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A Review of the Government Sponsored Offensive Biological Programs, Weaponized Biological Pathogens and their Countermeasures

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A Review of the Government Sponsored Offensive Biological Programs, Weaponized Biological Pathogens and their Countermeasures

by

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A Review of the Government Sponsored Offensive Biological Programs, Weaponized Biological Pathogens and Their Countermeasures

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Since the beginning of the 20th century humanity's capacity for warfare and death has evolved at an ever increasing rate. The horse was quickly replaced by the automobile and the rifle by the machine gun. The use of biological weapons (bioweapons) took the same leap forward from medics fighting battlefield illness to strategic weapons of mass destruction. The two largest research programs, the Soviet and United States of America (US), operated for decades at the height of their scientific fields developing and stockpiling biological weapons with the capability to kill thousands more cost-efficiently than any weapon previously designed. All of these weapons were developed from naturally occurring human pathogens and most of the research is still classified.

Many of these pathogens account for only a minimal number, if any, of disease cases each year within the US. The rarity of many of these diseases makes it difficult for medical personnel to diagnosis. The delay in diagnosis and

treatment can affect the outcome for the patient and drastically increase the risk of an outbreak.

This capstone covered a selection of the bioweapons produced by these two programs, their historical importance, clinical symptoms, and the available countermeasures in the case of exposure. It then opened a discussion on the selection criteria modern bioweapons programs may utilize in the 21st century and the role many of these pathogens play as public health risks. Finally it addressed many of the new developments and policies implemented by the US to tackle and minimize these risks.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Biological Defense: Biodefense

Biological Select Agents and Toxins: BSATs

Biological Terrorists: Bioterrorist

Biological Weapon: Bioweapon

Biological Weapons Convention: BWC

Biosafety level: BSL

Biosecurity Engagement Program: BEG

Centers for Disease Control and Prevention: CDC

Defense Threat Reduction Agency: DTRA

Democratic Republic of the Congo: DRC

Department of Health and Human Services: DHHS

Eastern equine encephalitis virus: EEV

Ebola virus: EBOV

Genetically modified organism: GMO

Homeland Security Presidential Directive: HSPD

Human Immunodeficiency Virus: HIV

Infectivity Dose: ID

Intra-muscular: IM

Intra-venous: IV

Investigational new drug: IND

Large cell variant: LCV

Lipopolysaccharide: LPS

Marburg virus: MARV

Mutually Assured Destruction: MAD

National Institute of Health: NIH

National Notifiable Diseases Surveillance System: NNDSS

National Security Council: NSC

Non-human primates: NHPs

Office of Cooperative Threat Reduction: OCTR

Plaque forming unit: pfu

Polymerase Chain Reaction: PCR

Prisoners of War: POWs

Shipboard Hazard and Defense: SHAD

Small cell variant: SCV

Strategic National Stockpile: SNS

Union of Soviet Socialist Republics: USSR

United States Department of Agriculture: USDA

United States of America: US

United States Postal Service: USPS

Venezuelan equine encephalitis virus: VEEV

War Reserve Service: WRS

Western equine encephalitis virus: WEEV

World Health Organization: WHO

World War I: WWI

World War II: WWII

CHAPTER 1: INTRODUCTION

"Mankind already carries in its own hands too many of the seeds of its own destruction. By the examples we set today, we hope to contribute to an atmosphere of peace and understanding between nations and among men."
–President Nixon on shutting down the US bioweapons program. 1969

Some of the earliest reports of biological weapons (bioweapons) date back to the 14th century. The first recordings of intentional bioweapons use occurred in what is now Feodossia, Ukraine. A Tartar army, while besieging the city Kaffa, catapulted the corpses of soldiers who had died from plague over the city walls in the attempt to spread the disease within the city.[1,2] In this time period it was not well understood what caused the disease and how it was spread from person to person; only the correlation that exposure to the dead increased the chance of becoming sick. With the development of germ theory and microbiology in the late 1800's by Pasteur and Koch came a better understanding of infectious disease. This understanding helped shift the use and development of bioweapons from chance to a science.[3-5] Germany, during World War I (WWI), was identified as the first government sponsored program to develop and utilize bioweapons. The United States of America (US) and Union of Soviet Socialist Republics (USSR) have since been recognized as having the most advanced and developed government sponsored bioweapons programs in history. In the last century, at least 22 biological agents, including bacteria, toxins, fungi, and viruses, have been 'weaponized'.[3,6] Each pathogen had a specific target: some were selected for enemy soldiers, others for civilians, and others for agriculture and livestock. The specific method of 'weaponizing' these agents was varied, and in many cases still classified, but the end result was the development of bioweapons with the capability to cause significant health and economic damage to an enemy's civilian and military populations.

The US biological defense (biodefense) program was initiated prior to the start of World War II (WWII) with the primary goal of protecting US soldiers from a biological outbreak, be it naturally occurring or an intentional release. After the signing of the Biological Weapons Convention (BWC) and destruction of the US stockpile of bioweapons, the biodefense, industry was brought to the forefront of infectious disease research with increased funding and manpower which was no longer focused on bioweapons research. The focus of the newly revitalized biodefense industry was to develop novel countermeasures and vaccines against bioweapons, specifically pathogens which were weaponized in the USSR and other enemy states. Since the 1970s, the biodefense industry continued to focus on threats of bioweapons. It also expanded into developing countermeasures against emerging or reemerging pathogens and potential pandemic biological threats which have a significant public health risk.

While the modern biodefense industry continues researching and developing novel countermeasures to pathogens, it also recognizes the need for an effective system to distribute the countermeasures when necessary. In 1998 the Centers for Disease Control and Prevention (CDC), in collaboration with other US government offices (Department of Health and Human Services, Department of Homeland Security), was put in charge of developing a system to acquire, store, and distribute countermeasures to the public in the case of an attack or pandemic. This system was named the Strategic National Stockpile (SNS).[7]

This capstone characterizes the known bioweapons developed during the Soviet and US sponsored programs as well as the available countermeasures that can be utilized to neutralize these threats in the event of an attack or outbreak occurs. It will also present a number of known uses of biological weapons for ill intent which have occurred in the US since the turn of the 20th century. Finally the discussion will address some of the biosecurity and public health issues facing the modern biodefense industry.

CHAPTER 2: METHODS

An extensive amount of literature is available on the subjects of bioweapons and biodefense, ranging from hearsay and rumors to peer reviewed publications. This section addresses how the large amount of available information was narrowed to focus on the aims of this review paper. The first set of sources employed for this review was primary peer-reviewed research articles applicable to specific pathogens and vaccines. The articles were identified via the search engine PubMed, where keywords are used to search for relevant articles. The keywords used for the search include: Bioweapons, Biodefense vaccines, Biodefense Dual-use, and Bioterrorism countermeasures. Following each search, the relevant articles were placed into 1 of 4 major categories; Vaccines and Therapeutics Development, Policy, Biological Agents, and Other. A graphical representation of the search terms is shown below (Figure 1), all searches were completed in May and June of 2013 and many of the search term results produced overlapping results, i.e. identified the same articles as other terms. Identification of each pathogen was completed in a similar search manner but with the selection set for "review papers" only (Table 1). Select references were identified within each review article and accessed for specific information on desired pathogens. In certain cases, PubMed was capable of returning an article title but not the published article itself. In these cases, Google Scholar or UTMB Illiad reference request were utilized to obtain the complete published article.

The second source of references utilized for this review was published texts on the subject. A number of books have been published on the US, Soviet, and other countries government sponsored bioweapons programs. Investigational journalists and scientific writers wrote many of the published books on these topics; however, the scientists themselves, especially in relation to the Soviet program, were also involved in the writing of some of the books. Identification of these sources was completed from

published journals, Google search engine, Amazon book search, and personal communication with experts as described in the section below. The two books that provided some of the most important information on US and Soviet bioweapons programs were 'The Soviet Biological Weapons Program' by Leitenberg and Zilinskas and '21st Century Textbooks of Military Medicine.' These books also provided a significant number of references utilized in the writing of this manuscript not found during the PubMed database search. Finally, the authors reported a large number of first person accounts and interviews which were not found in other sources.

The third set of sources was personal interviews and the recommendations of scientists and researchers experienced in the field for identifying additional articles and books. These interactions occurred in multiple formats including one-on-one interviews, class discussions, conference meetings, and special seminars or panels with the experts. Similar to the book section, these conversations and discussions provided additional published papers and articles that were utilized in this review.

This review combines declassified reports, independent books on the subject, and published peer-reviewed articles to provide a concise review of the subject of bioweapons, and begins a discussion on the US response and preparation to future biological threats.

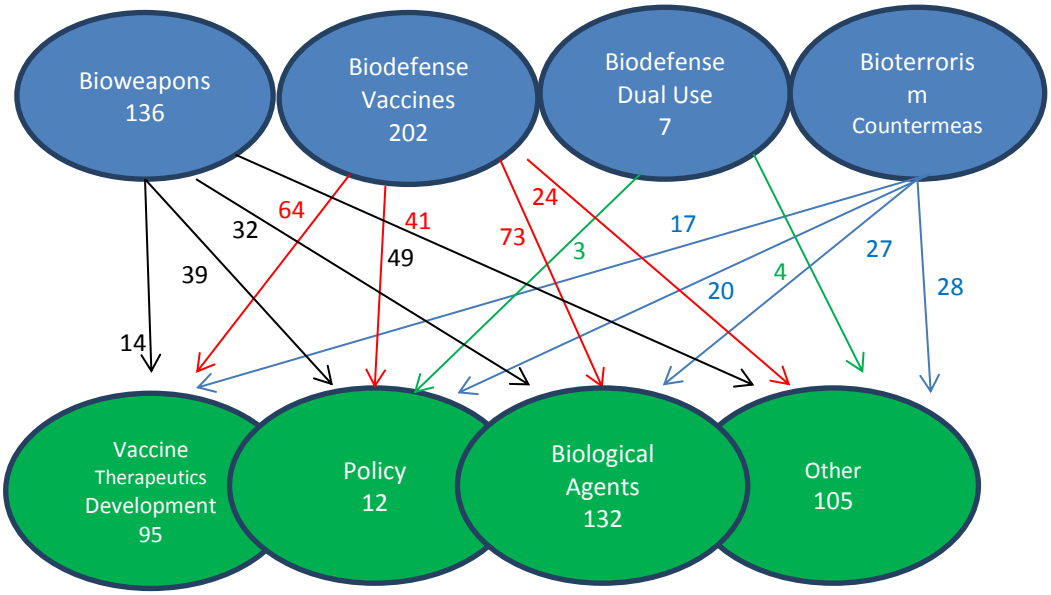

Figure 1: Key Phrases Searched for on PubMed.

CHAPTER 3: GOVERNMENT SPONSORED BIOWEAPONS PROGRAMS

3.1 THE UNITED STATES OF AMERICA BIOWEAPONS PROGRAM

The US sponsored one of the two largest bioweapons programs both in monetary funding and dedicated research personnel. The first step in the development of the US bioweapons program came about on the eve of the US entering WWII. In 1941, a US military committee identified the bioweapons threat and the vulnerability of the US if such an attack occurred. By 1942, President Roosevelt approved the War Reserve Service (WRS) to begin research and development against potential biological attacks to protect US civilians and troops. The original mission of the WRS was defensive in nature but it evolved to include offensive biological research and development programs. No offensive product from WRS was ever utilized against enemy soldiers during WWII but it was the first steps for a large scale offensive US bioweapons program.[8]

With the start of the Korean and the Cold Wars in 1950, the US military expanded research on identifying the threat bioweapons posed to troops and civilians. In parallel with the ideology of mutually assured destruction (MAD) in the nuclear arms race, the military desired the capability to counter any Soviet bioweapons attack with a bioweapons reprisal. With this goal, the modern US bioweapons program was founded with the opening of a large scale production facility in Pine Bluff, Arkansas.[6,9] By the time the US bioweapons program was shut down, it had 'weaponized' seven different pathogens and stockpiled three agricultural pathogens (Table 2).[2] The weaponization process for the bioweapons was varied for each pathogen and is still classified.

Throughout the 1950's, the US bioweapons program completed over 200 field tests of biological agents under Project 112/SHAD.[10] Many of these tests were completed at sea, away from civilian populations, but a number were completed on US soil and in populated cities.[11-13] These tests concluded that biological weapons were extremely cost

efficient, that they were was significantly less expensive per-kill than that of other conventional weaponry.[14] By the late 1960's public unrest with unconventional weapons, including bioweapons, was growing. This unrest was one factor which forced President Nixon to question the viability and necessity of the bioweapons program, both from political and military viewpoints.[15,16] In 1969, President Nixon ended the US bioweapons program, renouncing the use of bioweapons and directing the military to destroy all stockpiles of pathogens made during the program. The cancelation of the bioweapons program shifted funding and research toward the field which initially spawned the US program, biological defense (biodefense).[17] In 1972, the Biological Weapons Convention (BWC) was established as an international agreement prohibiting the development of biological pathogens (including toxins), which have no justifiable prophylactic, protective, or peaceful purpose.[18] It also calls for the destruction of the weapons and delivery systems which could utilize these bioweapons.[18] Both the US and USSR signed the BWC in 1972 and ratified it in 1975 within their respective governmental bodies.

3.2 THE UNION OF SOVIET SOCIALIST REPUBLICS BIOWEAPONS PROGRAM

The Russian program started with a similar goal as the US program, to develop countermeasures against infectious diseases in order to protect their soldiers in the field. Unlike their US counterpart, from the beginning of the 20th century to 1920, the Soviet Union had already fought in 3 major wars and suffered significant losses due to disease. This experience underscored the necessity of developing vaccines to protect their soldiers.

The bioweapons program in the USSR was initiated in 1926, soon after the end of WWI and can be described as having two major phases of development, classical and modern.[19] The classical phase began prior to WWII and lasted into the late 1960s and focused on identifying and stockpiling bacterial pathogens. During the early 1970's

multiple breakthroughs in technology occurred including Polymerase Chain Reaction (PCR), synthetic DNA, and gene sequencing. These advancements led to the development of entirely new fields of research including molecular genetics and bioengineering. These new fields of research allowed the Soviet program to shift into the modern era phase of bioweapons research.

By 1928, the offensive side of the Soviet biological research program was initiated. Early research focused on weaponizing *Bacillus anthracis*, botulinum toxin, *Vibrio cholera*, *Yersinia pestis,* and *Rickettsia typhus.*[19] By the early 1940s, the research and production facilities of the Soviet bioweapons program were only matched by that of the Japanese. Conflicting reports identified the use of *Francisella tularenesis* as an offensive bioweapon during the battle of Stalingrad. Some reports note a 10-fold increase in tularemia cases of German soldiers on the front line, while others describe the increase occurring in Soviet soldiers.[19,20] Following the end of WWII, Soviet forces captured a number of Japanese scientists who worked under Unit 731, Japan's premier bioweapons group. These scientists provided the Soviet program a significant amount of sensitive research on different lethal biological pathogens and their effects on human test subjects. The clinical data on human pathogenesis and disease development helped propel the Soviet bioweapons program into the Cold War. The classical era of Soviet bioweapons research incorporated basic science in characterizing pathogen culture/growth, disease development, and stockpiling. By the end of this first phase, the Soviet bioweapons program had researched and weaponized 11 different pathogens (Table 2).

With the advent of the new field of 'biotechnology,' Soviet researchers were able to elucidate many of the genetic factors of pathogenesis and utilize this knowledge to rationally modify numerous pathogens. The discovery of novel viral pathogens, including Marburg and Ebola viruses, also provided potentially new bioweapons due to their high mortality rates and short incubation time. The modern second phase of the Soviet bioweapons program was organized under the civilian organization Biopreparat with the

codename ‘Fermenty’ (Enzymes). The goal of the second phase was to utilize modern technology and research, specifically molecular biology and genetic engineering, to update the bioweapons stockpile from the first phase.[19]

Research under Biopreparat included open air testing of aerosolized pathogens, vaccine development, and antibiotic resistance adaptation to the bacterial agents. Research on viral agents included identification of infectivity dose, disease development, and identifying methods of stabilizing the pathogen for aerosol exposure. At the time of the collapse of the USSR in 1991, the researchers at Biopreparat had developed 8 different bioweapons and studied an additional 15 pathogens with the potential for weaponization.[5]

3.3 Other Bioweapons Programs:

While the two largest government sponsored programs have been briefly described above, other countries also developed their own bioweapons programs in the 20th century. Historically, 12 countries have been identified as having an active bioweapons program. These 12 are the US, USSR, Japan, Germany, France, Iraq, Iran, South Africa, North Korea, United Kingdom, Canada, and Syria. An additional 17 countries, including modern Russia, are believed to have research programs which could be used to generate stockpiles of bioweapons (Figure 2).[4] These programs ranged from stockpiling only a single bioweapon, such as Canada, to researching and stockpiling more than 10 bioweapons in the case of Japan and Iraq.[4]

While many of these programs never resulted in the intentional release of bioweapons, the Japanese bioweapons program holds an infamous reputation for their willingness to utilize civilians and prisoners of war (POWs) as test subjects in lethal biomedical research.[9,21] By the middle of WWII, Japanese bioweapons research and development was believed to have been the largest and most advanced program in the

world.[19,21] Throughout the Japanese occupation of China, researchers at Unit 731, the primary Japanese bioweapon research group, utilized POWs and Chinese civilians to test bioweapons and perform biomedical research. They performed live pathogen release experiments on villages to determine exposure and mortality rates for diseases like plague and anthrax.[21] The research completed by the Japanese bioweapons program during WWII was condemned by the Allied Nations. Notwithstanding these condemnations, both the US and USSR utilized the Japanese research in their own programs after the end of WWII.

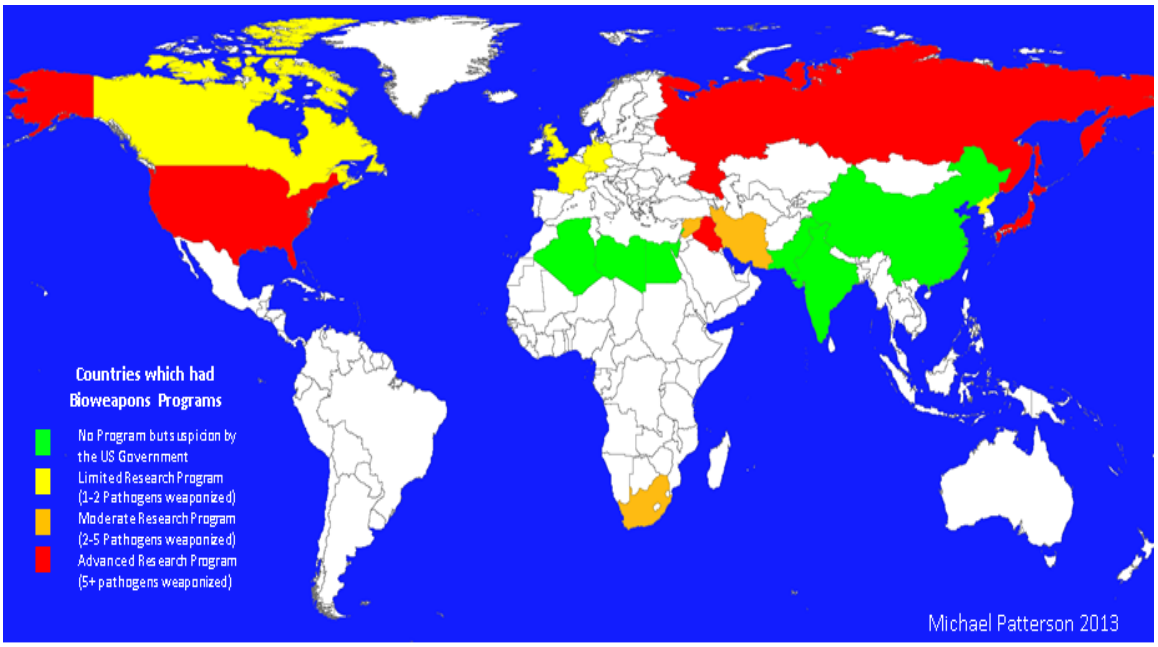

Figure 2: Map of Countries with Bioweapons Programs.

Countries in red: US, USSR, Japan and Iraq. In orange: Iran, South Africa, and Syria. In yellow: Canada, France, Germany, North Korea and United Kingdom. In green: Algeria, China, Egypt, India, Israel, Libya, Pakistan, Russia, Sudan and Taiwan.

CHAPTER 4: VALIDATED BIOWEAPONS

The difference between a bioweapons program and a biodefense program can be ambiguous. Many of the procedures used to study a pathogen with the goal of making a countermeasure can also be utilized to make a bioweapon. These procedures are commonly referred to as 'dual-use.' Early bioweapons programs utilized the basic understanding of trait-selection, similar to that of breeding horses by breeding only the strongest or fastest horses. Likewise only the most virulent strains were selected but without a strong understanding of the genetics involved. This methodology is also applicable in a biodefense setting when selecting for an attenuated and less virulent strain for developing a vaccine. The advent of molecular genetics, PCR, and synthetic genetics has made possible the rational modification of pathogens, either to attenuate for vaccine development or to increase virulence for bioweapon design.

All current National Institutes of Health (NIH) funded research must now address the issue of dual-use in an effort to ensure that all research has a minimal risk of increasing the virulence of the pathogen being studied. Recently, influenza research thrust the issue of dual-use into the international spotlight, identifying mutations linked to animal-to-animal transmission.[22] As research techniques and methodologies are developed to take advantage of new and more powerful research equipment, identifying the scientific line between biodefense and bioweapons may become more difficult. This lack of clarity will leave a single characteristic to distinguish a biodefense program from a bioweapons program in modern research: the intent of the researcher.

While the scientific steps in modifying a pathogen into a bioweapon are not easily accessible, the developmental goals for these modifications can be extrapolated. Both the US and Soviet program selected pathogens to fulfill three different roles within their program. The first targeted characteristic was a high mortality pathogen, which could

rapidly affect a target population. The second role to fulfill was a high morbidity and low mortality, which could rapidly debilitate a target population without a significant number of deaths. The third role was economic; targeting the agriculture and livestock industries could significantly weaken a nation's economy without directly infecting a population. Eleven identifiers for selecting pathogens to fulfill these roles have been described elsewhere.[23] These identifiers are:

1. Aerosolization – Aerosolization allows for easy dispersal of a pathogen over a wide area with a measurable exposure time. Without this route of exposure, it is difficult to ensure a large number of people are infected at the same time.
2. Stability – The more stable an agent, the better chance for infectious exposure. Many viral and some bacterial agents are unstable, especially when exposed to UV light.
3. Pathogen infectivity – The infectivity dose (ID) is the number of pathogen particles necessary to cause an infection. The lower the ID, the more targets that may become infected with a single attack.
4. Pathogen virulence – The virulence of a pathogen is directly correlated to the capability of the organism to cause disease including death. The virulence of a pathogen will identify the role it may fulfill.
5. Incubation period – The length of time a pathogen requires to grow to a significant load to cause the intended disease. A shorter incubation time equates to less time for the target to identify the exposure and provide a countermeasure.
6. Immunity of the target population – If a large percentage of a target population is immune to the pathogen through previous exposure or through vaccination; that population may not be affected by an attack from that specific pathogen.
7. Available countermeasures – Similar to immunity, if a population has easily available and accessible countermeasures, a pathogen may not be capable of fulfilling its role in an attack.
8. Transmission capability – The capability of a pathogen to replicate and infect additional targets is unique to biological weapons. A higher transmission capability may result in a larger population infected but may also make invasion difficult by an unprotected force. A lower transmission capability may result in the pathogen failing to infect enough targets to fulfill its primary goal.

9. Identification of the pathogen – Many pathogens have specific clinical symptoms or physiological indicators which assist in the identification. If a pathogen has been modified or is difficult to identify, it may extend the time period between pathogen exposure, diagnosis and countermeasure delivery.
10. Protecting non-target populations – Immunization of non-target populations, especially of the attacking nation, may greatly reduce the chance of an unintentional outbreak following the release of a bioweapon.
11. Inexpensive mass production – The cost of researching, producing, and storing biological weapons may be significant. The capability to quickly and safely grow different pathogens may be a factor in the selection process. Cost analysis identified biological weapons as potentially significantly less expensive than other weapons, such as chemical weapons (CW), in cost to death ratio.[14]

Some of these identifiers are inherent in certain naturally occurring pathogens, such as Ebola, with a high virulence/mortality rate.[24] Other factors may be modified with the insertion of additional antibiotic-resistance genes, as reported with the Soviet research on *F. tularensis* and *B. anthracis.*[20,25] The US Department of Agriculture (USDA), Department of Health and Human Services (HHS), and the CDC have identified a list of pathogens which pose a significant threat to human and animal life and list them under the designation 'Select Agents.' Many of these pathogens have been identified as potential or actual bioweapons. This list of identifiers helps law enforcement and regulators develop a risk assessment of hazardous pathogens in the event of an attack or release.[26]

A description of many of the bioweapons generated by the US and USSR bioweapons programs, including a selection of the modern countermeasures available for each pathogen, is described below. The bioweapons are separated based upon the desired effect each pathogen has on a target population: mortality, morbidity, or agriculture based. It will not address pathogens that were researched but not officially stockpiled or weaponized, nor will it address the biological toxins that were weaponized, such as

Botulism toxin, staphylococcal entertoxin B, and Ricin. Table 3 in Appendix A provides a reference guide for the pathogens reviewed below.

4.1 LETHAL BIOWEAPONS

4.1.1 *Bacillus anthracis*

The etiological agent for anthrax, *B. anthracis,* is a gram-positive, rod shaped bacteria that grows under both aerobic and anaerobic environmental conditions. Anthrax is a naturally occurring disease reported in both humans and animals as far back as the 15th century BC.[27,28] *B. anthracis* exposures occur annually in most developing countries while the disease rarely occurs in humans in developed countries, such as the US. Most cases of anthrax are reported in herbivores that consume the bacterial spores while grazing.[28] Humans are primarily exposed through consumption of infected animals or contact with contaminated animal products, such as wool or pelts.

Virulence is dependent upon two major factors, the polysaccharide capsule and an extracellular toxin produced by specific strains.[29,30] The production of the toxin is dependent upon the presence of 2 or 3 specific genes. These genes encode for specific proteins that individually do not cause disease but in combination can cause localized edema or death.[27] Patients can be infected with anthrax in one of three ways depending upon the route of exposure. The most commonly reported exposure method in humans is cutaneous, followed by inhalation and gastrointestinal. Cutaneous exposure occurs most often in the occupational environment. This exposure occurs commonly when a worker has an open wound which is exposed to the bacterium or is bit by an insect after it has fed on an infected animal. Reported mortality rate for cutaneous anthrax is around 20% if left untreated. Inhalation anthrax occurs when a person breathes in spores and is the most lethal, with an estimated mortality rate of 50-90% even with quick antibiotic treatment at the time diagnosis. Gastrointestinal anthrax is also highly lethal, between 25-60%

mortality rate, but extremely rare, it requires consumption of contaminated food or drink.[27]

Anthrax is recognized as one of the most widely researched diseases across all of the national bioweapons programs. To date, an estimated 13 countries have researched and stockpiled anthrax for use as a bioweapon.[4,5] The earliest report of *B. anthracis* being used as a bioweapon was during WWI when a German operative infected equines waiting to be shipped from the US to France.[9] *B. anthracis* was a popular pathogen for bioweapons programs for multiple reasons. As a naturally occurring pathogen, it was easy and inexpensive to collect pathogenic spores from the wild. Spore formation occurs at a specific stage in the bacterial life cycle in which environmental stress becomes too great for the bacteria to survive. The spores are highly resistant to UV light, temperature changes, pH changes, common disinfectants, and can remain infectious for decades following release.[31]

Both the US and Soviet programs generated stockpiles of weaponized anthrax for use as a bioweapon. Minimal data exists on the virulence modifications made for the 'weaponized' form of anthrax within the US program. Thousands of cow patties contaminated with anthrax spores were generated and sent to the UK during WWII, but they were never utilized in an offensive manner.[32] Studies requisitioned by the US Senate developed multiple models predicting significant loss of life and economic loss following an attack with aerosolized anthrax on a US city.[33] Reports on the Soviet program are more available, especially the genetic modifications completed in the late 1980's following the advent of molecular genetics in the second phase of the program. Early selection studies identified or generated strains resistant to antibiotics. Studying these strains, the Soviet researchers were able to duplicate and insert the resistance genes while maintaining pathogen virulence. They were also able to identify and duplicate the genes linked to the toxic proteins and insert them into another bacterial strain. This made the diagnoses of an infection much more difficult for an enemy state.[19]

B. anthracis made an ideal first bioweapon for many of the national programs due to its high virulence, natural stability, capability to be aerosolized easily, low infectivity dose, short incubation time, and inexpensive production.

Countermeasures For *B. anthracis*

Antibiotic therapeutics are available for anthrax and recovery is dependent upon quick recognition of the disease by a physician or foreknowledge of exposure. Due to the quick incubation time, especially of inhalational anthrax, rapid initiation of antibiotic treatment plays a key role in a positive outcome for the patient. Multiple antibiotics have been shown to be effective in treating anthrax but speed in diagnosis is important in ensuring efficacy of treatment. The two recommended antibiotics are intravenous (IV) infusion of ciprofloxacin (500mg every 12 hours for up to 60 days) and/or doxycycline (100mg every 12 hours for up to 60 days).[34] Additional antibiotics that have been shown to be partially effective in preventing significant disease include: rifampicin, vancomycin, imipenem, chloramphenicol, aminoglycosides, penicillin, ampicillin, clindamycin and clarithromycin. A licensed vaccine under the name BioThrax™ is approved for use in the US. It is an attenuated and non-encapsulated strain of *B. anthracis.* It was first licensed primarily for military use in 1970 and requires 6 doses within the first 18 months and annual boosters thereafter, impeding acceptable vaccination rates among the general populace.[35]

4.1.2 *Francisella tularenesis*

Francisella tularenesis is the etiological agent for tularemia, a disease described less than a century ago.[36] It is an aerobic, gram-negative, intracellular bacterium that is unable to generate spores but is stable for weeks in water and soil. Two major strains are

reported to cause lethal disease, biovar *tularensis* and biovar *holartica* (also known as biovar *palaearctica*), the former having a case fatality rate up to 7% if left untreated. Cases of tularemia have only been reported in the northern hemisphere. Tularemia is maintained in nature through arthropods feeding on infected animals and then spreading the bacteria to uninfected animals during a second feeding.

The most common route of exposure for humans is through the arthropod vector.[37] An alternative and less common route is through aerosolized exposure of an infected animals excreta or secreta. Both routes have been described as having a low infectivity dose, as few as 50 infectious particles. On average, clinical disease develops 3-5 days after exposure. Initial symptoms include fever, malaise, and fatigue similar to many febrile illnesses.[37] Further disease development commonly follows six different pathways: ulceroglandular, glandular, oculoglandular, oropharyngeal, pneumonic, and typhoidal.[38]

Both the US and Soviet bioweapons programs generated stocks of *F. tularenesis* for use as a bioweapon. While the case mortality rate is low, the advantages of the low infectivity dose, stability in the environment, and the proven capability to aerosolize the agent lead to *F. tularenesis* to be selected as a bioweapon. The US program was able to select for a resistant strain to a single antibiotic in the 1950's but it could still be treated with the alternative antibiotics.[19] The Soviet program focused on developing multiple antibiotic resistant strains and initial attempts were successful in providing resistance but pathogenicity was greatly reduced, decreasing its viability as a bioweapon. Further research in the mid-1980s by the Soviet program was successful in inserting multiple resistance genes, which generated a multi-resistant and highly-pathogenic strain suitable for use as a bioweapon. Their success with *F. tularenesis* was the initial breakthrough which allowed the Soviets to insert resistance genes into other bioweapons.[19,20]

Countermeasures For *F. tularenesis*

The preferred antibiotic treatment for a single or limited number of cases is streptomycin (1g intramuscular (IM) daily for 12 days) or gentamicin (5mg/kg IV or IM daily for 10 days). In situations with a large number of cases, ciprofloxacin (500mg orally once daily for 10 days) or doxycycline (10mg orally once daily for 21 days) are recommended due to limited availability of IV and IM antibiotics. A live-attenuated vaccine is used extensively in Russia and to a more limited extent in other countries for individuals at a high risk of exposure.[39] It is not recommended as a post-exposure prophylactic.

4.1.3 Filoviruses

Two members of the virus family *Filoviridae*, Marburg virus (MARV) and Ebola virus (EBOV) are highly virulent pathogens, which can cause viral hemorrhagic fever. The first reported cases caused by MARV occurred in 1967 in Germany where laboratory workers dissecting green monkey organs imported from Uganda became ill. The overall mortality rate of the six reported human outbreaks of MARV is 88%.[24,40] Similar in disease development to MARV, EBOV was first isolated in an outbreak in Zaire, later named the Democratic Republic of the Congo (DRC), in 1976. Four distinct strains of EBOV have been identified with three of them reported to cause human disease. The Reston strain was identified in a colony of cynomolgus monkeys imported into the US in 1988 which caused significant disease in the animals but no reported disease in the humans.[41] The other three strains have a reported case mortality rate of 70%. While EBOV has a lower reported mortality rate in humans than MARV, it has had nearly three times as many reported cases.[40]

Filoviruses are enveloped, negative-sense, single-stranded RNA viruses with a distinctive bacilliform shape when viewed with an electron microscope. The natural

reservoir of filoviruses has been very difficult to identify but recent publications point towards fruit bats as the natural host.[42,43] Spread from the natural reservoir is most likely from exposure to bat excreta. Multiple mammal species have been shown to be susceptible to filovirus infection. Some human outbreaks, such as the first outbreak of MARV in Germany, have been through contact with infected non-human primates (NHPs). Other cases of human-to-human transmission have occurred through needle sharing, sexual contact, direct contact with an infected deceased individual and breast feeding.[24,44] Researchers have also identified that pigs can become lethally infected and spread the infection to NHPs with a lethal outcome. Domestic canines have been shown to develop neutralizing antibodies against EBOV but no development of clinical disease.[45,46]

The incubation period of MARV is 3-14 days with initial clinical presentation of a febrile disease, similar to many other viral infections. Symptoms can include fever, headache, diarrhea, vomiting, and myalgia.[44,47-49] As the disease progresses the development of a maculopapular rash, skin lesions, skin sensitivity, petechiae, increased vascular permeability and bleeding from mucus membranes have all been reported in humans. The convalescent phase can last months, with live virus able to be isolated during this time from the urine and ocular fluid of infected patients.[50-52]

No reports were found from the US bioweapons program on the weaponization of a filovirus, most likely due to the isolation of MARV two years prior to the closure of the program. The Soviet program continued into the early 1990's, giving them the opportunity to study both viruses with the goal of weaponization. Both viruses have high mortality rates and neither can survive for an extended period in the environment. These traits make them an ideal weapon for target elimination from a specific region which is quickly followed by invasion by friendly troops as the pathogen will not stay infectious in the environment for long.[19] Most of the Soviet research focused on weaponizing MARV instead of EBOV in part because of its reported higher mortality rate, slightly better

stability in the environment, and the ease of growing large quantities of the virus.[19,20] Once highly concentrated liquid stocks were generated, the researchers focused on generating dry stocks of MARV that would allow for easy aerosolization. Attempts to weaponize the virus through genetic modification during the second phase of research were unsuccessful, as were all attempts to formulate an efficacious vaccine. Research on EBOV was initiated a few years after MARV with the primary focus of characterizing the disease and developing a vaccine. While this research did help to elucidate some of the genetic factors of virulence, a protective vaccine was never developed.[19]

Countermeasures For Filoviruses

The standard of care for infected patients includes administration of fluids and electrolytes, blood transfusions when necessary, and antibiotics for secondary infections.[40] Treatment with immune serum and neutralizing monoclonal antibodies has been shown to be effective in animal models but clinical trials in infected humans have not been completed.[47,50,53,54] No FDA approved vaccines are available to the public but multiple candidates are in clinical trials that show immunogenicity and are safe in humans. Many of these vaccines utilize the expression of the viral surface glycoprotein as the primary antigenic target.[55] Unlike treatment for other hemorrhagic viruses, ribavirin has not been shown to be effective in inhibiting clinical disease development.

4.1.4 Smallpox virus

Smallpox is one of the oldest reported infectious diseases in human history, first identified as far back as 10,000 years in Asia and Egypt.[56] It eventually spread to Europe and the Western hemisphere, contributing to the collapse and conquering of the Incan and Aztec empires in South America. The earliest reports of smallpox used as an intentional

bioweapon was during the French and Indian war, 1754-67, when British soldiers 'gifted' Native Americans with contaminated blankets.[2,6] Throughout the history of smallpox as a human disease 10s to 100s of millions of people are estimated to have died from the disease. Smallpox is unique; it is the only virus eliminated from the natural world through medical intervention. This eradication was accomplished through the cooperation of numerous countries, especially the US and USSR, the availability of an efficacious and stable vaccine, and the lack of a natural animal reservoir. The last reported natural exposure case of smallpox occurred in 1977 in Somalia.[57]

Two closely related viruses cause smallpox: variola major and variola minor. Clinically, the smallpox virus can be subdivided into four subtypes; ordinary smallpox, attenuated smallpox, flat smallpox and hemorrhagic smallpox. The most common subtype, ordinary, occurs in around 90% of cases with a mortality rate around 30%.[38,58] The incubation time of smallpox is 7-17 days, averaging 10 days post infection. The prodromal/febrile stage generally lasts 48-72 hours with symptoms including fever, malaise, headache, and back pain. Following the febrile phase, a rash normally appears along with enanthema on the face, hands and forearms. After an additional 24-48 hours, the rash develops into lesions and pustules that start to crust and fall off after 8-9 days leaving depressed scars.[59] The patients are usually infectious 1-2 days prior to enanthema development.[59,60] The three other subtypes of smallpox are much rarer and are either less pathogenic, as with the attenuated form, or nearly 100% fatal, as seen in hemorrhagic smallpox.[58]

The US bioweapons program never developed a weaponized form of the smallpox virus. The Soviets are reported to have started studying smallpox as a potential bioweapon in the late 1960s. However, their results are not well reported.[19] The highly pathogenic form of smallpox stockpiled by the Soviets is believed to have been acquired from a 1959 outbreak in India, not through serial passaging.[19] The methodology of producing large quantities of pathogenic smallpox through serial passaging, with chicken

eggs as a growth host, was developed during the classical era of Soviet bioweapons research. This method of growth required a substantial infrastructure and a large number of chickens to produce the eggs, making it inefficient and costly. During the modern phase of the Soviet bioweapons research, scientists worked to develop techniques for virus propagation in cell culture with the goals of eliminating the need for eggs and reducing the cost and infrastructure required. Successful in this endeavor, researchers were able to utilize cell culture to generate a highly concentrated and virulent stock of smallpox without genetically modifying the virus. At peak production, the generation of up to two tons of infectious and aerosolizable smallpox could be generated annually, more than enough to infect every person in the world.[19,20]

Countermeasures For Smallpox

Methods for countering smallpox have existed for centuries with limited success. The earliest form, variolation, was the intentional exposure of a patient to smallpox through contact to either a dry scab or the pus from a lesion.[56] This form of exposure often resulted in a mild form of the disease with a significantly decreased mortality rate. The first vaccine utilized an attenuated pox virus, cow pox, which proved partially immunogenic against smallpox. The modern smallpox vaccine strain, based on the vaccinia virus, was introduced in the mid-19th century with higher immunogenicity and fewer side effects.[57] The first generation vaccines, such the vaccine Dryvax which was first produced in the late 19th century, were administered through bifurcated needle punctures of the epidermal layer. A second generation vaccine, ACAM2000, was approved by the FDA in 2007.[61] Utilizing cell-culture instead of cows, ACAM2000 can be grown faster and with less infrastructure than Dryvax.

The availability of alternative therapeutics for smallpox is limited. Immune serum from recently vaccinated individuals has been utilized to treat smallpox infections but this

requires access to an immunized population and/or storage of the immune serum[62]. Cidofovir has been shown to inhibit virus growth *in vitro* and *in vivo* but it has not been utilized in human cases.[62,63] In 2012, the SNS purchased 1.7 million doses of a novel therapeutic Tecovirimat (ST-246), which has been shown to be efficacious in inhibiting smallpox disease development in animal models.[64]

4.1.5 *Yersinia pestis*

Yesinia pestis is a gram-negative, anaerobic bacterium that is the etiological agent of the human disease plague. The bacteria are transmitted by flea bites and are normally maintained within an enzootic cycle of different rodents to fleas. Many of these rodents are less susceptible to the bacteria than humans. The switch from the enzootic cycle to an epidemic cycle involving humans can occur for a multitude of reasons. One reason is a change in the bacteria virulence resulting in an increased mortality in the rodent host. When this occurs the flea may seek an alternative food source. Another factor can be increased rodent populations and failed pest control. When humans or other animals are living in close proximity the chances of exposure to an infected flea increases.[65] Plague is believed to have caused more than 200 million deaths in recorded history, with a significant percentage of those deaths during the outbreaks in the 6th, 14th, and 20th centuries.[9,66]

Y. pestis requires very few living organisms to infect a mammalian host and research has identified that a single live bacterium could be enough to cause a lethal infection when exposure occurs through the oral (aerosolized particles), intradermal, or intravenous routes.[67] While a single bacterium could be lethal, it is more likely the actual infectivity dose is between 100-20,000 organisms.[67] The incubation period for disease development after exposure is dependent upon the route of exposure. Intradermal

exposure has an incubation period of 2-6 days while aerosolized exposure incubation time can be as short as 1-3 days.[9]

Clinical disease development in humans infected with *Y. pestis* can be divided into three categories, bubonic, septicemic and pneumonic. In cases of bubonic plague, the bacterium grows primarily within the lymph nodes. Patients develop symptoms, which can include malaise, headache, vomiting, cough, and upper abdomen pain. Hours following symptom development, patients can develop buboes, swelling and painful sores at the lymph nodes, which blacken following tissue necrosis and become suppurative. Untreated bubonic plague has a case fatality rate ranging from 50-60%.[9,38] If treated with antibiotics, the mortality rate drops to 1%.[68] Septicemic plague occurs when the bacterium primarily grows within the bloodstream of a patient instead of the lymph system. Diagnosis of this form of the disease is more difficult, and treatment is often initiated later than with bubonic plague. Partially due to this reason, septicemic plague has a higher mortality rate of approximately 50% when compared to bubonic plague. Pneumonic plague is an infection of the respiratory system and is transmitted by aerosolized bacterium. It is the only form of plague which can spread through nosocomial exposure.[9] Disease progression is extremely rapid and clinical signs are difficult to distinguish from other respiratory disease. Pneumonic plague is almost always lethal without treatment. Modern antibiotics can reduce the mortality rate 50% but quick diagnosis and treatment is extremely important.[38]

The USSR initially started research on *Y. pestis* with the desire to make a viable vaccine against the bacterium. From that research, an immunogenic, whole-cell killed vaccine was developed and produced against plague. By the end of the first phase of Soviet research in the 1960s, research progressed towards isolating more pathogenic forms of the bacteria for use as a weapon. While the success of this selection process is not known, Russian laboratories currently classify *Y. pestis* comparable to that of Ebola, a biosafety level (BSL) 4 pathogen in the US, while US researchers identify it as a BSL3

pathogen. This difference in biosafety may identify a difference in the virulence of the stock strains between the two countries.[19] During the second phase of research in the 1980s, Soviet scientists made two attempts to further weaponize *Y. pestis* using different methodologies. The first attempt was to introduce antibiotic resistant genes in a similar manner as the resistance studies with anthrax. While successful at introducing antibiotic resistance genes into the pathogen, a significant reduction in pathogenesis resulted diminishing the viability of the bioweapon.[19] The second attempt to genetically modify *Y. pestis* was to include the genome of Venezuelan equine encephalitis virus (VEEV) within the genome of the bacteria, which would be expressed as an infectious virus at the time of antibiotic treatment for plague. The success of this research has not been reported.[19] No official report of US researchers studying plague as a bioweapon has been identified.

Countermeasures For *Yersinia pestis*

One of the most important factors in a positive outcome for plague is early diagnosis by a physician. The disease develops so rapidly from febrile to systemic, the quicker an antibiotic can be administered, the better the expected outcome for the patient. The primary antibiotic recommended for wild type plague is streptomycin (30mg/kg IM daily for 10 days). In the US, gentamicin is the recommended alternative therapeutic (5mg/kg IM or IV daily for 10 days).[68] Other effective antibiotics include doxycycline, ciprofloxacin, levaquin, and chloroamphenicol.[68,69] A whole-cell killed vaccine was utilized by the US military during the Vietnam War, but it is not currently FDA approved for use in civilian populations.[70,71]

4.2 INCAPACITATING BIOWEAPONS

4.2.1 *Brucella suis*

Brucella suis was first isolated and described in 1914 from samples acquired from an aborted fetus of a sow.[72] *B. suis* is one of four proteobacteria known to cause the disease brucellosis in humans. Brucellosis has been a recognized disease of humans for at least 300 years, with earlier descriptions of similar disease ranging back as far as Hippocrates.[73] Each of the four proteobacteria has a predilection to a specific mammal but all can infect humans with a low infectivity dose (10-100 organisms).[74] The bacteria are small, nonmotile and nonsporulating pathogens.[73] Different from many other pathogenic bacteria, *B. suis* does not generate exo- or endotoxins as normal virulence factors nor does its lipopolysaccharide (LPS) layer generate a normal alternative complement response.[75] Lacking these traits make it difficult for the immune system to recognize and achieve a quick immunological response.[75]

Humans are most often infected through consumption of contaminated animal products such as dairy, animal tissue, direct contact with infected animals, or exposure to aerosolized infectious particles.[75] Disease can progress in two primary presentations, acute or subacute. The acute phase takes 2-4 weeks for disease to develop in humans while the subacute phase can take up to a year before any symptoms develop.[76] Symptoms include fever, malaise, and weakness with complications from infection including arthritis, central nervous system (CNS) disorders, vomiting, and death. The mortality rate is very low, under 2% of those infected, while the relapse rate of the disease is closer to 10%.[75]

Both the US and Soviet programs weaponized strains of *B. suis* for use as an incapacitating bioweapon. The US program grew up large stocks of the bacteria in 1942 and prepared bombs for aerosolized release during WWII but they were never utilized. Field tests completed on animals in 1945-46 proved the effectiveness of aerosolized

spread of the pathogen but all stockpiles were destroyed in 1969.[11,73] Recent modeling of the impact of an aerosolized attack of *B. suis* on a US city identified an economic impact of nearly $500 million but minimal loss of life.[33] The Soviet program also developed a weaponized form of *B. suis* with a similar intent as their US counterparts for designing an incapacitating agent. Stockpiles and bombs for aerosolized release were generated during the first phase of the program. Attempts to introduce antibiotic resistance in the second phase in a similar manner as anthrax failed.[19] *B. suis* is unique within the two bioweapons programs. It has an extended incubation period without sporulation and can cause high morbidity in both humans and livestock, making *B. suis* an ideal civilian and economic bioweapon.

Countermeasures For *B. suis*

The development and identification of countermeasures for *B. suis* is difficult due to the difference between *in vitro* and *in vivo* infections. During an animal or human infection, the bacteria can be found within macrophages which necessitates antibiotic penetration to ensure elimination of the infection.[75] The World Health Organization (WHO) has two recommended treatment guidelines. Both regimens utilize doxycycline for 6 weeks in combination with either streptomycin for three weeks or rifampin for 6 weeks (100mg orally twice daily for all 3 antibiotics).[75] Other antibiotics shown to be efficacious are gentamicin, ciprofloxacin, and tetracycline. No vaccines for brucellosis have been approved by the FDA for use in humans. The utilization of the USDA approved vaccine in livestock and other brucellosis eradication programs have greatly reduced annual losses farmers suffered in categories including milk production, spontaneous livestock fetus abortions, and reduced breeding efficiency. The monetary loss in 1952 prior to vaccination was estimated at $400 million, while current losses due to disease are estimated at under $1 million anually.[76,77]

4.2.2 *Coxiella burnetii*

Coxiella burnetii is the etiological agent of Q fever, an infectious human disease first recognized, concurrently, in slaughterhouses in Australia and the US in the mid-1930s.[78] The bacterium is an obligate, intracellular, gram-negative pathogen with limited motility. While it is nonsporulating, it has two differing cell cycle forms, a large cell variant (LCV) which is metabolically active and a small cell variant (SCV) which is highly resilient to environmental changes, even outside a host, while still remaining infectious.[79] The growth cycle of the bacteria is slow, around 8 hours per cellular division, and occurs primarily within the low pH environment of the eukaryote phagolysosome.[80,81] The environmentally resistant SCV form requires exposure to 70% ethanol, 5% chloroform, or 5% formalin for 30 minutes before becoming inactivated; 10% bleach is not effective at killing all organisms.[82] Pasteurization and gamma irradiation have also been shown to be effective methods of inactivation.[83,84]

A zoonosis, Q fever is found in nearly every country in the world and has a very broad range of animal hosts. Exposure to infected ungulates and ticks are two of the most common routes of human infection in a rural setting.[85] A third route of infection is through aerosolized particles of *C. burnetii* which can remain infectious months after the bacteria leave an infected animal.[86] The infectious dose is believed to be under ten organisms, making infection very likely following exposure.[87] A proven route of infection in an urban setting is exposure to infected or contaminated cats and dogs.[88,89] Animals can become infected in the same manner as humans, and this may assist in delayed propagation of the disease following an attack while the animal host incubates the virus. Most infected animals are asymptomatic, other than an increase in spontaneous abortions, but they can still shed large quantities of infectious materials.[90] Cattle and goats have been shown to become chronically infected while sheep present with an acute

infection and clearance.[91] The chronic, asymptomatic infection of these animals makes eradication of the disease difficult.

As seen in animals, Q fever in humans presents clinically in 3 different forms: asymptomatic, acute and chronic. Most human infections are asymptomatic. The incubation period for acute disease can be a few days to a number of weeks.[92] The most common symptoms are fever, headache, and chills which can last up to 13 days.[93] Additional symptoms include cough, nausea, vomiting, myalgia, pneumonia, and rarely meningoencephalitis, but infection rarely results in death.[78] Diagnosing acute Q fever in humans is difficult as there are few unique characteristic symptoms. The chronic form of Q fever is uncommon but it has a higher mortality rate than the acute form. Patients with preexisting conditions, such as Human Immunodeficiency Virus (HIV) or immunosuppression, are more likely to develop chronic Q fever.[94,95]

The US and USSR are the only countries reported to have weaponized *C. burnetii*. Q fever is a disease with low mortality to morbidity ratio, with a low infectivity dose, high stability in the environment, easy aerosolization, and is accessible from naturally endemic regions for isolation. The US once stockpiled large quantities of *C. burnetii* and intentionally exposed volunteers to aerosolized *C. burnetii* to determine the capability of the pathogen as a weapon.[96] The Soviet program also stockpiled *C. burnetii* and, as previously mentioned, was rumored to have utilized the pathogen against the Germans during WWII.[20] Data on second phase genetic research completed by the Soviets studying *C. burnetii* is limited. This may be due to a failure to instill the same level of antibiotic resistance as they did in anthrax, or due to a lack of interest in further research due to the low mortality of Q fever.

Countermeasures For *Coxiella burnetii*

As most cases of *C. burnetii* are either self-limiting or asymptomatic, it is often cleared from a patient before a diagnosis can be made and antibiotics prescribed. For those who are diagnosed quickly, or who are known to have had an exposure, the two recommended antibiotics are tetracycline or doxycycline (100mg twice daily), administered for an average of 2 weeks.[97] In serious or chronic cases of Q fever, corticosteroids in combination with antibiotics have been shown to be effective; however, relapse commonly occurs once the antibiotic regiment is completed. Other antibiotics which have been shown to be effective are erythromycin, pefloxacin, rifampicin, and potentially ciprofloxacin.[97] While not approved for public use, a formalin-inactivated whole-cell vaccine has been shown to be immunogenic and safe. Australia utilizes a whole-cell vaccine which showed strong and long lasting immunity in 4,000 volunteers.[98]

4.2.3 Venezuelan equine encephalitis virus

A member of the family *Togaviridae* and genus *Alphavirus*, Venezuelan equine encephalitis virus (VEEV) was first isolated from a fatal equine case in 1936.[99] It was identified as a human pathogen during an outbreak in Columbia in 1952.[100] The virus is transmitted by mosquitoes, predominately *Culex*, and is maintained in a sylvatic cycle within a number of small vertebrate hosts and the mosquito. Humans and other animals become dead-end hosts through accidental exposure during the natural sylvatic cycle but these exposures to endemic strains do not normally result in an epidemic.[101] The change from a sylvatic cycle to an epidemic or urban cycle in equines and then humans is believed to be necessitated by significant mutations in the virus genome.[101] During these epidemic cycles, equines are extremely susceptible to the disease. Before succumbing, many horses act as amplifying hosts, generating high numbers of infectious virus particles in their blood, which, when fed on by mosquitoes, continues the epidemic cycle.

More than a dozen VEEV outbreaks with reported human disease have occurred since the initial outbreak in the 1950s, including one which reached southern Texas in 1970-71.[102,103]

Clinically, VEEV has the lowest mortality rate of the three New World encephalitic alphaviruses.[104] The other two viruses, Eastern equine encephalitis (EEEV) and Western equine encephalitis (WEEV), can have mortality rates up to 50%. With all 3 viruses, most deaths occur in children under 10, the elderly, or the immunocomprimised.[105-108] The incubation period in humans is 2-5 days followed with a rapid onset of febrile disease. Symptoms include fever, malaise, chills, and headache. Neurological impairment is rare but has been reported in roughly 5% of cases, mostly in young children. Venezuelan equine encephalitis virus has a mortality rate under 1% in humans.[109] The route of virus entry into the CNS is not well elucidated but research has identified the olfactory neurons as the most likely way into the brain, avoiding the blood brain barrier. The febrile disease clears within 4-6 days followed by a convalescence phase lasting a few weeks with minimal sequela.

Both the US and Soviet programs weaponized VEEV as a morbidity weapon against enemy targets. The low mortality rate, low infectivity dose (10-100 plaque forming units [pfu]), the natural route of aerosol infection through the olfactory neurons and the natural stability in aerosol form made VEEV an ideal morbidity bioweapon candidate.[110-112] In addition, a large quantity of VEEV could be grown quickly through cell culture, which made processing the virus much faster than many of the bacterial derived weapons. The US was the first program to study VEEV, both for the goal of developing a vaccine and for offensive capabilities. Stockpiles of the virus were generated in the US but no reports have identified any selection for a more pathogenic strain of the virus. An attenuated vaccine was developed soon after the closure of the US bioweapons program but no indications exist that the research between the offensive program and the vaccine are connected. The Soviet program initiated research on VEEV

following reports that the US program was studying the virus.[19] They were also able to develop a weaponized stockpile of VEEV during the first phase but genetic manipulation has not been reported from the second phase other than the insertion into a bacterial expression vector. Reports theorize that the Soviet researchers utilized one of the more virulent strains of isolated VEEV for their research and weaponization program.[19]

Countermeasures For VEEV

No FDA approved vaccines or therapeutics exist for VEEV for public use. Ribavirin has not been shown to be clinically effective in limiting virus growth or clinical disease.[113] Animal studies utilizing Ampligen, a potent IFN inducer, have shown a protective response against VEEV infection, but it has never undergone human trials.[114,115] Unlike the US program, the Soviet research program was reported to have successfully developed a vaccine against VEEV.[19] The vaccine generated by the Soviet program was either destroyed or lost and is unavailable for public use. The US biodefense program generated an attenuated strain of VEEV, TC83, by serial passaging the virus 83 times in guinea pig heart cells which has shown immunogenicity in equines and an 80% seroconversion rate in humans.[116,117] This vaccine is in use in Columbia and Mexico for equine vaccination; however, due to adverse side effects, it has investigational new drug (IND) status in the US and is only given to individuals at high risk for infection. Clinical studies have shown that TC83 can be transmitted by mosquitoes making it an unlikely candidate for national vaccine implementation.[118] An inactivated form of TC83, C-84, is utilized as a booster in horses and humans but has not been shown to be completely protective.[119] Other attenuated viruses utilizing chimeric combinations of alphaviruses have been shown to be immunogenic but they have not been approved by the FDA for public use as they have not completed clinical trials.[120]

4.2.4 *Burkholderia mallei*

The etiologic agent of glanders, *Burkholderia mallei,* has a reported history of hundreds of years. *B. mallei* is a gram-negative, obligate bacteria with a very low infectivity dose of 1-10 organisms.[121] It is nonsporulating and remains pathogenic in a natural environment for only a few weeks due to its susceptibility to the environment.[9] Glanders was first described by Aristotle around 350BC but the first identification of *B. mallei* occurred in 1882 when it was isolated from an infected horse.[122,123] Horses are highly susceptible to glanders and are believed to be the natural reservoir, but other four legged solipeds can become infected.[124,125] Humans and other mammals are accidental and dead end hosts.[126,127] Transmission of the bacteria occurs by absorption through the mucous membranes of the eyes, and nose and or mouth during inhalation.[9] This most often occurs through direct contact with an infected animal, the bedding or excrement of an animal, or in a laboratory setting. Glanders has been eliminated in most countries around the world.

Glanders in humans can be very difficult to diagnose due to the variety and non-specificity of symptoms, and the near eradication of the disease in most countries. Prior to the discovery of viable antibiotics, glanders, while rare, was nearly always fatal with a mortality rate close to 90%.[122] Six different manifestations of glanders can occur in humans: nasal, localized, pulmonary, septicemia, disseminated and chronic. The difference in manifestation is believed to be based more on route of exposure and the length of time between exposure and diagnosis. All six forms may develop if the disease is left untreated.[9] Localized disease normally occurs early in an infection and is characterized by pustules or abscesses. Dissemination to the gastrointestinal tract generally occurs after a localized or cutaneous infection, symptoms include fever, exhaustion, chills, headache, malaise, diarrhea, and cramping.[124] Aerosolized infection can result in lesion formation within 1-5 days of exposure. The nasal or ocular region can

become inflamed with discharge, and the face and lymph nodes become edematous. Further development into a respiratory infection can lead to pneumonia, pulmonary abscesses, cough, dyspnea, and chest pain.[9] Once a diagnosis is confirmed in both humans and animals, health authorities must be notified per health department guidelines.

B. mallei was one of the first biological weapons utilized in the 20th century and was studied as a potential pathogen by both the US and Soviet programs. The US program gained a significant amount of their knowledge on the bacteria from captured Japanese researchers from Unit 731 but never weaponized or stockpiled the pathogen as a prospective bioweapon. The Soviet program studied *B. mallei* during both phases of their program, generating stockpiles of the bacteria for aerosolized release in the first phase and studying antibiotic resistance in the second phase. Intentional release of *B. mallei* in Afghanistan during the 1982-84 war has been reported. However, this was prior to the development of an antibiotic resistant strain and would have been accomplished using the weaponized stockpile from the first phase.[20] During the second phase of research, *B. mallei* was studied along with the other bacteria for methods of introducing antibiotic resistance without a loss of virulence. Second hand reports confirm this was completed but official confirmation of success has not been published.[19]

Countermeasures For *Burkholderia mallei*

Most knowledge on currently available countermeasures for glanders is based on laboratory research due to the low number of natural human cases, most being from laboratory exposures. Recommended antibiotics include sulfadiazine, tetracyclines, ciprofloxacin, streptomycin, gentamicin, and other sulfonamides.[9] Antibiotic treatment should last for 2-12 months, dependent upon the diagnosis of infection and the resistance of the strain. For extreme cases of septicemia, the antibiotics ceftazidime, imipenem, or meropenem are the recommended treatments. No vaccines are available, even in IND

form, for humans or animals. Testing, reporting, and antibiotic treatment was the primary method of eliminating glanders from the US. No evidence of immunity following infection has been demonstrated as both horses and humans have been reinfected following clearance of the bacteria. While glanders is only a category B select agent, the lack of a vaccine and known resistance to antibiotics makes it a plausible candidate as a bioweapon for use against livestock and humans.[127,128]

4.3 AGRICULTURE BIOWEAPONS

4.3.1 Rice Blast, Rye-Stem Blast, Wheat-Stem Blast

While many of the previously mentioned bioweapons could also pose a significant threat to livestock as well as humans, a number of bioweapons were designed to specifically target only the agriculture sector. These included rice blast, rye-stem blast, and wheat-stem blast. Rye-stem and wheat-stem blast are both fungal infections from the same organism, *Puccinia graminis*. The fungus primarily spreads from crop to crop as urediniospores which are blown by the wind and can travel thousands of kilometers.[129] The organism is a diverse, obligate pathogen requiring a very specific strain of rye or wheat to infect. Wheat blast (*P. triticina*) is the most common of the pathogens. In the US, 40-60 different strains of the fungi are identified annually, all with variable virulence rates.[129] The US suffered from multiple epidemics of wheat-stem blast from the early to mid-20th century, which may have been connected to future research and stockpiling as a bioweapon.[130] The fungi spores can infect any portion of the plant found above ground, most often landing on the leaves and forming pustules. The infected plants produce fewer seeds and, when infected with more virulent strains, can die from the infection.

The third fungal bioweapon, *Magnaporthe grise* or *M. oryzae*, infects rice crops and was stockpiled by the US at a much lower tonnage than the other two bioweapons.[131] Also able to form spores, *M. grise* can travel thousands of miles by wind to infect new

crops. Infection results in a similar disease and loss of crop as the stem-blasts described above. Rice blast was stockpiled in 1966, which has led some to theorize it was a weapon specifically targeted for countries within Asia.

Both the US and Soviet national programs developed methods of culturing and stockpiling anti-agriculture bioweapons. The US first started in collaboration with other members of the Allied nations during WWII. This research collaboration studied multiple animal and agricultural pathogens but most of this work was never implemented into a production program. The US continued their program after WWII and by the mid-1950s had generated a stockpile of 3 different fungal agents.[19,132] Economic analysis at the time showed around 70% of the Soviet calorie intake was based on grain crops, making them a strong target for elimination during a non-nuclear offensive.[133] Delivery systems including bombs and specialized cruise missiles were developed for long range air release of the pathogens but the weapons were never utilized.[32,134,135] All US stockpiles, including delivery systems, were destroyed after the 1972 signing of the BWC.

The Soviet program was initiated only after learning about the US program in the mid-1950s. Very little information is available from the Soviet program, code-named Ekologiya (ecology), with much of the information coming from Dr. Ken Alibek, either from his direct experience or what he learned while at Biopreparat.[20,136] Reports by Dr. Alibek identified a number of agricultural weapons that were stockpiled including rice, rye-stem, and wheat-stem blast fungi. Research by the Soviets into other pathogens included Foot-and-Mouth disease, African Swine Fever, and Rinderpest but none of these pathogens were stockpiled. Instead, the Soviet program had select farms capable of producing the pathogens quickly at a time of emergency. According to Dr. Alibek, the agricultural bioweapons were destroyed and the research stopped prior to international recognition of the Soviet anti-personnel bioweapons program. The agriculture division of the program was closed as it targeted civilian populations with limited military purpose.[136]

Countermeasures for anti-crop bioweapons

Three routes for the prevention of infection and spread of the anti-crop bioweapons have been described: 1) early recognition and destruction, 2) chemical, and 3) genetically modified organisms (GMOs) resistant to fungal infection. The first method is recognition and destruction of infected crops. This must occur quickly after infection as any released spores may travel thousands of kilometers downwind and infect new crops. Loss of containment at the primary infection site may mean any form of containment will be impossible. The second countermeasure is chemical, multiple fungicides have been developed which are effective against the fungi that causes blast. The fungicides used worldwide include Probenazole, Tricyclazole, Azoxystrobin and Propiconazole.[137] Identifying the specific strain infecting the crops is difficult, which may lead to application of the wrong fungicide, which in turn can lead to resistance. The third countermeasure has come with the revolution of molecular genetics, GMOs, which are resistant to fungi infection. Genetically modified organisms have been shown to be effective at resisting specific strains of fungi; however, these strains and others not yet identified may overcome the resistance designed into GMO plants, on-going research will be necessary to ensure the protection of crop production. [137]

CHAPTER 5: DESCRIPTION OF A BIOWEAPONS ATTACK

5.1 DESCRIPTION OF A BIOWEAPON ATTACK

Millions of cases of infectious diseases occur in the US annually with hundreds of millions of cases worldwide. Not all cases are representative of an outbreak but many can be correlated to a temporal beginning and end. Naturally occurring infectious disease outbreaks can present in many different forms. The annual influenza season affects millions of people each year and, dependent upon the virulence of the specific strain, is associated with thousands of deaths annually.[138] Other diseases, such as plague or viral hemorrhagic fever, occur only once or twice in a year but result in a massive response by the CDC and local health infrastructure to contain any spread.[139] The National Notifiable Diseases Surveillance Systems (NNDSS) of the CDC have a total of 66 notifiable diseases as of 2010. These are diseases which US states are required to report once a diagnosis is confirmed.[139]

The recognition and control of a disease outbreak is a complex process. The first step is the identification of an outbreak, which is accomplished through recognition of a novel clinical disease, an abnormal increase in the number of cases for a disease, or the change in the clinical presentation in a disease. Once identification of an outbreak has occurred, and a report has been sent to the CDC, two steps must occur. First, the outbreak must be controlled and secondly, the cause of the outbreak must be investigated. Control can be accomplished through pharmaceutical administration, a social change such as quarantine, or in many cases, the outbreak may burn itself out before any special changes are implemented. When investigating an outbreak, identification of the first cases, temporal range of these cases, most likely routes of exposure, incubation period of the disease and location the outbreak occurred are all paramount to an accurate response. At

the completion of an investigation, investigators should be able to propose medical or social changes that can reduce the chances of a future outbreak.

In the case of a biological attack, a similar but more delineated pattern of responses will occur. The distinction between a bioattack and a novel virulent epidemic can be very difficult to distinguish. A few distinct features which may assist in identifying a bioattack are described below:

1. Abnormally high mortality rate – This is not always a consistent identifier, as seasonal strains of influenza have shown varying degrees of virulence, but a high mortality rate must always be investigated.
2. Increase in the number of cases – A significant change from the normal background level of a specific disease can sometimes be linked to a biological attack. This can be in the case of rare diseases, such as smallpox, or in more common diseases with a greater number of cases than commonly reported.
3. Reports of an abnormal disease – Certain diseases are uncommon, such as EBOV or MARV, especially in the US. Even a single case needs to be reported and a number of them at the same time are suspicious.
4. An unusual distribution of disease– A temporal or physical focal point may correlate to disease development. Cases downwind from a single location or occurring close together at a specific time may be signs of an intentional release.
5. Multiple outbreaks – This can again be seen in seasonal or novel outbreaks but the simultaneous outbreak in multiple locations must be investigated as the possibility of an intentional exposure or attack is possible.
6. Abnormal zoonotic disease – Deaths in animal populations corresponding to disease in humans may provide evidence of a biological attack.
7. Declaration of the attack – In certain cases, the attacking party may claim responsibility for an attack. This will need further investigation to confirm the party(ies) making the claim are truthful.
8. Laboratory identification – Specific strains of diseases may no longer be in circulation or are limited to specific geographic regions. Evidence of intentional transportation of the disease or release from a laboratory may be identified through pathogen genetics.

These identifiers will not always correspond to a biological attack but the confirmation of all or many of them may provide further evidence that such an attack occurred.

5.2 BIOLOGICAL ATTACKS IN THE US THROUGHOUT THE 20TH CENTURY

The first intentional use of bioweapons in the US occurred during WWI. A German agent stationed in the US intentionally infected equines set for transportation to the front line with anthrax and *Burkholderia*. He grew cultures of both bacteria in guinea pigs and infected the animals through IM injection or poured it into their feed.[140] At the time, no investigation into disease outbreak in animals took place and a modern investigation is not possible. This initial attack identifies the ease in which it is possible to grow large stocks of infectious pathogens and release them at an intended target even in a war-time environment.

The second use of biological weapons in the US occurred in The Dalles, Oregon in 1984. Investigations started with the reporting of an outbreak of a food borne illness in a large number of individuals. Salmonella was confirmed through laboratory testing as the causative pathogen. While investigating the first cohort of cases, a second outbreak of foodborne salmonellosis occurred. Epidemiologists identified a number of puzzling features connected with the outbreak. The development of illness was connected with 10 different restaurants and different types of food; outbreaks can normally be linked to a single location or single food type. Interviews with the restaurant workers also reported they became ill within the same time frame as the customers; and no cases preceded the initial outbreak.[141] Closure of all salad bars in the region halted the outbreak but, by that time, 751 cases have been identified including 45 hospitalizations. Further investigation identified a cult in the area was responsible for the bioattack. Evidence collected during a criminal investigation indicated that members of the cult ordered the Salmonella strain

through the mail, cultured it and intentionally contaminated the salad bars. The motivation behind the attack was entirely political.[142]

The third and most well-known bioattack on US soil occurred in 2001 when letters filled with fine anthrax powder were sent through the USPS. The first case of inhalational anthrax was identified in Florida and was initially diagnosed incorrectly.[143] Upon identification of two more cases in Florida and one in New York, investigators found that anthrax was being mailed out to different locations in the US. By the end of the attack, 22 cases of anthrax had occurred resulting in 5 deaths. These attacks resulted in an unprecedented number of reports by the public fearing exposure from letters which drastically stressed the public health response system.[144] Evidence suggests that anthrax researcher Dr. Bruce Ivins was behind these attacks but many still remain doubtful. Importantly, this attack identified the inherent risks any national or international package delivery system possesses as the attacker(s) was able to utilize the USPS to deliver *B. anthracis* across multiple states without the need of a complex delivery system. It also identified the inherent delay in recognizing an unusual biological pathogen and the initiation of a federal response.

The final series of bioattacks utilized the same delivery mechanism as the anthrax mailings but instead sent ricin toxin. Ricin residue was identified in a post office as well as US Senator William Frist's D.C. office in 2003 and 2004. In 2013, two additional attacks occurred which utilized ricin as a bioweapon. The first pair of letters were addressed to Senator Wicker and President Obama but intercepted before delivery and tested positive for ricin. A third letter was received by a county Judge and also confirmed to contain ricin. Two months later, an additional pair of letters containing ricin residue were addressed to President Obama and Mayor Bloomberg. These letters were also intercepted before delivery. These cases confirm that the USPS is still being utilized as a delivery tool of bioweapons; however, the security mechanisms implemented following

the anthrax attacks were effective in identifying the letters prior to delivery to political targets.

CHAPTER 6: DISCUSSION

6.1 WEAKNESSES IN THE METHODOLOGY

All three methods utilized in the discovery phase of this capstone have specific weaknesses that must be addressed. First, only a single database, PubMed, was used for the peer review article search. Google Scholar and Illiad were only utilized to locate articles other sources had already identified. While PubMed is a large database and contains many of the papers published since the middle of the 20th century, it is not exhaustive. Two shortcomings are that it lacks non-English journal publications and that older papers from out-of-print journals are not consistently available. Another weakness was the large number of articles that were returned for each keyword searched. The availability of an abstract and a strong citation history were important for selection by the author. Papers without an abstract or a strong citation history were not utilized due to their perceived weakness by the author. The large number of publications returned in certain keyword searches may also have obfuscated important publications. Finally, when numerous review articles were identified, the newer article was generally chosen which may have led to a loss of important information the author may have overlooked.

The second set of sources was published texts which lack validation and peer review. In both cases, the authors may have been focused on a specific agenda and ignored or misrepresented the facts to suit their bias. The information is also dependent upon the authors or the interviewees correct recollection of events sometimes decades old. Finally, as much of the data is still classified, many of the people involved may not have been truthful in their reports or were unable/unwilling to be interviewed.

Likewise, the final method of data acquisition, interview or direct interaction with the source, was dependent upon the memory and experience of the interviewee. Many of these programs are decades old and documentation is limited. It is also dependent upon

the capability of the interviewer to phrase the questions correctly to receive an objective answer. A lack of knowledge or inability to correctly articulate a question may have resulted in a loss of important data.

The most difficult section to find official reports and histories was the national bioweapons programs, especially the US program. While the US program was closed in 1969 after only 27 years in operation, it was able to complete a significant amount of research and testing with a variety of pathogens. Tests exposing civilian and military volunteers to live pathogens were completed, and summarily classified making retrieval of this information for this review difficult. Some of the declassified data was only released following media investigations and reports. The most recent major declassification of data occurred in 2000 when an investigative reporter teamed up with CBS News to release a 6-year report on the US Project 112/SHAD (Shipboard Hazard And Defense).

This capstone provides a brief description of the two largest government sponsored bioweapons programs to have ever existed. It identifies the initial biodefense goals of each program and their subsequent shift towards bioweapons. The capstone also presents information on a number of the biological weapons developed by these programs, a description of the clinical disease development and what modern countermeasures are available in the US. It does not address all of the bioweapons believed to have been developed by either program, nor does it address the bioweapons generated more recently by other national programs. While this information is available elsewhere and in other formats, the combination of historical significance, clinical development, weaponization, and countermeasures for bioweapons in both programs is not available in an abridged format.

6.2 THE THREE P'S

The reasoning behind the paradigm shift from biodefense to bioweapons is not well documented but the evidence points towards the cost effectiveness of the biological weapons.[14] Unlike normal armaments, once acquired, bioweapons can be grown cheaply, requiring only a method of dispersal or release. The cost of studying a pathogen and developing a vaccine can be extremely high, as seen with modern pharmaceuticals; alternatively, there are many naturally occurring pathogens that are already shown to cause high morbidity and mortality. During WWII and the Cold War, this cost effectiveness may have been critical in deciding whether to develop a large-scale bioweapons programs.

Documented application of these bioweapons on US soil was summarized identifying the different levels of technology, funding and education. The wide variety of attacks identifies some of the difficulties the US faces in preparing and implementing policies preventing future attacks. Since the 2001 anthrax attacks, there has been a significant increase in funding for research in biodefense and biosecurity. A portion of the funding is targeted towards biosecurity and health preparedness on a national scale as the US government recognizes the threat of naturally occurring biological pathogens as national security threats similar to intentional bioweapons. To ensure the security and health of the US populace from biological attacks, the US government focuses on three core concepts: policy, preparedness, and prevention.

6.2.1 Policy

The US has been involved in policy implementation since the signing of the BWC in 1972. Without government recognition and policy in prohibiting bioweapons, research and development may have continued. While the BWC specified that the signatory states agreed to stop all development, evidence from the Soviet program shows it was not fully effective. Many of the issues identified during the era of the bioweapon programs remain

relevant even with the changes in technology. Currently, 179 countries are signatories to the BWC, all of which have agreed to not pursue any biological research or development for offensive agents nor the delivery mechanisms to release potential biological agents. While many countries have ratified the BWC, oversight and enforcement to ensure compliance are limited. Attempts to form an oversight committee in 2001 failed, in part due to the refusal of the US to allow access to research facilities due to the fear of exposing national defense or industry secrets.[145] The ratification of an oversight committee would assist in preventing future programs from existing in secret but may do little for identifying individuals or organizations.

Along with scientific oversight, this committee may also judge the intent of the researcher through their grants and publications. Through congressional mandate, the DHHS was required by the Antiterrorism and Effective Death Penalty Act of 1996 to establish a list of biological and toxic pathogens which could threaten public health.[146,147] This policy is administered by the CDC, in coordination with the DHS and US Department of Agriculture (USDA), through the Select Agent Program. The CDC is charged with tracking the use for research of Biological Select Agents and Toxins (BSATs), their transport between facilities, storage at research facilities, and the federal clearance of the researchers handling the pathogens. This act has significantly reduced the chances that a pathogen can be obtained by an unauthorized individual. Reducing access to BSATs makes it more difficult for legitimate scientists to perform research with the pathogens, potentially weakening the preparedness and competitiveness of the nation.

Presidential directives and orders have also been written to address many of these issues. In 2007, Homeland Security Presidential Directives (HSPD) 21 and 18 were issued calling for the biosecurity industry to implement a method of acquiring and distributing countermeasures in the event of a biological epidemic or attack. In 2004, HSPD 9 called for improved methods for ensuring the security of the US agriculture industry and food supply from biological pathogens. Executive Order 13486 calls for the

strengthening of federal laboratory biosecurity, increased monitoring of research laboratories to ensure they were following correct biosecurity procedures and limiting and tracking access to BSATs.

These policies have a few primary foci. Executive order 13486 requires a background check on researchers, annual inspections and reviews, and detailed tracking of the pathogens. What the policies do not address is that these pathogens are also found naturally in the wild. A malevolent individual does not need access to a laboratory to obtain many of these pathogens; they need only travel to an endemic region and possess the capability and knowledge to isolate the pathogen from the environment. This capstone previously described 11 factors which could be used to identify a bioweapon. While still valid for modern selection, non-state entities commonly have different motives than a government program and may select pathogens differently. In addition to these 11 factors, the author of this review proposes an additional set of 5 modern factors for selecting pathogens, which take into account the change in political climate and technology which has occurred since the collapse of the Soviet Union. These factors are:

12. Fear – The capability of destabilizing a target population through fear of the pathogen following an outbreak or intentional release. A pathogen with high visibility impact, such as scabs or paralysis, but minimal spread or mortality may induce an equal response as a pathogen with a high mortality or infection rate. Smallpox possesses the potential for both high mortality and visibility while chickenpox would be an example of lower mortality but relatively high visibility.
13. Economic destabilization – Another method of destabilization would be through targeting the economic backbone of a society. As both the US and USSR stockpiled agriculture bioweapons, they recognized the importance of non-human targets, such as crops and farm animals, and the impact the elimination of these targets could have on a nation.
14. Pathogen availability – All of the pathogens, except for smallpox, can be found in the wild, but some are more difficult to acquire than others. Anthrax can be found annually in rural settings in many countries, including the US, while Ebola is localized to a few countries and outbreaks are uncommon.

Access to research facilities may negate some of these difficulties but such facilities are most likely secured in a manner to prevent such access.

15. Method of dissemination/release –Aerosol is still one of the most effective methods of pathogen release; many bioweapons were designed around aerial or missile release which individuals or smaller groups will not have available. Pathogens which can be generated and stored outside of a large laboratory may take precedence over those pathogens which can be aerosolized.
16. Knowledge – The availability of published research on many pathogens ranges from a few dozen articles to thousands. Most of this data are available for free or for a minimal fee online. This availability may be a driving force in the selection of a specific pathogen, especially by an instigator not interested in research but in use/misuse.

6.2.2 Preparedness

The US biodefense industry recognizes the inherent difficulty in preventing all biological outbreaks, both natural and malevolent. The next core concept in ensuring national protection is preparedness. The SNS, an organization within the CDC, has the sole mission of preparing pharmaceutical countermeasures for distribution during a national emergency in which pharmaceuticals or other protective equipment may be necessary to reduce morbidity and mortality. To obtain this goal, the SNS has the capability to purchase and store large stockpiles of pharmaceuticals and vaccines in specific regions across the nation. In the event of an outbreak or attack, the SNS is capable of distributing those stockpiles to most major cities within a short time period. The capability of the SNS to deliver pharmaceuticals was tested during the H1N1 pandemic of 2009 when they released 25% of their stock of antiviral medicine within weeks of the pandemic.[148] This pandemic showed the SNS was capable, but it was not stressed due to the relative lightness of the outbreak. Whether the SNS is capable of handling a pandemic on the scale of the 1918 flu or an intentional biological attack remains to be seen. The SNS does not publish a list of the stockpiled pharmaceuticals but presumably they contain a selection of antibiotics and vaccines which are efficacious against the most likely pathogens. Another potential weakness of this strategy of

preparedness is how rapidly and effectively the pharmaceutical industry can respond. Studies completed by the US bioweapons program in the 1960s identified exposure and death occurring in a significant percentage, greater than 30%, in fewer than 8 hours when NHPs were exposed from kilometers away.[149] From these results the delivery of countermeasures on a national scale would be impossible to occur in time to prevent death in exposed targets.

In order to identify when the SNS stockpile needs to be released, up-to-date information about an outbreak needs to be available. This requires a national surveillance system which can distinguish aberrations in health data from normal background activity. A number of these systems are in place, ranging from active air sampling and laboratory testing to syndromic analysis of social media and daily emergency room health reports. This is a very difficult objective to obtain as background health data will change based upon spatial and temporal factors. While detecting aberrations during a natural epidemic may be possible, a targeted bioattack may affect too many people within too short of a time for surveillance to be effective as a preventative strategy. As a system, BioWatch surveillance units passively collect air samples from different locations within more than 30 major US cities.[150] These samples are analyzed daily but the units are inherently flawed as they are immobile devices requiring the correct meteorological conditions and lucky placement for the detection of specific bioweapons to be possible. The development of automated and mobile detection devices may greatly improve the capability of detecting an aerosolized pathogen.

6.2.3 Prevention

The final core concept of importance is prevention. This concept focuses on two key areas; internally on high containment laboratories within the US and externally on laboratories and scientists in other countries. Many laboratories in the US now work with

high containment pathogens which could be utilized during a bioattack. Increased security and tracking of BSATs at these laboratories ensures only authorized researchers have access to the pathogens. In addition, background checks and insider threat training ensures the researchers are scrutinized before access is granted and any emotional or physical changes are reported. While increased security around laboratories is laudable, most of these pathogens can be acquired elsewhere by a determined individual or group, circumventing all security mechanisms implemented at these laboratories. Increased training and non-invasive observation of researchers can be of great benefit, as it helps the researchers better understand the public health risks of these pathogens and ensures the emotional and physical stability of anyone working within the laboratories.

The Clinton administration, based upon a report by the National Security Council (NSC), in 2000 recognized HIV as a national security threat, the first natural infectious disease to receive such recognition.[151] Within the same report identifying HIV, the NSC also recognized the threat other pandemic and emerging infectious diseases posed to the general population. The Biosecurity Engagement Program (BEG), run by the Office of Cooperative Threat Reduction (OCTR), focuses on enhancing international disease surveillance through collaborations with laboratories in other countries and providing funding for improving biosecurity and ensuring research positions at laboratories in countries which once housed bioweapon development facilities.[152]

The surveillance portion of OCTRs mission is to promote collaborations between American laboratories and international researchers to track and investigate new diseases that may become a global threat. The second mission of the OCTR is to assist with funding and improve biosecurity in laboratories in foreign nations with a history of bioweapon development. Funded in part by the Defense Threat Reduction Agency (DTRA), grants are offered to international laboratories for a variety of preventative measures. From funding the installation of a new security fence, to salaries for Russian scientists who once worked in Soviet bioweapons programs, the primary mission is to

prevent the proliferation of biological pathogens and/or the research skills necessary in handling the pathogens.[19]

All three of these core concepts, when unified, help protect the public from biological pathogens. However, they are not all encompassing nor do they make the government omnipotent in its capability to prevent an outbreak or attack. Continued advances in surveillance will greatly assist in identifying and potentially controlling an outbreak but will not prevent it. With increasing globalization and international travel, a national surveillance program will not assist in identifying international risks which can easily come to the US. To reduce the risk of a biological attack as much as possible, understanding and recognizing the capability of bioweapons, continued research and development of countermeasures and vaccines, and improved international cooperation in surveillance and policy are all necessary. While completely eliminating the threat of an individual antagonist may not be possible, they may be identified earlier and therapeutics provided more quickly to significantly reduce morbidity and mortality through global cooperation.

6.3 FUTURE DIRECTIONS

Studying the methodologies and capabilities of the historic bioweapons programs will provide modern scientists and healthcare professionals a stepping-stone in preparing for future biological attacks or emerging infectious disease outbreaks. The threat of a biological attack from the Soviet Union is no longer primary motivation of the US biodefense program however, the increasing threat from non-state or biological terrorists (bioterrorists) is believed to be much greater than in previous decades.[153] All of the reported biological attacks within the US in the previous decades have been implemented by individuals or small groups, not state sponsored agencies. These groups probably do not have the monetary backing or research infrastructure comparable to that of the

historic bioweapons programs. During many of the attacks, the goals were simple or non-existent, with little evidence of strategic long term planning of additional attacks. All of these factors suggest that the biosecurity and preparedness initiatives developed to defend against state-run programs may not be relevant or effective against bioterrorists.

One issue within the policy and prevention core concepts is the increased level of biosecurity at national laboratories, which helps to ensure that access to stored pathogens is limited. However, it does not address the issue that many of these pathogens are naturally available. Unlike the contest of the Cold War, non-state agencies do not have large intelligence agencies, if any, and they are to infiltrate a national laboratory to steal a naturally occurring pathogen. The Japanese cult Aum Shinrikyo attempted to acquire EBOV by traveling to Zaire during an outbreak instead of attempting to steal it from a containment laboratory.[9] The direct and indirect costs for increasing biosecurity are substantial. The increased security makes it more difficult and expensive for legitimate scientists to study these pathogens. It also limits the number of facilities capable of researching these pathogens. By impeding research through costly and possibly ineffective security measures, government policy may delay the development of effective countermeasures.

The primary method of pathogen dispersal has also changed. Both the Soviet and US programs developed specialized missiles and bombs for the release of bioweapons. In the last decade, the primary method of attack has been through the USPS. Following the anthrax mailings in 2001, the USPS installed new biological detection systems (BDSs) that collect ambient air from around letters while they are being sorted and automatically test it for specific pathogens. The USPS also detected Ricin shipped in letters in 2013 presumably with a BDS or similar system. These systems operate passively without inhibiting the delivery of mail by USPS and appear to be effective in identifying specific pathogens. Further utilization of passive or semi-passive sensors, such as BioWatch, may be one of the most effective ways of detecting a pathogen prior to clinical disease

development. BioWatch is currently in its third generation as a national surveillance tool for detecting aerosolized pathogens in major cities within the US. Further development of these systems could include increasing the number of pathogens the system can detect and improving the mobility of sensor systems like BioWatch. A major criticism of BioWatch is that the detection of a pathogen is dependent upon meteorological conditions and initial placement of the sensors which autonomous mobility could overcome.

The final and perhaps most important factor in preparing for and preventing a biological attack is research. Understanding the determinants of pathogenesis and attenuation is a necessary first step towards the development of effective countermeasures. This can only be accomplished through basic bench science by trained scientists. Three items which help to ensure this research is completed; consistent funding, training of students who will become the next generation of scientists and development of new infrastructure and laboratories. New pathogens continue to emerge around the world, and, while many are not endemic to the US, globalization and increased international travel provides routes of transmission which can lead to outbreaks in the US. Many of these diseases have the potential of becoming a significant biological threat, as bioweapons and pandemic diseases. As such, the US government must ensure it has the capability to research these diseases for decades to come.

Appendix A

TABLES

Table 1: List of PubMed Search Phrases.

Key Search Term	Number of Articles	Number of articles selected for reading
Anthrax Vaccines	161	11
Anthrax Countermeasures	18	4
Anthrax Biodefense	21	4
Anthrax Bioweapon	9	2
Francisella tularensis Vaccines	49	6
Francisella tularensis Countermeasures	1	0
Francisella tularensis Biodefense	3	1
Francisella tularensis Bioweapon	3	0
Marburg Vaccines	75	1
Marburg countermeasures	3	0
Marburg biodefense	3	1
Marburg bioweapon	0	0
Filovirus Vaccines	59	3
Filovirus Countermeasures	6	1
Filovirus Biodefense	9	2
Filovirus Bioweapon	0	0
Smallpox Vaccines	508	13
Smallpox Countermeasures	14	1
Smallpox Biodefense	22	8
Smallpox Bioweapon	5	0
Yersinia pestis Vaccines	40	5
Yersinia pestis Countermeasures	0	0
Yersinia pestis Biodefense	5	1
Yersinia pestis Bioweapon	6	1
Brucellosis Vaccines	63	1
Brucellosis Countermeasures	0	0
Brucellosis Biodefense	0	0
Brucellosis Bioweapon	0	0
Coxiella burnetii Vaccines	29	3
Coxiella burnetii Countermeasures	0	0
Coxiella burnetii Biodefense	1	1
Coxiella burnetii Bioweapon	3	1
Venezuelan equine	35	5

encephalitis Vaccines		
Venezuelan equine encephalitis Countermeasures	3	1
Venezuelan equine encephalitis Biodefense	10	3
Venezuelan equine encephalitis Bioweapon	0	0
Burkholderia mallei Vaccines	4	2
Burkholderia mallei Countermeasures	1	1
Burkholderia mallei Biodefense	1	0
Burkholderia mallei Bioweapon	1	0
Puccinia triticina	1	1

The list of terms searched for on PubMed and the relevant articles selected from the search.

Table 2: Updated List of US and USSR Bioweapons

Bioweapons Stockpiled by the US	**Bioweapons Stockpiled by the USSR**
B. anthracis (Anthrax)	*B. anthracis* (Anthrax)
F. tularenesis (Tularemia)	*F. tularenesis* (Tularemia)
Botulism toxin	Marburg virus
B. suis (Brucellosis)	Variola Major (Smallpox)
C. burnetii (Q Fever)	*Y. Pestis* (Plague)
Venezuelan equine encephalitis virus	*B. suis* (Brucellosis)
Staphylococcal entertoxin B	*C. burnetii* (Q Fever)
Rice Blast	Venezuelan equine encephalitis virus
Rye-stem Blast	*B. mallei* (Glanders)
Wheat-stem Blast	Botulism toxin
	Wheat-stem blast*
Biological Pathogens Researched by the US	**Biological Pathogens Researched by the USSR**
B. suis (Brucellosis)**	Ebola virus**
Variola Major (Smallpox)**	Machupo virus
Alphaviruses	Junin virus
Junin virus**	Lassa virus**
Machupo virus	Japanese encephalitis virus**
Hantavirus	Russian spring-summer virus
Y. Pestis (Plague)**	Yellow fever virus**
B. mallei (Glanders)**	Typhus
Yellow fever virus**	*Burkholderia pseudomallei*
Psittacosis	Psittacosis
Chikungunya virus	Rinderpest
Dengue virus	African swine fever virus
Rinderpest	Rice blast
Ricin	Ricin
Botulism toxin	Staphylococcal entertoxin B
	Legionella

This list identifies the biological pathogens stockpiled and researched by each government program. Modified and updated from select lists. [4,9,19]

*The Soviet program developed a mechanism to quickly produce anti-crop bioweapons but never stockpiled

**Research on these pathogens is believed to have been predominately focused on developing vaccines and not with the intent of developing an offensive agent.

Table 3: Table of Bioweapons

Pathogen	Common clinical symptoms	Type	Countermeasures*	Endemic Region	Infective Dose
B. anthracis	Three Clinical Presentations	Bacterial-Lethal	Antibiotics and Vaccine	Worldwide	
Cutaneous	Blister and local swelling of lymph nodes				Low**
Gastrointestinal	Nausea, Fever, Vomiting, Diarrhea, Sore Throat				Low**
Inhalation	Febrile Illness, Chest Pain, Shock, Meningitis				8,000-50,000 spores
F. tularensis	Fever, Malaise, Fatigue, Headache	Bacterial-Lethal	Antibiotics and Vaccine	Northern Hemisphere	10-50 organisms
Marburg	Fever, Headache, Diarrhea, Vomiting, Myalgia, Bleeding	Viral-Lethal	Immune Serum	Africa	1-10 pfu
Smallpox	Fever, Malaise, Headache, Back pain, Enanthema, Exanthema	Viral-Lethal	Vaccine	CDC and Vector/Russia	10-100 pfu
Y. Pestis	Malaise, Headache, Swelling and Necrotic Buboes	Bacterial-Lethal	Antibiotics	S. and N. America, Africa, Asia	100-500 organisms
B. suis	Fever, Malaise, Weakness, Exhaustion, Arthritis, CNS impairment, vomiting	Bacterial-Incapacitating	Antibiotics	Worldwide	10-100 organisms
C. burnetii	Fever, Headache, Chills, Nausea, Myalgia, Pneumonia, Meningoencephalitis	Bacterial-Incapacitating	Antibiotics	Worldwide	1-10 organisms
Venezuelan equine encephalitis	Fever, Malaise, Chills, Headache, Encephalitis	Viral-Incapacitating	In research	S. and N. America	10-100 pfu
B. mallei	Pus Nodules, Pneumonia, Pulmonary	Bacterial-Incapacitating	Antibiotics	Worldwide	1-10 pfu

	Abscesses, Chest Pain				

This is a list of the bioweapons researched and stockpiled with humans as the primary target. It includes information on the available form of countermeasure, the pathogens endemic region, and the pathogens estimated infective dose.
*FDA approved and available countermeasures in the US
**The dose in humans is not known but estimated to be low

Figure 3: Timeline of Important Events in the History of Bioweapons.

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Peer Reviewed Publications:

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