas contaminated with anthrax spores. Other chemical disinfectants have been suggested through the extrapolation of results from standardized laboratory disinfection experiments, such as the Kelsey-Sykes capacity test and the United States Association of Official Analytical Chemists' method (14); however, neither of these tests specifically examines the disinfection of anthrax in agricultural situations. The inherent difficulty with agricultural disinfection lies in the fact that chemicals that appear promising in the laboratory are often found wanting when used in the field. For example, while a 4% solution of formaldehyde will destroy a suspension of 10^8 anthrax spores/mL in 2 h under laboratory conditions (15), disinfection of a spore-contaminated animal waste slurry requires that the entire slurry be maintained at a concentration of 4% formaldehyde (roughly 100 kg of formaldehyde per square meter of slurry) for 4 d in order to achieve disinfection (16).

Peracetic acid, a peroxide-based disinfectant, is another compound that, under laboratory conditions, appears promising for anthrax disinfection (15), both because of its sporucidal properties (17) and because it has the additional benefit of creating an acidic environment that may facilitate calcium leaching from spores and loss of spore viability. However, given that changing environments can have a dramatic impact on the power of disinfectants, in the case of agricultural anthrax, it would seem prudent to withhold final judgment on the efficacy of peracetic acid or any other disinfectant, until it is possible to perform controlled bacteriological studies on the efficacy of such disinfectants on bacterial spores in their "natural environment;" that is, surrounded by soil and organic materials in which there are variations in pH and temperature. Additionally, although bacterial spores are believed to be physically and biochemically similar, it would be beneficial to perform these experiments on *B. anthracis* directly, rather than "surrogate" *Bacillus* spp., in order to produce a scenario that best approximates that of an anthrax outbreak in the field

In conclusion, although, at present, there is little evidence that proves the efficacy of any chemical for agricultural anthrax disinfection, there *is* strong evidence that we ought *not* to use lime (or any other calcium containing compound), as the

calcium residue that results actively encourages spore preservation. Consequently, as a result of the information presented here, the Canadian Food Inspection Agency (CFIA) no longer recommends the use of lime as an agricultural disinfectant when dealing with anthrax. For more information on anthrax please consult the official CFIA webpage (18).

Anthrax is a reportable disease in Canada; therefore, the CFIA District Veterinarian should be contacted immediately, if a case of anthrax is suspected. The District Veterinarian will then provide information on appropriate carcass disposal and disinfection procedures.

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References

1. Blancou J. History of disinfection from early times until then end of the 18th century. Rev Sci Tech 1995;14:31–39.
2. Maris P. Modes of action of disinfectants. Rev Sci Tech 1995;14: 47–55.
3. The Center for Food Security and Public Health [page on the Internet]. SPH Main Menu, Biological Risk Management, Veterinarians Menu, Disinfectant Resources, Disinfectants 101, Disinfection 101 (Glenda Dvorak) c 2005. Available from www.cfsph.iastate.edu/BRM/resources/disinfectants/Disinfection101Feb2005.pdf Last accessed October 15, 2008.
4. Constable PD, Gay CC, Hindcliff KW, Radostits OM. Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats. 10th ed. Edinburgh: Saunders, 2007:816–819.
5. Madigan MT, Martinko JM. Brock Biology of Microorganisms. 11th ed. Upper Saddle River: Pearson Prentice Hall, 2006:87–91.
6. De Vos V, Turnbull PCB. Anthrax. In : Coetzer JAW, Tustin RC, eds. Infectious Diseases of Livestock. 3rd ed. Vol 3. Oxford: Oxford Univ Pr, 2004:1788–1811.
7. Dragon DC, Rennie RP. The ecology of anthrax spores: Tough but not invincible. Can Vet J 1995;36:295–301.
8. Jubb KVF, Kennedy PC, Palmer N, eds. Pathology of Domestic Animals. 4th ed. Vol 2. San Diego: Academic Pr, 1993:240–243.
9. Foerster HF, Foster JW. Endotrophic calcium, strontium, and barium spores of *Bacillus megaterium* and *Bacillus cereus*. J Bacteriol 1966; 91:1333–1345.
10. Rode LJ, Foster JW. Influence of exchangeable ions on germinability of bacterial spores. J Bacteriol 1966;91:1582–1588.
11. Rode LJ, Foster JW. Quantitative aspects of exchangeable calcium in spores of *Bacillus megaterium.* J Bacteriol 1966;91:1589–1593.
12. Rowley DB, Levinson HS. Changes in spores of *Bacillus megaterium* treated with thioglycolate at a low pH and restoration of germinability and heat resistance by cations. J Bacteriol 1967;93:1017–1022.
13. Kaufmann AF. Observations on the occurrence of anthrax as related to soil type and rain fall. Proc Int Workshop Anthrax. Winchester, England, April 11–13, 1989.
14. Turnbull PCB. Guidelines for the Surveillance and Control of Anthrax in Humans and Animals. 3rd ed. World Health Organization: Emerging and other Communicable Diseases Surveillance and Control, 1998.
15. Spotts Whitney EA, Beatty ME, Taylor Jr. TH, et al. Inactivation of *Bacillus anthracis* spores. Emerg Infect Dis 2003;9:623–627.
16. Bohm R. Resistance, survival, sterilization and disinfection of *Bacillus anthracis.* Proc Int Workshop Anthrax. Winchester, England, April 11–13, 1989.
17. Jefrey DJ. Chemicals used as disinfectants: Active ingredients and enhancing additives. Rev Sci Tech 1995;14:57–74.
18. Canadian Food inspection Agency [page on the Internet] Subjects — Animals, Animal diseases, Anthrax (date modified 2008-06-18). Available from http://www.inspection.gc.ca/english/anima/heasan/disemala/anthchar/anthchare.shtml Last accessed October 15, 2008.