Using a qPCR Technique to Monitor Germination of *Bacillus anthracis* Spores in Soil

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Bacillus anthracis is a Gram-positive, endospore forming, rod-shaped bacterium that causes anthrax, a fatal disease of livestock (primarily cattle, goats, and sheep). This bacterium sporulates under unfavorable growth conditions to produce spores, which are the infectious form of *B. anthracis*. Spores are highly resistant to environmental stressors including extreme temperatures, desiccation, UV and γ-irradiation, oxidation, and harsh chemical treatments. Spores are found worldwide and remain in a dormant, non-reproductive stage for decades to centuries in the soil. However, the introduction of specific nutrient stimuli (germinants) may cause endospores to germinate into vegetative cells. It is known that *Bacillus anthracis* efficiently germinates inside a living host, but it is possible that nutrients in the soil may cause germination to occur outside of a living mammalian host.

The goal of this study is to determine whether *B. anthracis* spores can germinate in soil environments. Quantitative polymerase chain reaction (qPCR) will be utilized to quantify DNA from viable *B. anthracis* cells. Absolute quantification of gene expression using SYBR Green dye will be applied to real-time PCR. The Sterne vaccine strain of *B. anthracis* that is attenuated for virulence will be utilized. The PCR amplification target is the protective antigen (PA) gene that resides on the pXO1 virulence plasmid. If the spores germinate, DNA is extracted from the resulting vegetative bacterial cells. Environmental conditions favoring spore germination can then be examined. If spores can germinate in soil, followed by replication and re-sporulation, it would contribute to persistence of the spores in soil and potentially greater exposures to grazing animals.