Biological warfare: the history of microbial pathogens, biotoxins and emerging threats





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Abstract. Bioterrorism is the deliberate misuse of a pathogen (virus, bacterium or other disease-causing microorganisms) or biotoxin (poisonous substance produced by an organism) to cause illness and death amongst the population. Bioterrorism and biological warfare (biowarfare) are terms often used interchangeably. However, bioterrorism is typically attributed to the politically motivated use of biological weapons by a rogue state, terrorist organisation or rogue individual whereas biological warfare refers to a country's use of bioweapons. Although rare, bioterrorism is a rapidly evolving threat to global security due to significant advancements in biotechnology in recent years and the severity of agents that could be exploited. The pursuit of publicity plays a vital role in bioterrorism. The success of a biological attack is often calculated by the extent of terror resulting from the event, psychological disruption of society and political breakdown, rather than the lethal effects of the agent used.

What is a biological agent?

Biological weapons are defined as a biological agent (such as a pathogenic organism or toxin) that produces effects through proliferation within or intoxication of a target host with the intent to incapacitate, harm or kill[1]. Many pathogens have the potential to be a biological weapon. However, most experts believe *Bacillus anthracis* (anthrax), *Variola* spp. (smallpox), *Yersinia pestis* (plague), *Clostridium botulinum* (botulism) and *Francisella tularensis* (tularaemia) are the most likely agents to be employed in an act of warfare as they hold the most dangerous potential in their effect (Table 1)[4]. Characteristics of an effective biowarfare agent include their availability, virulence, degree of expertise required for weapons production, and the subsequent ease of dispersion (Figure 1)[2,5].

History of biological warfare and bioterrorist attacks

While rare, the deliberate use of biological weapons with the intent of causing mass terror and significant harm has a long history well before microbial pathogenesis was understood. Historically, past usage of biological warfare was predominantly characterised by the weaponisation of pathogens for sabotage, whereas the form of biological warfare most feared today is its use as a method of mass destruction, inflicting catastrophic devastation and loss through mass hysteria and subsequent economic damage[6]. Due to the increased threat of bioterrorism, it is crucial to understand the historical application of pathogenic organisms to evaluate the potential use of biological weapons in the future[7].

Table 1. Biological organisms and toxins of relevance to biological warfare.

Agents/Diseases	Incubation period[2]	Symptoms	Use in biowarfare[3]
Bacteria			
Anthrax (*Bacillus anthracis*)	1–6 days	Fever, malaise, respiratory distress, skin ulceration	WWI; WWII; USSR (1979); Japan (1995), USA (2001)
Plague (*Yersinia pestis*)	2–3 days	Fever, chills, respiratory and gastrointestinal distress	14th century Europe
Tularaemia (*Francisella tularensis*)	1–21 days	Fever, headache, swollen glands, respiratory distress	WWII
Cholera (*Vibrio cholerae*)	4 h–5 days	Watery diarrhea, vomiting	WWII
Food poisoning (*Salmonella*, *Shigella*)	6–72 h	Fever, headache, stomach cramps, diarrhea	WWII; USA (1990s)
Glanders (*Burkholderia mallei*)	10–14 days	Fever, muscle aches, respiratory distress, rash	WWI; WWII
Typhus (*Rickettsia prowazekii*)	7–14 days	Abdominal pain, fever, rash	WWII
Viruses			
Smallpox (*Variola* spp.)	7–17 days	Fever, malaise, pustular centrifugal rash	18th century USA
Haemorrhagic viruses: Ebola, Marburg, Lassa, Machupo	2–21 days	Fever, chills, muscle pain, vomiting, diarrhea	USSR bioweapons program
Encephalitis: Alphaviruses	2–10 days	Fever, headache, drowsiness	WWII
Toxins			
Botulinum (*Clostridium botulinum*)	1–5 days	Difficulty swallowing, facial weakness, nausea, paralysis	Mexican Revolution (1910s)
Ricin (*Ricinus communis*)	18–24 h	Fever, respiratory distress	Umbrella assassination (1978), USA (2003, 2013)

Biological warfare during the past millennium can be divided into three main eras: (1) prior to the germ theory (up until the late 19th century); (2) the emergence of microbiology (late 19th century–1945); and (3) the modern era of molecular and reconstructed organisms (1945–present) (Figure 2)[7]. However, the historical study of biowarfare is problematic, and any conclusions must be treated with caution due to:

- the lack of reliable microbiological and epidemiological data surrounding the alleged attacks;
- difficulties discerning a biological attack from a naturally occurring disease outbreak;
- secrecy surrounding biowarfare programs;
- the deliberate use of allegations for propaganda and hoaxes; and
- potential discord due to modern misinterpretation of ancient accounts.

It was not until major wars of the 19th century that science was harnessed in earnest for the application of biowarfare (Table 2). During the Cold War, the Soviet Union (USSR) had the most extensive and sophisticated covert biological weapons program ever developed by a nation[8]. Despite this, with the exception of the Japanese field trials (involving the release of infected fleas from aircrafts over Chinese cities to initiate plague epidemics) and intentional use of biological agents (anthrax) by Rhodesian troops, there are no well-documented biological attacks by nation-states[9]. A defining step in the modern era of biological warfare occurred after World War II, when individuals, small groups of activists and non-state parties gained access to potentially dangerous organisms to inflict harm on a wider population[3].

The dual-use dilemma

In life sciences, the dual-use dilemma describes scenarios where materials, equipment and scientific research can be used for both peaceful and malicious purposes[10]. Rapid advancements in biotechnology has extended the use of dual-use technologies to a growing number of individuals and organisations, making external monitoring and verification of dual-use sciences nearly impossible. The primary concern of dual-use research is the deliberate misapplication of biological sciences to cause

Figure 1. Common characteristics of biological agents that influence their potential as weapons.

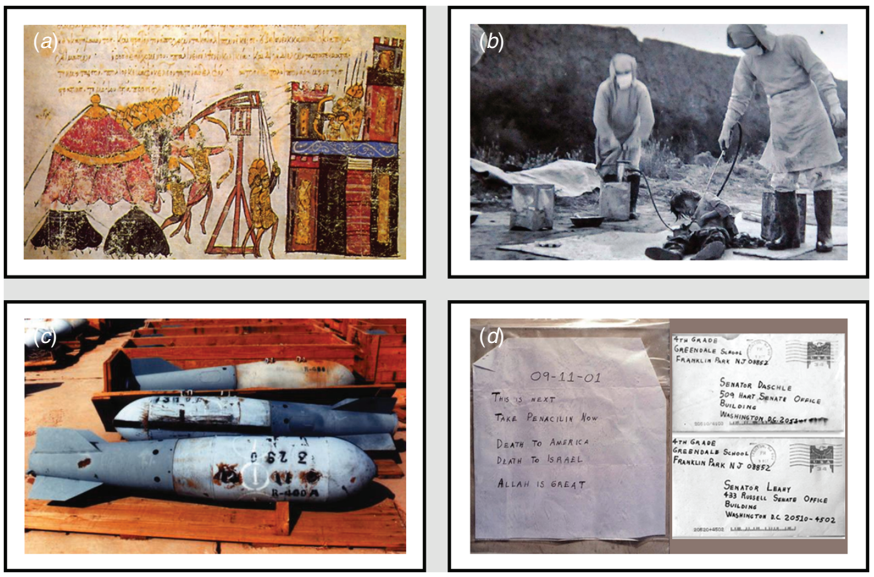

Figure 2. Examples of biological warfare during the past 1000 years. (*a*) Depiction of a citadel siege where a tactic of ancient biowarfare was to catapult plague-infected bodies into the city by Rashid-al-Din (https://commons.wikimedia.org/wiki/File:Mongol_siege_Jami_al-Tawarikh_Edinburgh.jpg)/licensed under Public Domain. (*b*) Unit 731 personnel during WWII (November 1940) conduct a bacteriological trial upon a test subject in China by Unit 731 (https://sl.wikipedia.org/wiki/Slika:Unit_731_victim.jpg) =/licensed under Public domain. (*c*) Two R-400A bombs at an Iraqi military airbase with markings indicating they were intended to be filled with botulinum toxin by FAS (https://fas.org/irp/cia/product/Iraq_Oct_2002.htm)/licensed under Public domain. (*d*) The anthrax-laced letters addressed to Senator Daschle and Senator Leahy in November 2001 by Mirror Vax (https://en.wikipedia.org/wiki/2001_anthrax_attacks)/licensed under Public Domain.

significant harm to public health and safety[11]. Alarmingly, very few pathways differentiate developing biological weapons to developing a vaccine, until there is the resolve to inflict injury (Figure 3)[12].

For example, in 2002, researchers artificially synthesised a 'live' polio virus with blocks of DNA purchased via mail-order to emphasise the ease in which terrorists could produce biological weapons[13]. In 2012, researchers genetically modified the bird flu

Table 2. History of biological warfare and bioterror attacks during the past millennium.

Date and agent	Description of biological warfare event	Morbidity and mortality
1346 Plague	The crude use of cadavers was employed during the siege of Caffa when plague-infected bodies were catapulted into the besieged Crimea Peninsula by the Mongol army.	Unknown[A]
1763 Smallpox	During the French and Indian War, the British plot to spread the smallpox virus constitutes the first well-documented instance of the intentional spread of an infectious disease. Native Americans were given smallpox-infected blankets, with William Trent, commander of the local militia forces noting: 'I hope it will have the desired effect'.	Unknown[A]
1910 Botulinum toxin	Supporters of Pancho villa are alleged to have buried canteens filled with water, green beans and slivered meat to produce botulinum toxin for use against Mexican Federal Troops.	Unknown
1916–1918	During WWI, it is believed that the German Army used anthrax and glanders to infect livestock of Allied Forces.	Unknown
1930–1940s Typhoid, paratyphoid, cholera, plague and anthrax	Japan's use of biological weapons from 1932 until the end of WWII represents the single most important instance of biological warfare undertaken by a state-party. The program known as 'Unit 731' engaged in mass cultivation of pathogens. Experiments originally conducted on POWs were subsequently passed into field trials, where plague-infected fleas were airdropped on the civilian population of China.	>10 000 casualties
1964–1966 Shigella and typhoid	Dr Mitsuru Suzuki contaminated food to infect people with typhoid and dysentery.	200–412 infections and 12 deaths
1978 Ricin	The US alleges that USSR agents stabbed Bulgarian exile, Georgi Markov, with an umbrella that injected a ricin-encapsulated pellet.	One person died
1978–1980 Anthrax	During the Rhodesian Civil War, anthrax was deliberately introduced by Rhodesian Military Forces resulting in the largest recorded outbreak of anthrax in humans.	11 000 infections and 182 deaths
1979 Anthrax	An aerosol of anthrax was accidentally released from a covert USSR biological warfare facility in Sverdlovsk.	94 infections and 64 deaths
1984 Salmonellosis	The largest bioterrorist attack in US history was an attempt to influence a location election by the Rajneeshee cult in September 1984. Members intentionally poisoned salad bars at ten restaurants throughout Oregon, resulting in a community-wide outbreak of salmonellosis.	751 documented cases, of which 45 required hospitalisation
1991–1995 Botulinum toxin and anthrax	Cult members of the Aum Shinrikyo made at least seven known attempts to disseminate biological agents including Botulinum toxin and anthrax in Japan, albeit unsuccessfully due to failed disseminations and the weaponisation of an avirulent *Bacillus anthracis* strain.	None
2001 Anthrax	The 2001 anthrax attacks in the US became the deadliest bioterrorist event in modern history. Several letters containing anthrax were mailed to government officials and the media, costing the government upwards of $27 million.	17 cases and five deaths
2003–2004	Three letters containing ricin were mailed to government offices in the United States by an unknown perpetrator.	None
2013	Two separate attacks by different perpetrators occurred in April and May where three letters containing ricin were addressed to government officials and the President of the United States Barack Obama.	None

[A]Although the intent was clear, it is not known if these deliberate attacks prior to the germ theory caused the spread of disease, or if outbreaks resulted from other interactions.

virus H5N1 to become airborne and easily transmissible among ferrets, presenting the risk that a human-transmissible strain of H5N1 could kill millions of people if produced. Controversial cases like this have led parts of the scientific community calling for greater caution and government regulations to prevent the misuse of biological research. For example, in November 2012 the *Defence Trade Control Act 2012* was established to control the transfer of military and dual-use goods and technology to ensure the export of such sensitive technologies are consistent with Australia's security interests and international obligations.

Public health and biosecurity: preparedness, surveillance and response

Public Health is defined as the science of preventing disease, prolonging life and promoting health through organised efforts

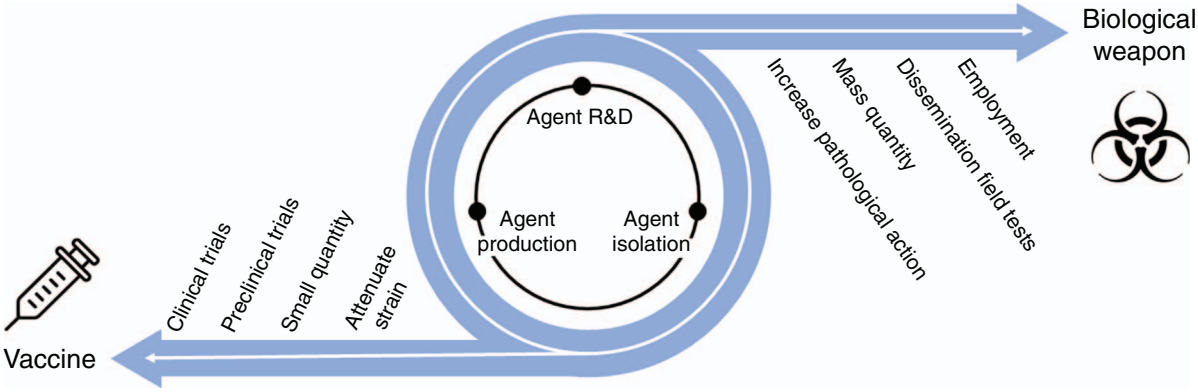

Figure 3. Dual-use nature of biological agents and technology.

of society[14]. Although the probability of an attack is difficult to predict, bioterrorism is not a hypothetical threat and presents a significant challenge for public health. Critical factors that may influence the magnitude of a public health emergency include:

- terrorist intent;
- the destructive power of a biological agent; and
- society's vulnerability to many biological agents.

A successful assault could initiate epidemics of some magnitude, and with a degree of lethality unprecedented in modern history[1]. Unlike conventional terrorist acts which can be readily identified and limited to a specific geological region, a covert biological attack could remain undetected for an extended period of time, be widely spread depending on the pathogenicity of the organism, and may not be immediately recognised as a deliberate attack[15].

Most procedures developed to respond to a bioterror act are the same as those necessary to respond to natural outbreaks of infectious diseases in the environment including early detection, a comprehensive investigation and an effective response. Intensive international cooperation, government preparedness and medical defence against pathogenic organisms must be a central pillar of safeguarding national security, where the ultimate goal is to prevent suffering and loss of life[16]. In addition, to improve biosecurity for both natural epidemics and intentional attacks, international cooperation should include joint biopreparedness exercises involving various countries to develop necessary skills and systems to deal with unexpected outbreaks of disease. Command, control and coordination of multi-agency operations at federal, state and city locations will ensure:

- hospitals are equipped to deal with the sudden influx of patients;
- rapid diagnoses can be made;
- stockpiles of critical supplies including vaccines, antimicrobials and equipment are rapidly dispatched; and
- sufficient emergency personnel can be swiftly deployed.

Syndromic surveillance

Syndromic surveillance is an integral component of biopreparedness that uses the acquisition of automated data to monitor individual and population health indicators in real-time[17]. The primary aim of syndromic systems is to identify illness clusters, focussing on the early symptoms (prodrome) period before confirmed diagnoses are reported in order to mobilise a rapid response and reduce the potential burden of disease[18]. Syndromic surveillance primarily measures the incidence of clinical symptoms reported; however, it also utilises other data sources such as emergency department patient volume, emergency calls, unexplained deaths, insurance claims, clinical laboratory ordering volumes, school/work absenteeism, over-the-counter pharmaceutical sales and increases in internet-based health inquires[18]. For example, if a bioterrorist act involved the deliberate release of *Yersinia pestis*, a syndromic surveillance system might detect an influx in the number of influenza-like illnesses and thus act as an early warning tool of a covert biological attack. Real-time analyses of relevant data make syndromic surveillance valuable for the rapid detection, monitoring and investigation of bioterrorist-related disease outbreaks[19].

Biological and toxin weapons convention

The 1975 Biological and Toxin Weapons Convention (BTWC) was the first multilateral disarmament treaty banning the development, production and stockpiling of bacterial and toxin weapons of mass destruction[20]. Although the BTWC has 187 State-Parties and Signatory States, history tells us that virtually no country with the means to develop weapons of mass destruction of any nature has refrained from doing so[3]. The 1979 accidental anthrax outbreak in Sverdlovsk (USSR), and the 1990–1991 discovery of the Iraqi biological warfare program during the Persian Gulf War (Table 2) highlight that international treaties like the BTWC are ineffective in the absence of operative inspection provisions. There has been some criticism that a central flaw of the treaty is that there are no references to the world of research, or any references to what may be considered offensive or defensive activities in an investigative context that prohibits nations from conducting, assisting or authorising research aimed at biological warfare[8,20]. There is an immediate need for formal measures and enforcement protocols to ensure compliance and prevent systematic violations of the convention.

21st century bioterrorism and the evolving threat to biosecurity

Perhaps the most dangerous threat using biological warfare is the application of genetic engineering to influence the pathogenicity and capacity of biological agents to be used as weapons[21]. Synthetic biology is an evolving interdisciplinary field in which engineering principles are applied to biology[22]. Although the knowledge gained from synthetic biology is not a direct threat to biosecurity, risks associated with the potential exploitation of this technology have emerged in recent years.

During the eighth review of the BTWC in 2016, it was acknowledged that advances in synthetic biology had expedited the development of biological weapons[22]. Although some effort is being made to put in place global safeguards, there is no regulated plan to deal with the threat of a bioterror attack, including the exploitation of synthetic biology. A key challenge is how to establish standards, policies and regulations without restricting the continued growth of biotechnology. To mitigate potential harm, scientists should play an integral role in the strategic and systematic collaboration between public health, intelligence communities and national security to ensure a coordinated defence against the misuse of synthetic biotechnology. As part of Australia's biosecurity efforts, the Security Sensitive Biological Agents (SSBA) Regulatory Scheme was developed to regulate the handling of harmful biological agents on the list of SSBAs. The scheme aims to limit the opportunities available for deliberate acts of bioterrorism to occur and provides a legislative framework for the control and handling of SSBAs.

Conclusion

For centuries, biological agents have been used for warfare or terrorist activities by governments, non-state organisations and individuals. The threat of a biological attack remains a serious concern for local and international security. Although the state of biopreparedness is improving, many important challenges concerning the consequences of a biological attack remain. Governments must be flexible with the ability to adapt to changes in the global security environment, and all countries must strive to make a coordinated effort to develop appropriate policies, operations and preventive countermeasures for possible future attacks.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors thank Commonwealth Health for support for the biosecurity QAP.

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Biographies

Dr Alexa Kaufer graduated with a Bachelor (Honours) of Forensic Biology in Biomedical Science in 2015. She received her PhD in parasitology from the University of Technology Sydney in 2020, investigating the use of kinetoplast DNA molecular systematics, species identification and diagnostics of trypanosomatid parasites. She began her role as a scientist at the Biosecurity Department of the Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) in 2019.

Dr Torsten Theis is a Senior Scientist at the Biosecurity Department of the Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP). He received his PhD in Molecular Microbiology from the Berlin University of Technology (Germany). A post-doctorate fellow at University Colleges London (UK), University of Sydney and University of Technology Sydney, he investigated different aspects of the multidrug resistant phenotype of *Staphylococcus aureus*. Dr Theis joined RCPAQAP in 2010, and is currently responsible for all program aspects that require PC3/PC4 laboratory work, including researching, evaluation, and preparation of specimens to be used in the Proficiency testing programs (PTP) offered by the Biosecurity Department of the RCPAQAP. He liaises with leading specialists in the fields of public health, forensics and counter-bioterrorism, researching current issues and authoring educational material and reports. In collaboration with the Laboratory Strengthening and Biorisk Management department at the World Health Organization, Dr Theis is the RCPAQAP project lead for the development of PTPs for the detection of arboviruses, coronaviruses, and agents responsible for viral haemorrhagic fevers.

Dr Katherine Lau was awarded her PhD in Medicine for her research on the biology and molecular biology of new recombinant viruses of HIV from the University of Sydney in 2009. She then completed her postdoctoral research training at the Kolling Institute of Medical Research, University of Sydney in 2013. Dr Lau's main expertise is primarily on the application of advanced molecular biology technique in virus research. Her passion has always been to incorporate the principle and technical knowledge into her roles for a better disease and diagnosis outcome. Dr Lau has been with the Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) Biosecurity since 2013. At present, she is responsible for the Viral Specimen Program, including ongoing research, development, and evaluation of new and existing Proficiency testing programs (PTP). In 2018, Dr Lau led and developed a pilot PTP for detection and analysis of a bacterial isolate using Whole Genome Sequencing (WGS), in collaboration with the Communicable Diseases Genomic Network (CDGN).

Joanna Gray has a Bachelor of Applied Science majoring in Microbiology from the University of Technology, Sydney. She has approximately 40 years' experience in microbiology and quality management, a black belt in six sigma and lean, a broad range of experience in training and other business improvement roles in the pharmaceutical and medical industries. For the past 20 years Joanna has been an accredited Evaluator for Business Excellence Australia who annually host the Australian Business Excellence Awards. Since 2009, she is RCPAQAP Biosecurity Program Manager – Molecular Infectious Diseases providing Australia, and a select number of overseas laboratories, with proficiency testing surveys and educational modules for Security Sensitive Biological Agents (SSBAs), other potential agents and emerging communicable diseases threats to Australia. The Biosecurity program is funded by the Australian Government's Department of Health. Since 2019, Joanna has taken on the added responsibility for the Molecular Genetics programs as RCPAQAP Manager – Molecular Genetics.

Professor William Rawlinson, AM FAHMS BSc(Med) MBBS PhD (Cantab) GCM FRACP FRCPA FASM FFSc, is Director of Serology and Virology Division (SAViD), Director Organ and Tissue Donor screening laboratory, Director NSW State Reference Laboratory for HIV, Chair Biosecurity Quality Assurance Program (QAP) RCPAQAP, Deputy Chair Serology Quality Assurance Program (QAP) RCPAQAP, Chair National Verification Committee for Measles and Rubella. William Rawlinson is a clinician scientist researching viral pathogenesis, particularly respiratory viral infections, congenital infections, and enteroviruses in type 1 diabetes mellitus. He established, and oversees, serology and virology clinical research programs, statewide transplant donor screening, and national quality programs for serology and biosecurity. He is conjoint professor at UNSW with over 400 publications and over 18 000 citations from his publications in basic research, diagnostic and clinical virology.

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