KEYNOTE ADDRESS

THE ANTHRAX CAPSULE: ROLE IN PATHOGENESIS AND TARGET FOR VACCINES AND THERAPEUTICS

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The poly-γ-D-glutamate capsule of Bacillus anthracis, the causative agent of anthrax, is a well-established essential virulence factor, conferring antiphagocytic properties on the Bacillus. After a review of capsule structure and its role in pathogenesis, I will present our research on targeting the capsule for the development of medical countermeasures, first using the capsule as a vaccine, similar to successful efforts with other bacteria, and secondly, by developing a novel therapeutic against the capsule. Our experiments demonstrated that a capsule vaccine is protective in the mouse model and its efficacy could be enhanced by conjugation to a protein carrier. In initial experiments using high challenge doses, a capsule conjugate vaccine was not protective in the rabbit but did show some protection in the non-human primate. Subsequent experiments showed complete protection in the non-human primate against lethal aerosol challenge. This suggests the capsule may be useful for inclusion in a protective antigen-based vaccine. We are also developing the use of the B. anthracis capsule depolymerase (CapD), a γ-glutamytranspeptidase, as a therapeutic. CapD contributes to virulence by attaching the capsule to the cell wall peptidoglycan and releasing low-molecular weight capsule. We demonstrated that in vitro treatment of the encapsulated anthrax bacillus with recombinant CapD enzymatically removed the capsule from the bacterial surface making it susceptible to phagocytic killing. Initial experiments in vivo showed that CapD, and a more stable circularly permuted variant, could successfully treat experimental anthrax infections. Such an approach to target the capsule virulence factor might be of value in the treatment of infections due to multidrug-resistant strains.

S1.1

EFFECTIVENESS OF A LONG ACTING STERILANT (PATHOSTER® 0.5%) AGAINST SPORES OF BACILLUS ANTHRACIS AND VEGETATIVE FORMS OF BURKHOLDERIA MALLEI, BURKHOLDERIA PSEUDOMALLEI, FRANCISELLA TULARENSIS, YERSINIA PESTIS, BRUCELLA ABORTUS AND BRUCELLA MELITENSI

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Peracetic acid is considered a potent biocide. It has the advantages of remaining effective even in the presence of organic residues, and it decomposes into nontoxic and non-mutagenic substances (acetic acid and oxygen) and provides excellent disinfection in a short period. This research reports the results of the antimicrobial effectiveness and the long stability of a product containing 0.5% stabilized peracetic acid (Pathoster® 0.5%). Pathoster® 0.5% consisting of two components: the activator (75mL) and the stabilizer (625mL). The sterilizing solution ready for use contains 0.46% peracetic acid, 2.2% of hydrogen peroxide, buffer solution, corrosion inhibitors, non-ionic surfactants, complexing and stabilizing agents. The formulation shows a good compatibility with the materials exception for aluminum, copper and corresponding alloys and natural gums. In this research was evaluated the antimicrobial effectiveness and stability against spores of Bacillus anthracis and vegetative forms of Burkholderia mallei, Burkholderia pseudomallei, Franciscella tularensis, Yersinia pestis, Brucella abortus and Brucella melitensis. After the reconstitution the product was stored at room temperature and every day the effectiveness was evaluated after the following exposure times: 5, 15, 30 and 60 minutes. The efficacy parameter was established as the complete absence of growth of the bacteria after the exposure to the product. The tests were carried out every day.
S1.2
AN ECONOMIC EVALUATION OF A LIVESTOCK ANTHRAX VACCINATION PROGRAM IN HIGH-RISK REGIONS OF THE COUNTRY OF GEORGIA

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Background: In 1995, legislation was passed requiring the prevention of epizootic diseases in Georgia through mandatory livestock anthrax vaccinations and other mechanisms. In 2007, responsibility for prophylactic livestock vaccinations shifted from the government to livestock owners. Between 2010 and 2012, there was a substantial reduction in anthrax vaccinations, a three-fold increase in livestock cases and a four-fold increase in human cases. The government resumed responsibility in 2013. We analyzed the economic feasibility of a government-funded livestock anthrax vaccination program in the predominantly affected regions of Kvemo Kartli and Kakheti.

Methods: We used an Excel model to analyze program costs (livestock vaccination administration) and benefits (averted livestock and human cases) from a government perspective. Cost per human case averted was the primary outcome. Based on a prior study, we assumed a 1:1 ratio for animal to human cases, and calculated a minimum correction factor of 3.8 to account for livestock case underreporting. We performed sensitivity analyses to determine how changes to model inputs affected results. Sensitivity analyses were guided by subject matter expert opinion.

Results: We estimated first-year vaccination costs, at 100% coverage, in high-risk regions was 194,451 Georgian Lari (GEL)/$86,743 US. This translated to 0.46 GEL/$0.21 US per animal vaccinated (assuming 2 vaccines/animal in year 1). Vaccine, salary and transportation accounted for 80% of program costs. Accounting for underreporting, 46 livestock cases and 46 human cases would be averted. The cost per human case averted was 4,227 GEL/$1,902 US. Simultaneously, decreasing vaccine cost by 40% (from 0.08 GEL/$0.05 US to 0.05 GEL/$0.03 US), and increasing anthrax prevalence by 150% (from 0.011% to 0.027%) changed the cost per human case averted to 1,431 GEL/$644 US.

Conclusion: These data can aid the government of Georgia in decision-making regarding continued funding of livestock anthrax vaccination programs.

S1.3
A RESAZURIN-BASED RAPID ANTIMICROBIAL SUSCEPTIBILITY TEST FOR BACILLUS ANTHRACIS

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Background: In the event of an outbreak or deliberate release of a bacterial bioterror agent such as Bacillus anthracis, antibiotics will be distributed for treatment or post-exposure prophylaxis of affected populations. For an effective public health response, it is essential to ensure that the identified isolate is susceptible to antimicrobial agents before those are distributed. However, conventional antimicrobial susceptibility tests (AST) require 16-20 hours incubation, and interpretation of the results depends upon a subjective, visual inspection for growth. Here we report the development of a more rapid AST that also replaces visual interpretation with quantitative fluorescence detection by a plate reader.

Methods: Susceptible and resistant control strains of avirulent B. anthracis were inoculated into conventional broth microdilution 96-well plates to a final concentration of 2 - 5 x 10⁸ CFU/mL, and