

MEETING ABSTRACTS

ANTHRAX COUNTER MEASURES 2013 INTERNATIONAL CONFERENCE PROCEEDINGS ROYAL UNITED SERVICES INSTITUTE, LONDON 4 FEBRUARY 2013



Session on 'How to Respond?' in progress. From left to right: John-Francois Duperre (Public Health Agency of Canada), Dr Bob Spencer (UK Health Protection Agency / Public Health England), Dr Gerald Kovacs (US Biomedical Advanced Research and Development Authority), Dr Luciana Borio (US Food and Drug Administration), Greg Burel (US Division of Strategic National Stockpile – hidden from view), Thomas Craik (Metropolitan Police) and Simon Churchill (London Fire Brigade). © Crown copyright 2013.



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Guest Editorial

Anthrax Counter Measures

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Anthrax is still a high threat biological agent, with historical precedence of potential terrorist use. A 1 kg release of anthrax preparation could contain more than 10^{14} spores (~5 billion lethal doses), while atmospheric dispersion models have predicted that a 100 kg release could result in between tens of thousands to several million deaths in the absence of a public health response [1-3].

While lack of human-to-human transmission may contain attacks in pockets, multiple and coordinated attacks have now become the terrorists' *modus operandi* (e.g. London, Mumbai). Unlike bombs and chemical weapons, anthrax attacks can also be covert, therefore successful containment is heavily dependent on rapid identification after the smallest (possible) number of early cases, immediate and accurate statistical assessment of its geographic extent based on case histories, and a rapidly targeted prophylaxis strategy that considers both antibiotics and vaccine. Moreover, persistence of spores could lead to continued post-event threat, lack of guarantee that total decontamination has been achieved, fear of repeat attacks, concerns and disruptions to normal life, and tremendous loss to the economy. Indeed, a recent poll examined a 'worst-case scenario' (e.g. inhalational anthrax discovered without an identified source and the entire population of a U.S. city or town asked to receive antibiotic prophylaxis within 48 hours) and discovered possible barriers for racial/ethnic minorities, including greater concern about pill safety and multiple attacks [4, 5].

The aforementioned considerations were among those that underpinned the recent 'Anthrax Counter Measures 2013' international conference held at the think-tank Royal United Services Institute, London, on 4 February 2013. Convened in collaboration with the Health Protection Agency (Public Health England since April 2013) and with the support of the UK Home Office, this event was part of a series of three 'Medical Security and Resilience' conferences organised by the Institute. It brought together academic, government and third sector organisations to debate key issues in anthrax counter measures under four themes: 'What might happen? How will we know? How to respond? What does the future look like?'. Each question was explored in the form of presentations by invited speakers, followed by a panel discussion. Experts invited to this event represented a range of disciplines and organisations from the UK and North America, and presented novel approaches and fresh suggestions to the discussions [6-8]. This was the fourth international event on anthrax to be co/convened by the Health Protection Agency and its predecessor

organisations, which have been at the forefront of research in this area for over seven decades [9-11].

Peer-reviewed abstracts of fifteen oral papers and nineteen posters presented in this conference are being published as proceedings in this issue of the Military Medical Science Letters journal (ISSN 0372-7025) [12]. As co-convenor, the following points/trends from the conference's deliberations and related journal publications are of interest to this author:

- Of the different modes of infection, current focus is understandably on the latest (and fourth) mode of injectional anthrax, especially its clinical manifestations and investigation of recent outbreaks in Europe [7, 8]. A number of abstracts (e.g. Brooks; Burton, et al.; Cuthbertson, et al.; Hawkey, et al.; Latham, et al.) in this issue [12] deal with this topic. However, inhalational anthrax continues to drive biodefence considerations like animal models, emergency preparedness, etc. (Ibid., abstracts of Baillie; Egan, et al.; Hatch, et al.; Lansley, et al.; Leach; Vipond; Williamson). Surprisingly, knowledge about inhalation anthrax is suboptimal among the public as seen from a recent poll [5].
- Following the Amerithrax attacks, the CDC's Strategic National Stockpile is being strengthened by U.S. government agencies and their initiatives. For instance, the FDA's Medical Counter Measures initiative and Animal Rule, combined with BARDA's efforts, have played a major role in raxibacumab becoming the first licensed product under 'Project BioShield'. Discussions with U.S. experts, as seen from the abstracts of Borio; Khan; Kovacs; Morris in this issue [12], were naturally centered on this recent milestone (raxibacumab was granted FDA approval on 14 December 2012), and how to accelerate products from laboratory to field in the future, in addition to making the most out of the currently licensed antibiotics and vaccines. The conference also covered current and emerging trends in anthrax detection and diagnostics (e.g. see abstracts of Osborne; Silman, et al. in this issue [12]).
- Another major theme was how to bridge the gap between science and frontline response to anthrax, and there were interesting perspectives shared on this challenge by UK experts with their North American counterparts (c.f. abstracts of Burel; Uhthoff in this issue [12]). One conclusion was that this would require greater inter-disciplinary and cross-departmental collaboration, for instance, the need to integrate reverse epidemiology [e.g. 4] with syndromic surveillance and dispersion models, and communicate this effectively to the frontline as and when appropriate. Such an approach could enable a rapid and targeted prophylaxis strategy involving not just antibiotics, but also vaccines and therapies.

Finally and surprisingly, no accurate accounting of the economic impacts associated with the Amerithrax attack exists even a decade after the event, although Concordia University researchers have now estimated the total environmental decontamination cost for all

affected sites to be US\$ 320 million (2001 dollars; range: 290-350). Their research is still going on to work out the overall burden by estimating the 'costs associated with medical expenditures, consumer response, investigative costs, and prevention, as well as the broader economic impact using models of economic interaction or impacts of business shutdowns or diversions' [13]. With other infectious diseases such as dengue (another category A pathogen), it has already been shown that the latter could be substantial, especially if the affected location is of importance to tourism or business [14].

As governments around the world are re-examining public spending, the United States is preparing an annual 'National Health Security Preparedness Index' to demonstrate value for taxpayer money and identify gaps [15], while its 'Shelf-Life Extension Program' is increasingly appealing to other countries as well [e.g. 16]. If fiscal pressures continue, it could encourage like-minded friendly governments to share resources and stockpiles, leading to other benefits such as increased resilience and cooperation. The recently formed Medical Counter Measures Consortium (MedCMs) is a step in that direction to enable partnerships between the health and defence portfolios of the 'quad countries' (Australia, Canada, UK and USA) [7, 8].

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US National Health Security: Anthrax – Preparing for the Unimaginable

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Anthrax poses a serious threat as a bioterrorism agent. During the 2001 U.S. anthrax attacks, which resulted in 5 deaths and direct economic costs exceeding \$1 billion, officials faced considerable challenges in curbing the attack, informing the public, and making recommendations about testing, treatment and prevention. Significant progress in anthrax preparedness has been made since 2001. The U.S. Centers for Disease Control and Prevention (CDC) supports federal, state and local preparedness efforts, administers programs including the Strategic National Stockpile (SNS), and establishes strategy and policy to drive innovation. The Laboratory Response Network, Epi-X and Health Alert Network enable rapid information sharing to expedite emergency response. SNS provides a national repository of crucial medical assets for Category A biological threats (including anthrax) as well as chemical, radiation and pandemic influenza emergencies. Since its inception in 1999, the role of SNS has expanded to include countermeasures and medical devices, policy guidance, subject matter expertise and other functions. State and local officials provide critical oversight, guidance and planning for SNS implementation. Recently, the CDC Anthrax Management Team (AMT) has convened an interdisciplinary team of public health experts to evaluate and refine anthrax preparedness and response efforts. By integrating communications, environmental, epidemiologic, laboratory, regulatory and other areas, the AMT is redefining CDC's capability to protect against and respond to anthrax bioterrorist attacks.

This paper was presented as the keynote presentation at the 'Anthrax Counter Measures 2013 International Conference', Royal United Services Institute, London, 4 February 2013.

UK Perspective on Anthrax Preparedness and Counter Measures

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In this presentation, the preparedness of the UK in terms of the availability of countermeasures to anthrax, was discussed, and the recommended regimens for use of vaccines and antibiotics was reviewed. The critical factors in response to anthrax include: response time after a suspected exposure, an estimation of the incubation period *in vivo* to the development of symptoms, and the efficacy of the antibiotic regimen used. The bacterium causing anthrax (*Bacillus anthracis*) is spore-forming, leading to its persistence in the environment and *in vivo*. Additionally, the asynchronous germination of spores carries the risk of late germination so that a long course (60 days) of an appropriate antibiotic (e.g. ciprofloxacin) is recommended to ensure the eradication of spores in an exposed person. In the UK, the licensed vaccine is Anthrax Vaccine precipitated (AVP) and comprises protein – predominantly Protective Antigen with traces of lethal factor (LF) and edema factor (EF) – precipitated onto aluminium sulphate, and is produced at Porton Down by the Health Protection Agency (Public Health England from April 2013). The AVP is licensed for prophylactic use through the UK's Medicines and Healthcare Regulatory Agency (MHRA) and has been in clinical use since the 1960's. This paper reviewed the available data on the vaccine's immunogenicity and utility as a prophylactic. The dosing regimen currently comprises of four priming doses (0, 3, 6 and 32 weeks) with an annual booster dose. Dstl and Public Health England are to continue collaborating on clinical trials to investigate a simplified 3-dose priming regimen.

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Canadian Perspective on Anthrax Preparedness and Counter Measures

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Pre-9/11, most of Canada's health preparedness and counter measures actions were based on "Cold War" scenarios with particular emphasis on a nuclear attack. The question "What might happen?" quickly shifted to "What did happen?" after the events of September 11, 2001, and the anthrax letters. Given that most countries were preparing for a state sponsored scenario, it is perhaps understandable that

the governments were ill prepared for a non-state event and thus resorted to a "What did happen?" mode to guide them through the post 9/11 anthrax preparedness. Fortunately Canada did revert back to "What might happen?" with the guidance of two independent threat and risk assessments (TRA). The respective TRAs of the Public Health Agency of Canada (PHAC) and the Global Health Security Action Group (GHSAG) highlighted anthrax, among a few other agents, as a significant risk. Given these findings, anthrax has shaped Canadian preparedness. Indeed PHAC has commenced using a new tool – the Strategic Asset Management (SAM) tool – to validate the procurement, divestment or maintenance of an individual emergency health asset. The current TRAs and the new SAM tool will direct current and future anthrax preparedness and counter measures in Canada.

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Death is Only the Beginning: The Complex Manifestations of Anthrax

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Traditionally, anthrax presents as cutaneous, gastro-intestinal or inhalational disease depending on the route of exposure. These forms can proceed to sepsis and death, with very high mortality in untreated cases from inhalational and gastro-intestinal anthrax. Recent infections in heroin users have presented with many of the classical features of anthrax including gross oedema, shock, pleural and pericardial effusions and bleeding. The pathology depends on the combination of the actions of lethal toxin, which destroys macrophages and effectively eliminates the immune defence against the bacteria, suppressing inflammatory responses, and oedema toxin which causes massive leakage of fluid into the intercellular space and shock. The bleeding is associated with a sudden catastrophic fall in platelets, without the disseminated intravascular coagulation seen in most haemorrhagic fevers. Our current studies suggest that a significant proportion of individuals do not progress through these stages, and asymptomatic or relatively trivial infections which self-limit often occur. The presence of concurrent infections which cause an inflammatory response also may limit the pathology caused by anthrax, presumably by reversing the anti-inflammatory response caused by the organism. Examples of these different forms of disease will be presented.

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Immune Response of Naturally Infected Individuals

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Anthrax is a disease caused by a bacterium called *Bacillus anthracis*. Concerns over the illicit use of this organism has resulted in considerable energy has being expended in efforts to develop medical countermeasures capable of protecting at risk individuals. While licensed human vaccines are available, their protective efficacy for humans has yet to be comprehensively demonstrated against the exposure route likely to be encountered during a bioterrorist attack. In contrast to the majority of laboratory animals, the human race are genetically diverse, and thus their susceptibility to disease and response to immunization with antigens (such as the protective antigen) is likely to be influenced by a multitude of host-defined factors [1]. In an attempt to characterise the human immune response to anthrax better, we are in the process of analysing the antibody and T cell responses of naturally infected individuals in eastern Turkey and the Caucasus were the pathogen continues to represent a clear and present danger to animal and human health.

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Can Real-Time Syndromic Surveillance Help Detect Anthrax Cases and Outbreaks?

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We describe the suite of real time syndromic surveillance systems managed by the Real Time Syndromic Surveillance Team including systems using national Telehealth, General Practice, and Emergency Department data. This Team is part of Public Health England since April 2013, and prior to that Health Protection Agency. We outline the syndromic surveillance service, and provide examples of the types of incident and support provided by the Team over recent years. As part of the evaluation of the syndromic surveillance systems prior to the 2012 Olympic and Paralympic Games we created a range of public health

scenarios covering potential incidents that the Health Protection Agency would require syndromic surveillance to rapidly detect and monitor. For the scenarios considered, including one involving anthrax, it is now possible to determine what is likely to be detectable and how incidents are likely to present using the different syndromic systems.

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Modelling Public Health Mitigation Strategies for a Deliberate Release of Anthrax

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The Emergency Response Department (ERD)'s embedded science and technology team develops capabilities to assist in preparedness and response to high impact threats to public health including the potential deliberate release of biological agents. Working with others across the Health Protection Agency (and Public Health England from April 2013) as well as government and relevant agencies, these include the integration of GIS and mathematical modelling into a toolbox of approaches to optimise public health mitigation strategies. Recognition of a deliberate release (trigger) could arise from a number of different avenues. Certainly, it would likely at some point be recognised by cases of serious illness reporting into health systems; the basis of syndromic surveillance. Failing other intelligence, the epidemiology from the earliest cases can be used to mathematically infer the "hazard area" that likely gave rise to the geographic disposition of cases. In collaboration with other groups, the ERD has developed such reverse epidemiology systems, which will be outlined. It is feasible that other forms of intelligence would provide a trigger and also an assessment of the hazard area. Knowing the hazard area is important but "health" need to know how to best target countermeasures. ERD has therefore developed tools that take any hazard area assessment, given as the probability of infection across grid cells in space, knowledge concerning dose response, incubation period distribution, population densities, etc. that optimise countermeasure distribution. These tools have been used in preparedness work that demonstrates that targeting antibiotics using such tools can save a considerable number of lives and that vaccine could provide some additional benefit, especially if compliance were an issue.

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New Strategies for Development of Anthrax Counter Measures

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The Pandemic and All Hazards Preparedness Act established the Biomedical Advanced Research and Development Authority (BARDA) as the focal point within the U.S. Department of Health and Human Services (HHS) for the advanced development and acquisition of medical countermeasures to protect the American civilian population against Chemical/Biological/Radiological/Nuclear (CBRN) and naturally occurring threats to public health. BARDA bridges the valley of death funding gap that exists between the early stages of product development and the acquisition of approved or approvable medical countermeasures for the Strategic National Stockpile (SNS). BARDA's CBRN program accomplishes this by supporting advanced research and development of medical countermeasures against CBRN threats and establishing stockpiles of vaccines, drugs and diagnostics against these threats. For example, on January 20, 2004, the Secretary of Homeland Security determined that anthrax is a material threat to the U.S. population sufficient to affect national security, and although clinical manifestations of the disease differ by route, inhalational anthrax is the most lethal. Therefore, HHS is pursuing a comprehensive strategy to address the threat of anthrax, and has made a substantial investment in the acquisition of medical countermeasures for the SNS – which includes acquisition of antibiotics, therapeutics and vaccines to meet immediate public health needs in the event of an attack.

Antibiotics remain a cornerstone of the response strategy to anthrax, and the SNS now has sufficient quantities of this antibiotic regimen for over 40 million individuals. The BARDA antitoxin program includes three projects. Two of these therapeutics are currently in the SNS and available for use in declared emergencies. In 2005, BARDA awarded contracts for the monoclonal antibody raxibacumab[®] and the polyclonal antibody Anthrax Immune Globulin (AIG). In December of 2012, the FDA approved raxibacumab under the Animal Efficacy Rule. The Animal Rule allows efficacy findings from adequate and well-controlled animal studies to support FDA approval when it is not feasible or ethical to conduct trials in humans. To date, over 60,000 doses of raxibacumab and over 10,000 doses of AIG have been delivered to the SNS. In addition to raxibacumab and AIG, the development of a third antitoxin, Anthim, is supported through a contract for advanced research and development. In addition to antibiotics and therapeutics, anthrax vaccines offer pre-exposure protection to those who are at risk from anthrax, and they may provide added protection when given with antibiotics as part of a post-exposure prophylaxis regimen. BARDA awarded a contract for the advanced research and development

of the currently licensed Anthrax Vaccine Adsorbed (AVA), to enable a label expansion to post-exposure prophylaxis. Soon after the Project BioShield Act of 2004, BARDA began working with NIAID to transition mid-stage products (beyond Phase 1 clinical testing) into its pipeline for anthrax vaccine development. To date, the Sparvax rPA subunit vaccine has transitioned to BARDA, and programs for the development of an adenovirus vector based vaccine and two rPA subunit vaccines have been awarded through BARDA's Broad Agency Announcement mechanism. However, current research with rPA-base vaccine has raised concerns associated with stability of the product, immunogenicity and efficacy. BARDA in conjunction with the AVA manufacturer has endeavored to improve the production capacity and testing of the AVA product, and is working to expand its stockpile of vaccine by conducting dose sparing studies, label expansion for PEP, as well as increasing manufacturing capacity. Through its June 2012 Broad Agency Announcement, BARDA is seeking to support projects for anthrax vaccines that provide advantages over AVA, including one or more of the following: (1) fewer doses to protection; (2) faster protective immune response; and (3) improved storage conditions (e.g. no cold chain). In addition, BARDA is placing special emphasis in supporting programs to expand availability of licensed anthrax vaccines for at risk populations, e.g. pediatric populations.

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Post-Amerithrax: Evolving Role of the FDA through the MCMi

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The 2001 anthrax attack in the United States presented numerous challenges and exposed gaps in the United States' preparedness for such events. Since the 2001 anthrax attack, the US government has made great strides in preparedness. However much work remains to be done and if an anthrax attack occurred today, the United States would still face many complex challenges. The US Food and Drug Administration (FDA) plays a vital role in protecting the United States from chemical, biological, radiological, nuclear (CBRN) and emerging infectious disease threats by: ensuring that medical countermeasures (MCMs) are safe, effective, and secure; providing scientific and technical assistance to MCM developers; facilitating access to MCMs through a variety of regulatory mechanisms; and working with US government partners to build and sustain MCM programs necessary to respond to public health emergencies. In 2012, FDA launched its Medical Counter-

measures Initiative (MCMi) as part of a larger US government initiative to increase its capacity to respond faster and more effectively to CBRN and emerging infectious disease threats with MCMs. MCMi is addressing key challenges related to the development and regulatory review of MCMs in three areas: enhancing the regulatory review process for the highest priority MCMs and related technologies; advancing regulatory science for MCM development; and modernizing the legal, regulatory, and policy framework to facilitate MCM development and access and to ensure an effective public health response.

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Coordinated Response by Federal, State and Local Authorities: Reflections and Lessons Learned

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Since its inception in 1999, the Centers for Disease Control's Strategic National Stockpile has deployed medical countermeasures (MCMs) in response to a broad range of public health emergencies throughout the United States. Through these 13 years of experience in deploying and maintaining the nation's stockpile of MCMs, CDC has captured lessons learned and identified opportunities for improvement across the spectrum of activities comprising MCM preparedness. A selection of these lessons learned and improvements are shared in this presentation for conference attendees to provide perspective on challenges unique to the United States and possible solutions to challenges that cross international boundaries and impact our partners around the world.

Lessons Learned in the Approval of the First Project BioShield Medical Countermeasure

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On December 14, 2012, the FDA approved raxibacumab, the first product developed with Project BioShield funding

to achieve this milestone. Several lessons were learned during this process that touch on regulatory and policy issues, including implementation of the FDA Animal Rule, the important differences between R&D and regulatory science, and the approval endgame. While no one expected the Animal Rule to provide advantage regarding time and money, the amount of effort required to develop a scientific animal model to a regulatory model was not anticipated. As the models developed, expectations regarding the data they could provide grew. A new expectation was unveiled at the time of the initial Biologics License Application, resulting in a Complete Response letter from the FDA and more work on the animal models. With the final approval of raxibacumab, the impact of the label and post-licensure commitments became clearer and it became apparent that products can outpace policies in development. As work on raxibacumab continues, the lessons about regulatory science and the approval process are already being applied to other programs with benefit.

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Current and Emerging Anthrax Diagnostics

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The Rare and Imported Pathogens Laboratory at Porton Down performs anthrax diagnostics on a wide range of environmental and clinical samples. A combination of culture and three-target anthrax PCR is employed in the investigation of various clinical and environmental sample types from drum-related and heroin-related anthrax cases, as well as in screening Royal Mail and general environmental samples. Patient sera are investigated using EIAs to measure antibodies, and anthrax toxins can be detected in body fluids. Serological assays are also being used to screen sera from heroin users retrospectively. Utilisation of the correct combination of tests on the appropriate sample type is vital. We present an update on the current approach to the investigation and diagnosis of anthrax dependent on the context and sample types available, and discuss how we are standardising diagnostic methodologies to optimise rapid and accurate results.

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Current and Emerging Anthrax Vaccines

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Anthrax continues to be considered a potential bio-terrorism threat in a number of countries including the UK and USA. Currently, the only anthrax vaccines licensed for human use are AVP (anthrax vaccine precipitated) licensed in the UK, and AVA (anthrax vaccine adsorbed) which is licensed in the US. More recently, there has been considerable efforts and investment to develop new anthrax vaccines. These efforts have particularly concentrated on the concept of using a single recombinant antigen, anthrax protective antigen (PA). Other vaccines in development include live or heat-killed *B. anthracis* spores. This presentation will describe a personal perspective on the status of the various vaccines, currently in development, as well as providing a comparison of current and potential new vaccines.

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The Role of Animal Models in Accelerating the Licensure of Counter Measures

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Anthrax is a potentially fatal disease in man, and events of recent times have highlighted the possibility for the agent to be used as a biological weapon through deliberate release. Whilst outbreaks of anthrax occur in several countries throughout the world, there are only a few areas in which natural outbreaks of inhalational anthrax occur, so it is not possible to test therapies using conventional clinical trials. To overcome these problems, the FDA published the 'Animal Rule' in 2002. This rule is designed to permit licensure of drugs and biologics that are intended to reduce or prevent serious or life-threatening conditions caused by exposure to biological, chemical, radiological, or nuclear substances. Due to limited proven post-exposure prophylaxis after inhalational exposure for use in humans, it is essential to have a robust animal model of this disease. A model system that represents human disease would enable the generation of data packages that could support the licensure of medical countermeasures against diseases caused by pathogens of high consequence, in this instance *B. anthracis*. The work presented details the assessment of orally administered antibiotics in a *B. anthracis* aerosol infection model.

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Processing of Clinical Samples for the Detection and Identification of *Bacillus anthracis*

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Background: In December 2009, the Rare and Imported Pathogens Laboratory identified *Bacillus anthracis* DNA in post mortem samples from an intravenous drug user [1, 2]. A similar case had been reported in Norway in 2000 [3]. This was the start of an outbreak of anthrax in drug users in the UK that peaked in 2010, with a second smaller outbreak in 2012.

Aims: To describe the methods used for the detection of *B. anthracis* DNA and culture of *B. anthracis* in clinical and associated environmental samples, for example drugs and associated items.

Experimental approach: Clinical samples were processed by homogenisation (if necessary) followed by manual DNA extraction and real-time PCR. The real-time PCR assay was developed in house and targets *B. anthracis* specific genes from pX01, pX02 and a region of the chromosome. Where appropriate, samples were cultured on non-selective and selective agar (PLET). Confirmation was done by PCR and testing with anthrax specific bacteriophage and penicillin, and by demonstration of M'-Fadysen's reaction. In order to try and identify the source of the contamination, items associated with the patients were tested using serial dilution and culture on non-selective and selective agar.

Results: Between 2009 and 2012, 1429 samples were tested by PCR. 156 samples were positive from 48 cases. During the same period, 563 samples were cultured with 53 positive isolates from 27 cases. No *B. anthracis* has been detected from associated items to date.

Conclusions: A system for processing clinical and associated environmental samples for the detection and identification of *B. anthracis* has been developed, and is described in this poster.

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Molecular Characterisation of *Bacillus anthracis* responsible for Outbreaks of Anthrax in Injecting Heroin Users

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Aims: To characterise, through molecular analysis, the genotype of *Bacillus anthracis* responsible for two outbreaks of anthrax in the UK and Europe (2009 and 2012) among injecting heroin users [1, 2].

Approach: The Rare and Imported Pathogens Laboratory based at Porton Down is at the frontline in detection of *B. anthracis* in clinical specimens. PCR and culture positive *B. anthracis* samples taken from injecting heroin users were obtained and used in three genotyping assays as previously described [3-5]. Next Generation Sequencing (NGS) was utilised comparatively, in order to discover novel single nucleotide polymorphisms (SNPs) within the *B. anthracis* genome to help strain discrimination [6].

Results: The analysis determined the same genotype to be causing disease in the heroin users. Phylogenetic inference revealed that the archetypal strain most closely resembled one previously found in Turkey [6].

Conclusions: It is likely that the heroin became contaminated with *B. anthracis* during transit through Europe; either from contact with spores present on animal skins, or from a contaminated cutting agent. The utility of NGS has been demonstrated for detection of subtle changes in the genome, which in turn helps to distinguish strains, derive closest known isolates, and permit the development of rapid outbreak-specific typing assays [6].

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Novel Methods Support Diagnosis of Anthrax Infection in Heroin Users during UK Outbreak

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Background: In 2009 and 2010 there was an unusual outbreak of anthrax infection in the UK. This is a disease that is rarely seen in the UK but during this outbreak there were more than 40 confirmed cases in heroin users. A number of these cases were fatal. The presentation of injectional anthrax may be different from that of classical anthrax (cutaneous, inhalational or gastrointestinal) and the presentation may vary from patient to patient [1].

Purpose: The purpose of this study was to use an ELISA method to enable detection of *Bacillus anthracis* toxin components (Protective Antigen 'PA' and Lethal Factor 'LF') and detection of antibodies to these components to supplement routine diagnostic testing and to inform treatment decisions.

Methods: During this anthrax outbreak, four separate ELISAs were used for the detection of immunoreactive PA, immunoreactive LF, anti-PA IgG antibodies, and anti-LF IgG antibodies. The sample types that were tested in the ELISAs as part of this anthrax outbreak included serum, EDTA blood, blister fluid, pleural fluid, pericardial fluid

and bile. The test samples (n=555) came from people who were at various stages of illness as well as from post mortem samples. The ELISA results were categorized as positive, negative or equivocal.

Results: In the majority of cases where the traditional PCR and culture diagnostic results were positive, the ELISA methods applied to the samples showed positive results for either immunoreactive PA or LF, or anti-PA or -LF IgG antibodies, or both. In only 9% of these positive cases was no positive ELISA results obtained. Eleven patients with PCR or culture negative results were however diagnosed as confirmed cases on the basis of positive ELISA results being obtained.

Conclusion: The data obtained from the ELISA testing is in agreement with the traditional diagnostic techniques and the novel test allowed diagnosis of numerous cases who could not receive a positive diagnosis based on traditional diagnostic techniques alone.

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Reducing Turnaround times for Confirmation of *Bacillus anthracis* in Blood Cultures

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Background and Aims: Blood cultures are among the recommended clinical samples taken from patients suspected of infection with ACDP hazard group 3 bacteria such as *Bacillus anthracis* – either due to a natural infection [1], or exposure via accidents, bioterrorism, etc. [2]. The use of serum separator tubes to concentrate bacteria from positive blood cultures has been described previously [3-5]. The aim of this study was to evaluate the processing of *B. anthracis* blood cultures with serum separator tubes prior to molecular and phenotypic characterisation tests.

Approach: Simulated blood cultures were used with horse blood inoculated with *B. anthracis* suspensions to enable a comparison of the diagnostic test results processed with and without serum separator tubes. The effect of pathogen concentration on time to detection was determined for eight strains of *B. anthracis*. Confirmatory tests were performed on ten strains of *B. anthracis*; five mixed blood cultures of *B. anthracis* and contaminating bacteria; and ten isolates (non-*B. anthracis*) referred to our laboratory during the 2009-2011 outbreak.

Results: An inverse linear relationship between concentration and time to detection has been demonstrated for all eight strains of *B. anthracis* examined. The confirmatory tests performed on *B. anthracis* and non-*B. anthracis* simulated blood cultures were comparable.

Conclusions: Relating the results from this study to a patient scenario, we infer that the time to detection was approximately 6-8 hours for patients with 10^6 cfu/ml *B. anthracis* circulating in their blood. Concentration of *B. anthracis* using serum separator tubes can provide adequate material for: (i) a simple wash step; (ii) heat inactivation for confirmatory PCR assays; and (iii) setting up phenotypic characterisation tests 24 hours earlier than using current blood culture processing protocols.

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Towards an ELISA based Assay to Measure the Protective Immune Status of Individuals Immunised with the UK licensed Anthrax Vaccine

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The current UK licensed anthrax vaccine (Anthrax Vaccine Precipitated, AVP) has a priming schedule in which

volunteers receive doses at 0, 3, 6 and 32 weeks. The ability of this vaccine to stimulate a protective immune response is thought to correlate with the production of toxin neutralizing antibodies (TNA). Measurement of TNA involves a cell culture assay, an approach which does not lend itself to mass screening. We therefore sought to identify individual protective linear epitopes which bind TNA and could thus form the basis of a future ELISA based assay to measure protective immunity. Using a panel of synthesized peptides with sequences based on published TNA binding sequences within Protective Antigen and Lethal Factor (PA and LF), we determined the ability of each peptide to bind antibodies from AVP immunised individuals collected at different time points during the immunization schedule. The magnitude of the peptide specific response varied between peptide and with time. Peptide 135, which encodes a region within the C terminal domain of PA, exhibited the strongest antibody binding activity (~20 % of the total PA antibody response) at weeks 10 and 32, but there was no detectable activity before or after these time points. Further work is required to fully characterise the protective response of AVP immunised individuals and to identify robust peptide signatures which could be used to assess protective status.

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Development of an *in vitro* Potency Assay for Anti-anthrax Lethal Toxin Neutralizing Antibodies

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The lethal toxin (LT) of *Bacillus anthracis* mediates the down-regulation of the inflammatory response leading to a reduction in the production of a number of inflammatory mediators – including transcription factors, chemokines and cytokines in various human cell lines. We used the immunomodulatory response to LT to develop assays to examine the effects of therapeutic monoclonal antibody (mAb) to treat LT intoxication. The reduction of interleukin-8 (IL-8) has been shown to be a sensitive marker of LT-mediated intoxication in human neutrophil-like NB-4 cells and IL-8 levels are restored to normality when toxin-neutralising (TN) antibodies are added. In this study we present an *in vitro* assay in the human endothelial cell line HUVEC jr2 that can measure the toxin-neutralisation activity of therapeutic antibodies in LT intoxication. These cells have the advantage that they are adherent, do not require a differentiation step, and used in a microplate format, can facilitate high throughput analysis. We also show that HMGB1, a histone-like protein with a cytokine func-

tion, is transported from the cell nucleus to the cytoplasm during LT intoxication.

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Investigating Immune Responses to Anthrax Vaccination: Developing a Human TNA

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Background: Immune responses to Anthrax Vaccine Precipitated (AVP) have been extensively characterised in mice using a cell based bioassay known as the Toxin Neutralisation Assay (TNA). This assay measures the ability of antibodies raised to *Bacillus anthracis* toxin components (protective antigen and lethal factor) to protect J774A.1 mouse macrophage cells against *B. anthracis* toxin activity.

Purpose: This study will examine the effect of using human sera in the assay, with the aim of developing a human TNA. Development of such an assay is important in assessing the human functional antibody responses to AVP immunisation.

Methods: Initially, the existing mouse TNA protocol was used to test human sera samples. Serum samples from seven volunteers of known AVP vaccination status were tested in the TNA to check the starting dilutions required for the assay. After testing these samples in a variety of plate positions, quality control and reference samples were determined, along with assignment of preliminary system suitability criteria. Subsequently, sera from a further 146 human AVP vaccinees were tested in a series of studies to assess the sensitivity, specificity, dilutional linearity and precision of the assay and to set further suitability criteria.

Results: Any non-anthrax antibodies present in serum do not interfere with assay performance and non-immunoglobulin human serum proteins do not interfere with the assay. The hTNA has an acceptable level of sensitivity and precision. Human serum samples exhibit a linear relationship in the hTNA.

Conclusion: This human TNA will be an important tool for investigating human immune responses to AVP, with potential for use in clinical trials.

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New life for an Old Vaccine: Implementation of Single Use Technology for Production of the UK Anthrax Vaccine

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Background: The UK-licensed Anthrax Vaccine Precipitated (AVP) has been produced using the same process for over 50 years. It would therefore be beneficial to improve and modernise the manufacturing process for AVP utilising large capacity, disposable wave bag (WB) technology.

Purpose: The purpose of this study is to assess the comparability of AVP produced using modernised and traditional methods.

Methods: WB were inoculated with *Bacillus anthracis* Sterne strain in parallel with manufacturing Thompson bottles (TB) and incubated until harvest pH and glucose criteria were met. The culture material was formulated into vaccine and a mouse vaccination study performed. The toxin neutralising capability of serum samples was then determined to indicate potency. Lethal Toxin (LT) activity of samples was determined by macrophage cell lysis assay and composition of culture supernatants examined by protein assay, SDS-PAGE, Western blotting and ELISA.

Results: The WB fermentation procedure appears to be highly consistent, and growth physiology was comparable with that observed in TB. The total protein levels were equivalent and antigen levels in bulk vaccines were comparable. The ratios of protective antigen to lethal factor in samples from both vessel types were comparable, which may be pivotal for vaccine function. The immune responses to vaccination with both types of vaccine appear to be equivalent.

Conclusion: These results suggest that AVP production could be transferred to WB to make manufacturing less labour-intensive, cheaper, and to enable production to be scaled up should the demand arise.

This paper was presented as a poster presentation at the 'Anthrax Counter Measures 2013 International Conference', Royal United Services Institute, London, 4 February 2013. It won one of the two poster prizes awarded at the conference by a panel of judges.

Potency Testing of UK Anthrax Vaccine: Validation of an *in vitro* Test for Batch Release

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Background: Following manufacture of the UK licensed anthrax vaccine (Anthrax Vaccine Precipitated, AVP), a range of batch release tests are undertaken, one of which is the guinea pig potency test. This is a challenge test, in which the endpoint is death following challenge and a relative potency of the test batch is calculated.

Purpose: A replacement potency test is being developed in the form of a Mouse Potency Test (MPT). Introduction of this MPT will result in significant cost savings and risk reduction; decreased use of animals, no requirement for lethal challenge, and reduced severity of the animal procedures.

Methods: Mice received dilutions of a test vaccine batch and a reference batch of AVP using a set immunisation schedule, before being bled. The serum was then analysed using the mouse Toxin Neutralisation Assay (mTNA) which was previously developed in house. System suitability criteria were set during an extensive optimisation study, and in this study the mTNA validation was completed by performing robustness and stability studies.

Results: The mTNA was assessed for precision, linearity and specificity in pre-validation and validation studies to demonstrate that the mTNA is fit for its intended purpose. The most recent MPT study showed that the vaccine doses used give a linear response so the test can be used to give an accurate estimate of relative vaccine potency. The test also showed the response is proportional to vaccine dose and that vaccines of varying potency can be successfully differentiated using this test.

Conclusion: The agency has developed, optimised and validated an *in vitro* mouse toxin neutralisation assay to be used in conjunction with an *in vivo* mouse potency test, for the release of the UK anthrax vaccine (AVP).

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Anthrax Vaccine Candidate Based on the Key Protective Regions of Protective Antigen and Lethal Factor

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Background and Aims: The pathogenicity of *B. anthracis* is dependent on the production of a polyglutamic acid capsule, and expression of a tripartite toxin comprising protective antigen (PA), edema factor (EF) and lethal factor (LF). Numerous animal studies have identified PA as the principal protective immunogen in the licensed US (AVA) and UK (AVP) human vaccines and have confirmed its protective efficacy [1]. Immunity is thought to be antibody mediated and there is a strong correlation between toxin neutralizing PA specific antibodies (TNA) and protection against anthrax [2-5]. Given the tripartite nature of the anthrax toxin it would be surprising if LF and EF failed to stimulate TNA. Indeed LF alone has been shown to confer protection in animal studies and in humans appears to be a more potent immunogen than PA. As a consequence it has been suggested that, in spite of the issue of transient side effects, vaccines such as AVP which contain both PA (7.5 mg/ml) and LF (2.5 mg/ml) could confer protection against strains of *B. anthracis* in which PA has been genetically altered [7,8]. In addition to conferring direct protection, LF has been shown to enhance the magnitude of PA specific antibody responses in mice [9]. This adjuvant effect resides in the non-toxic N terminal PA binding domain of LF (LFD1, amino acids 1-254), which has been used as a means of delivering antigens of up to 550 amino acids into the cytosol of antigen presenting cells [10]. Given the ability of this approach to deliver T cell epitopes surprisingly little is known about the human T cell response to both PA and LF following infection and vaccination other than it is relatively long lived [11].

Experimental Approach: In order to determine the contribution of LF to the protective immune response stimulated by AVP we characterized the antibody and T cell responses of immunised volunteers. Animal studies were undertaken to identify immunodominant and protective regions of LF and to determine the effect of co-administering with PA. Finally a fusion protein LFD1 + PAD4 (comprising of LFD1 and the C terminal cell binding domain 4 of PA) was constructed and assessed for its ability to protect mice against a lethal spore challenge.

Results and Conclusions: Humans immunised with the UK licensed anthrax vaccine (AVP) mount antibody and T cell specific responses to PA and LF. T cell response to LF (SI=28.08) was significantly higher ($p=0.0046$) than those seen for PA (SI = 1.49) even though the vaccine contains a lower level of LF (PA 7.5 mg/ml vs LF 2.5 mg/ml). Individual domains of LF differ in their ability to invoke toxin neutralizing antibodies. The N terminal domain of LF (LFD1) conferred complete protection against a lethal i.p. challenge with 100 MLDs of *B. anthracis* STI spores. The fusion protein comprising LFD1 + PAD4 also conferred complete protection against a lethal i.p. challenge with 100 MLDs of *B. anthracis* STI spores. LF is immunogenic in humans and is likely to contribute to the protection stimulated by AVP. A single vaccine comprising protective regions from LF and PA would simplify production and confer a broader spectrum of protection than with PA alone.

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Multi-agent vaccine candidate that confers protection against *Bacillus anthracis* and *Yersinia pestis*

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Background: *Bacillus anthracis* and *Yersinia pestis* are zoonotic organisms that can cause severe and sometimes fatal diseases in humans and animals. Due to the ease of dissemination and the potentially high rates of mortality, these organisms are classified as category A biothreat agents by the U.S. Centers for Disease Control and Prevention (CDC).

Aims: An alternative approach to vaccination against anthrax and plague is the use of a vaccine cocktail, comprised of virulence factors from both organisms. Vaccines comprised of multiple antigens from different organisms would confer several benefits, such as rapid immunization against multiple biological threats, reduced administration doses, and as a contingency provision if one component is compromised. Studies have demonstrated that when combined vaccine candidates comprised of recombinant PA, F1 and LcrV have been administered there are no detrimental effects on protective efficacy [1, 2].

Experimental Approach: In order to determine the feasibility of constructing a vaccine capable of conferring protection against plague and anthrax we engineered a fusion protein (MaF2) comprising of key protective regions from *B. anthracis* and *Y. pestis* protective antigens. In addition, the MaF2 sequence was incorporated in an expression plasmid (pDNA-MaF2). Immunogenicity of MaF2 and pDNA-MaF2 were evaluated *in vivo* in homologous and heterologous prime-boost immunization regimens.

Results: Both MaF2 and pDNA-MaF2 elicit significant immune responses against LF and LcrV, while the responses to the PA and F1 elements are minor. When presented as a protein MaF2 invokes stronger protection than when presented to the immune system as a DNA vaccine. A DNA prime/protein boost schedule invoked better protection than DNA only.

Conclusions: A single subunit-vaccine against both *B. anthracis* and *Y. pestis* would be attractive for use in biodefense. Production of such a vaccine is likely to be simpler and less expensive; administration to target populations would be easier; and it could be perceived by the public as a safer and more effective vaccine.

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Comparison of the Prevalence, Fine Specificity and Lethal Toxin Neutralization Capacity of Anthrax Toxin-specific Antibodies in AVA- and AVP-vaccinated Individuals

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Background: A primary immunogen in both currently licensed acellular anthrax vaccines (AVA, AVP) is the Protective Antigen (PA), and the most widely accepted correlate of vaccine efficacy is neutralizing PA-specific antibodies. However, animal studies have demonstrated that antibodies to Lethal Factor (LF) provide protection against spore challenge. AVA contains negligible LF, while AVP has about a 4:1 ratio of PA to LF.

Purpose: We have previously determined the prevalence, fine specificity, and neutralizing capacity of anti-PA and anti-LF in a large cohort of AVA-vaccinated individuals (n=1000). The aim of this study was to determine if antibody response to PA and LF, as well as neutralizing capacity, differs between AVA and AVP vaccinees.

Methods: The humoral response to PA and LF in an AVP-vaccinated population (n=14), as well as an expanded AVA-vaccinated population (n=1675), was characterized by ELISA, in vitro toxin neutralization, and linear B cell epitope mapping. In this ELISA-based solid-phase epitope mapping assay, overlapping decapeptides spanning full-length PA and LF and covalently linked to polyethylene pins were screened for serum antibody binding.

Results: AVP-vaccinated individuals have higher average titers to LF than AVA-vaccinated individuals (402+464 versus 11+67; p<0.001), and more AVP individuals have anti-LF (79% versus 4%). AVP vaccinees with high titer, LF-neutralizing antisera (n=11) bound novel linear B cell epitopes on both PA and LF not recognized by high titer, LF-neutralizing AVA vaccinees.

Conclusion: Although AVP- and AVA-vaccinated individuals mount similar responses to PA, AVP vaccination induces a humoral response to LF that is both more extensive and greater in magnitude than AVA vaccination.

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A Simple, Portable Biosensor System to Detect Anthrax in Soil and Environmental Samples within 17 Minutes

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Aims: There is a proven need for analysis of soil and other environmental samples for the presence of select biological agents, especially *Bacillus anthracis*, the causative agent of anthrax. Brownfield redevelopments often involve sites where there exists a high likelihood of historical contamination by anthrax spores in locations such as previous tannery sites, abattoirs and buildings older than 100 years which typically employed horse-hair plasters. Additionally, there are a large number of government and public sector-owned buildings which suffer on-going 'white powder' incidents.

Experimental Approach: Molecular assays involving PCR or other amplification assays are typically of little value in analysing these types of sample due to the high concentrations of inhibitors within the samples which result in the assays often exhibiting false negative results. In order to overcome these problems, the Health Protection Agency (Public Health England from April 2013) and Crown Bio Technology Limited have developed a biosensor assay designed to deliver rapid results at the point of use without the problems of matrix inhibition.

Results: Successful proof of concept of the assay has been obtained. The technology is rapid (17 minutes sample to result for anthrax), inexpensive, portable, sensitive and specific, and has added benefits such as high-discrimination and low-complexity. The biological agent assay system is a platform technology employing Crown Bio Technology's Qwik-X extraction system and a portable reader module. Proof of concept has been obtained with the assay format, which is now undergoing final development towards commercialisation.

Conclusion: The assay format has been successfully applied to the detection of anthrax spores in soil samples. Further development to improve sensitivity is needed. In addition to anthrax, this assay can be tailored to detect many different biological agents of interest, including, but not limited to, *E. coli* 0157, *Clostridium difficile*, Norovirus and *Cryptosporidium*. One of the major benefits of this biosensor assay is that site surveys may be undertaken rapidly and cost-effectively rather than the single 'rule-out' sampling regimen typically employed.

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Olympic Legacy: Testing the Fitness of Syndromic Surveillance Systems

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How much confidence can we have that our syndromic surveillance systems would give us early warning of public health risks during the Olympics? What would they detect, what can they not detect? A range of scenarios have been developed to cover the main incidents we could face during a 'mass gathering', such as the Olympics. Each scenario is being modelled and simulations carried out to test our statistical alerting systems. Modelling includes looking at the impact of visitors and population changes on our detection systems. The main factors affecting our detection ability are identified. Different sizes of incidents are considered and the timeliness of alerts so we can quantify how early and what size of event we can detect.

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Estimation of the Geographical Extent of a Covert Anthrax Release and Subsequent Evaluation of Potential Mitigation Strategies

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Aims: We propose a method for characterizing the time, location and geographical extent of a covert anthrax release based on data on the first few observed cases of the subsequent outbreak. We then combine this back-calculation

method with a number of published techniques that investigate mitigation strategies.

Approach: We investigate how previously reported levels of adherence with taking antibiotics might affect the total outbreak size compared to assuming full adherence. Post exposure vaccination is also considered, both with and without the use of antibiotics, following the last day of symptomatic onset of some number of observed initial cases (5, 10 and 15).

Results: Our analysis confirms that outbreak size is minimised by implementing prophylactic treatment after having estimated the exposed area based on 5 observed cases; however, imperfect (rather than full) adherence with antibiotics results in approximately 15% additional cases. Only the 'Vaccination+imperfect antibiotic adherence' outcome remains somewhat effective at very high inhaled doses because immunity is achieved whilst taking antibiotics for a proportion of individuals.

Conclusion: With no antibiotic resistance, a combined antibiotic and vaccination strategy is realistically optimal, given that it is likely there will be imperfect antibiotic adherence. However, if logistical constraints meant that vaccination could not be performed then rapid administration of antibiotics alone would still be a highly effective prophylactic treatment strategy. Our analysis shows that it is critical to have systems and processes in place to rapidly identify, geospatially analyse and then swiftly respond to a deliberate anthrax release.

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The UK Response to EQADeBa Exercises for the Detection of Highly Pathogenic Bacteria

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In 2006, the need to further improve diagnostic reliability was identified in response to results of a tender for External Quality Assurance (EQA) exercises. This led to the 'Establishment of Quality Assurances for Detection of Highly Pathogenic Bacteria of Potential Bioterrorism Risk' (EQADeBa) [1]. The Rare and Imported Pathogens Laboratory in Porton Down (formerly the Special Pathogens Reference Unit, and now part of Public Health England) co-ordinated the UK response to the EQADeBa exercises, and acted as a conduit between the other specialist reference services in the UK at Colindale and Weybridge. These EQADeBa exercises were used to demonstrate quality assurance for diagnostic assays to detect key highly pathogenic

bacteria covered within the remits of these three UK reference services. This poster presents the UK response to the EQADeBa exercises with respect to workflow, lessons learnt and benefits from participation. The lessons learnt over the three exercises fell into the areas of co-ordination, transportation, samples, testing, training and staff. Sharing of reference material and participation in a supportive network of laboratories were amongst the specific benefits from these exercises. The 'European Network for Highly Pathogenic Bacteria' created in the EQADeBa project has now combined with the 'European Network of P4 Laboratories' in an EU-funded joint action. This combined project is called QUANDHIP (Quality Assurance Exercises and Networking on the Detection of Highly Infectious Pathogens) [2], under which exercises have taken place during 2011 and 2012.

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Efficacy Testing of Orally Administered Antibiotics against an Inhalational *Bacillus anthracis* Infection in BALB/c Mice

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Aims: Whilst phase III trials in humans are possible for many common infectious diseases, ethical and safety restrictions do not permit such trials to assess vaccines, antibiotics and other novel therapies against anthrax. In order to facilitate relabeling drugs for use against inhalational infection, the Health Protection Agency were funded by NIAID to develop a reliable and reproducible aerosol model of inhalational anthrax in mice. These studies may contribute to a portfolio of information which may permit relabelled use of antibiotics against inhalational anthrax via the FDA's Animal Rule.

Methods: *Murine aerosol infection.* Stocks of *Bacillus anthracis* Ames (NR3838, BEI) were produced by fed batch culture in a bioreactor for approximately 26 hours. The spores were concentrated and washed by centrifugation, with final suspension in sterile distilled water to an approximate titre of 1E+10 CFU/ml. Female BALB/c mice, free of intercurrent infection and obtained from a commercial supplier (Charles River, UK) with an approximate body weight of 20 grams. Groups of mice were restrained in mouse tubes and challenged for 10 minutes with aerosolised *B. anthracis*. Aerosol particles with a diameter range of 0.5–7.0 µm, mass median aerosol diameter (MMAD) 1.2 µm, were generated and delivered directly to the snout of the animals using a 3-jet Collison nebuliser conjunction with an AeroMP-Henderson apparatus maintaining the aerosol at 65±5% relative humidity and a total system flow of 25L/min. A sample of the circulating aerosol was collected into 20 ml of sterile distilled water using AGI-30, sampling at 6.0 L/min. The presented dose was calculated using the assessed level of *B. anthracis* in the circulating aerosol and an estimate of each challenge group's respired volume, using Guyton's formula based on mean body weight. Bacteria were enumerated on trypticase soy agar (TSA). Plates were incubated at 37°C for 16–24h. Treatment. From one day post-challenge the test antibiotics or carrier control was administered by oral gavage (0.2ml volume). Treatment continued for a period of thirty days after which the animals were observed for a further fourteen days. Separate groups were sacrificed to permit assessment of the peak and trough circulating antibiotics levels achieved.

Results: Analysis of the combined Kaplan Meier curves with the Log-Rank statistic shows that compared to the negative control levofloxacin, amoxicillin and co-amoxiclav all confer significant protection ($p < 0.001$) from inhalational anthrax. The doses of azithromycin and clarithromycin used in this study did not protect mice ($p = 0.198$ and 0.815 respectively) from morbidity or mortality but this may have been due to inadequate dosage levels being achieved.

Conclusion: The agency has developed a murine aerosol infection model in BALB/c mice. The model is suitable for use in testing the protective efficacy of antibiotics and may have use for other prophylactics or novel therapies designed to confer protection against aerosolised *B. anthracis*.

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Virulence Confirmation of *Bacillus anthracis* Delivered to Cynomolgus Macaques by the Aerosol Route

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Aims: Whilst outbreaks of anthrax occur in several countries throughout the world, inhalational anthrax is an unusual occurrence. For these reasons it is not possible to test potential vaccines or therapies using conventional human clinical trials. Therefore, for an infection such as inhalational anthrax, much emphasis is placed upon animal models since efficacy testing in Phase 3 clinical trials is not possible for ethical reasons. The aim of this study was to develop a non-human primate (NHP) aerosol infection model in order to evaluate the efficacy of therapies or vaccines for human use.

Methods: *Aerosol Infection model.* *B. anthracis* Ames (NR-3838, BEI) was grown in a bioreactor fed batch culture for approximately 26 hours. Following sporulation, stocks were washed by centrifugation and stored in distilled water at an approximate titre of 1.0×10^{10} colony forming units per millilitre (cfu/ml). Two cynomolgus macaques, (M270B and I320C), were anaesthetised and placed in a plethysmograph (Buxco) throughout challenge to monitor respiration to an accumulated volume of 3 L. Aerosol particles with a mass median aerosol diameter (MMAD) of $1.2 \mu\text{m}$, were generated and delivered directly to the snout of the animals, via a face mask, using a 6-jet Collision nebuliser (containing 8.0×10^9 cfu/ml challenge material) producing 15 L/min in conjunction with an AeroMP-Henderson apparatus, which maintained the aerosol at $65 \pm 5\%$ relative humidity and a total flow of 25 L/min. A sample of the circulating aerosol was collected into 20 ml of sterile distilled water using a SKC Biosampler, sampling at 12.5 L/min and was enumerated onto tryptic soya agar (TSA). Sampling. Post-challenge, animals were sedated on alternate days and EDTA blood samples taken for bacteriological analyses. Daily manual checks were conducted throughout the study and at least twice daily at critical phases of the study (days 3-7 post infection). Clinical observations were recorded to determine a humane end-point at which the animals would be euthanized. At necropsy, animals were exsanguinated under terminal anaesthesia and samples of brain, lung, spleen, kidney, hilar lymph node and pharyngeal lymph node were collected. Bacteriology. Blood samples were serially diluted in sterile water onto TSA for enumeration of total bacterial counts (TBCs). Tissues collected at necropsy were processed using an automated tissue processor (Pre-cellys24) using CK14 beads and cycle of 6200 rpm, repeated 3 times for 5 s with a 30 s break after each repeat. This cycle was repeated between 1 and 3 times until samples were homogeneous. The homogenates were enumerated as above, for bacterial load determination.

Results: Both animals were successfully challenged with approximately 200 LD₅₀s. One animal (M270B) was euthanized on day 5 of the study whilst the other (I320C) survived until the end of the study, day 7. The presence of *B. anthracis* was demonstrated in the blood of both animals for the duration of the study. All tissues sampled from both animals, brain, lung, spleen, kidney, hilar lymph node and pharyngeal lymph node, showed the presence of *B. anthracis*.

Conclusion: The agency has developed an aerosol model of *B. anthracis* infection in cynomolgus macaques. The model is suitable for use in testing the protective efficacy of antibiotics and or prophylactics and novel therapeutics designed to provide protection against aerosolised *B. anthracis*.

Acknowledgement: These studies have been funded in whole with Federal funds from the National Institute of Allergy and Infectious Diseases (NIAID) contract number N01-AI-30062.

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Re-engineering the Manufacturing Process for the UK Anthrax Vaccine Using Disposable Technology

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Anthrax Vaccine Precipitated (AVP) was initially developed in the 1950's and has been successfully manufactured at Porton Down since UK licensure in 1979. The production process is highly labour-intensive consisting of static culture of the production strain in a large number of glass bottles, followed by downstream processing whereby vaccine components are precipitated using alum, prior to filling. Re-engineering AVP manufacture has centred around the use of disposable bag technology for microbial growth made possible by the low oxygen demand of *B. anthracis* in static culture. Growth in bags was shown to closely mimic that in glass bottles demonstrating similar pH, viable count and glucose utilisation profiles and attaining the criteria for harvest within 1-2 hours of that achieved during traditional manufacture. Vaccine generated using bag culture was shown to give similar proportions of the key vaccine components, Protective Antigen (PA) and Lethal Factor (LF) – to conventionally manufactured vaccine and was capable of generating comparable levels of toxin neutralising antibodies following vaccination. The feasibility of extending the application of disposable technology to the downstream processing stages will be discussed in this poster, thereby significantly increasing the scale of manufacturing allowing the only anthrax vaccine licensed within Europe to be potentially available for others.

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