ANIL AGGRAWAL'S
Internet Journal of Forensic Medicine and Toxicology
The world's first online journal devoted entirely to forensic medicine and toxicology · Published 1 January and 1 July

VOL. 20, NO. 2 · JULY – DECEMBER 2019
Downloaded from www.anilaggrawal.com · ISSN 0972-8074 (Online) · https://www.anilaggrawal.com/ij/vol_020_no_002/papers/paper001.html

An Overview Of Various Biological Warfare Agents

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CITE THIS ARTICLE

Purba MK, Agrawal N, Shukla S. An Overview Of Various Biological Warfare Agents. *Anil Aggrawal's Internet Journal of Forensic Medicine and Toxicology* [serial online], 2019; Vol. 20, No. 2 (July – December 2019): [about 16 p]. Available from: https://www.anilaggrawal.com/ij/vol_020_no_002/papers/paper001.html

ABSTRACT

Use of biological warfare agents is an emerging threat to mankind. In 21st century awareness and threat regarding these biological warfare agents is increasing.These agents are used in war or for terrorist attack. Various toxins and microorganisms are used as biowarfare agents to cause harm to humans, animals and plants. They are classified under various categories. They can be used in various forms as sprays, as explosive or food or water contamination. Biowarfare agents are difficult to identify and can be easily transported and delivered which makes them more favorable tool for destruction for terrorist operations or to gain political advantage. To detect such agents, the diseases associated with them should be recognized using various techniques. Protective measures should be taken according to the symptoms of the diseases related and their contagious nature. This review on the biological warfare agents will provide information on the biowarfare agents, their mode of transmission and also the detection systems available to detect them.

Keywords: Forensic science, biowarfare agents, common agents, characteristics, detection systems

INTRODUCTION

Those living organisms which are responsible of infecting and causing both illnessand death in people, animals and plants are biological agents.Use of toxins and infectious materials or organisms to infect and cause illness and death in humans, animals and plants is termed as biowarfare. The toxins and other infectious organisms used for biowarfare are known as biowarfare agents. These agents reproduce and divide inside host body. They can be lethal or non-lethal. These are classified under 4 categories, Biological warfare Bioterrorism, Biocrime, andBio accident [1][2].A Critical Agent List has been made by Centers for Disease Control and Prevention (CDC) in coordination with military, intelligence, medical and public health agencies which classifies all the possible biological warfare agents to be used in biological warfare or bioterrorism. This list is divided into 3 categories

which is category A includes the most preferred agents which cause mass casualty, create panic and require public health response. These weapons are most likely to be used in future attacks. Category B includes the second most preferred agents which include many agents which are responsible for contamination of food or water and can disperse easily. Category C includes those pathogens which can be easily accessed, produced and dispersed and cause high mortality [2].The biowarfare agents are further classified into various categorieswhich include Bacteria, Virus, Fungus, Rickettsia and Toxins. Various diseases are kept under these categories such as Anthrax, Plague,Brucellosis, Cholera, Clostridium Perfringens toxin, Staphylococcal Enterotoxin B, Melioidosis and Tularemia under Bacteria,Crimean – Congo Hemorrhagic Fever, Ebola Hemorrhagic Fever, Small Pox, Rift Valley Fever and Venezuelan EquineEncephalitis under Virus, and Trichothecene Mycotoxin under Fungus, Q Fever under Rickettsia and Botulinum toxin, Saxitoxin(derived from paralytic shellfish) and Ricin (cytotoxin derived from castor bean mesh) under Toxins [3].

CHARACTERISTICS

There are certain characteristics which are possessed by biowarfare agents and make them effective. These characteristics include high toxicity, fast action, capacity of survival outside the host for optimum time to establish itself in a victim, predictable in its impact, non-destructible with air, water and food purification methods, susceptible to treatment or vaccines available to the attacker but not to the victims, ability of efficient dispersion, stability after dispersion, manufactured on large scale, difficult to detect, capable to produce desired psychological affects [1] [2].

COMMON BIOWARFARE AGENTS

ANTHRAX

Anthrax is a zoonotic disease of humans and animals. It is caused by bacterium *Bacillus anthracis*. It is an anaerobic, gram negative, facultative, non-motile and spore forming bacterium. These bacteria enter in thebody through wounds in skins and may infect humans as aerosol or ingestion[3]. Anthrax is considered asan effective biological warfare agents because it produces spores that are highly stable and resistant to harshenvironmental conditions like heat, humidity, UV radiation and disinfectants[4]. It is classified into threetypes i.e. Cutaneous Anthrax, Inhalation Anthrax and Gastrointestinal Anthrax [3] [4] [5].

PLAGUE

Plague is an infectious and zoonotic disease which is caused by an enterobacteria *Yersinia pestis*. It is a facultative anaerobe. It is non-motile and gram negative bacteria. This infection is transmitted to humans through rodents and their fleas. This coccobacillus is transmitted to humans by the bite of the infected oriental rat flea or *Xenopsylla cheopis*[6]. Under natural conditions, plague can spread through air, by direct contact or by contaminated undercooked food. It hasthree syndromes i.e. bubonic plague, septicemic plague and pneumonic plague. The symptoms of these three syndromes depend upon the concentrated area of infection such as bubonic plague affects lymph nodes, septicemic plague affects blood vessels, and pneumonic plague affects lungs. In biological warfare scenario, the most types of syndromes that are spread through contaminated fleas are bubonic plague or pneumonic plague [3].

BRUCELLOSIS

Brucellosis is a disease which is caused by bacteria. It is also known as Bang's disease, Crimean fever, Gibraltar fever, Malta fever,Maltese fever, Mediterranean fever, Rock fever, or undulant fever [3].It is a zoonotic disease which is mainly caused by one of the four species: *Brucella melitensis, Brucella abortus, Brucella suis, andBrucella canis*. These bacteria are aerobic, gram negative, non-spore forming and non-motile coccobacilli[4]. Humans gets infected due to consumption of raw or undercooked food like meat or milk, inhalation of aerosols or contact with bacteria due to some abrasion. This infection is not contagious but it may spread from one human to other through breastfeeding.Incubation period of brucellosis is 3-4 weeks but can even vary from 1 week to several months [7].Cardiovascular, genitourinary, hepatobillary, osteoarticular, pulmonary, gastrointestinal and nervous systems may be some of the involved complications. Untreated patients may develop chronic brucellosis syndrome[8]. Later symptoms are complicated which include arthritis, vertebral osteomyelitis and rarely endocarditis. Fatality is about 6%.

CHOLERA

Cholera is an intestinal infection caused by bacteria *Vibrio cholerae.* It is a gram negative, short and curved bacteria. Cholera spreads through contaminated water and food [4]. The early symptoms of cholera are diarrhea and vomiting of clear fluid. Symptoms can be seen after one to five days of ingestion of contaminated food or water. Untreated and severe cholera may lead to life threatening dehydration and electrolyte imbalances and prove to be fatal in about half of the cases. Loss of large quantity of fluids from the body turns patient’s skin to bluish-gray. This is the reason cholera is also termed as Blue Death[4] [9]. Fever is not a common symptom in cholera. If there is fever then it may be because of some infection. Other symptoms include lethargy, headache, abdominal cramps, sunken eyes, dry mouth, cold clammy skin, wrinkled hands and feet, blood pressure drops, pulse raises and passage of urinedecrease with time. Due to fluid loss, muscle cramping, altered consciousness and even coma can be observed, especially in children[9].

CLOSTRIDIUM PERFRINGENS TOXIN

Clostridium perfringens is a very common anaerobic bacteria.There are five types of *Clostridium perfringens* A, B, C, D and E. Four different types of deadly toxins are produced by these strains of bacteria. These four different deadly toxins are: Alpha, Beta, Iota, and epsilon. Theta toxin is another toxin which is produced by these bacteria which damages blood vessels and enterotoxins present in intestinal cells [4].They sustain well in soil and gastrointestinal tract of healthy animals and humans. Nasal itching, pain, epitasis, runny nose and sneezing are symptoms which can be seen due to exposure of bacteria to upper respiratory system. Exposure to mouth and throat causes pain and blood tinged sputum and saliva. Difficulty in breathing, coughing and wheezing are some of the symptoms which can be observed when a person suffers from pulmonary or tracheobronchial toxicity [4].If the toxin affects the gastrointestinal tract of a person then he/ she is likely to experience nausea, vomiting, loss of appetite, crampy abdominal pain along with bloody diarrhea. Ocular exposure to this bacteria leads to eye pain, tearing, blurred vision, foreign body sensation and redness. These eye symptoms can be seen within few minutes of exposure whereas skin symptoms can be seen in minutes to hours. Exposure of these bacteria through any route may lead to systemic toxicity. This causes weakness, dizziness, prostration, loss of coordination and ataxia [10]. In fatal cases, irregular heartbeat, hypotension and hypothermia can be observed.

Swelling is present as a result of production of large amount of gases such as CO_2 and hydrogen produced by the bacteria. This is the reason why it is called Gas gangrene[4] [11].

STAPHYLOCOCCAL ENTEROTOXIN B

Staphylococcus aureus is a gram positive bacterium which produces an enterotoxin known as *Staphylococcus enterotoxin B (SEB).* This toxin is very stable and survives even after the bacterium which produces it is killed. SEB can be transmitted through inhalation and ingestion of contaminated food and is toxic in both cases [4]. The symptoms depend upon the route of inoculation of toxin into the body. If the toxin is inhaled then symptoms such as fever, chest pain, dyspnea and non- productive cough can be seen. Fever may last upto 5 days and coughs upto 4 weeks. If the toxin is ingested then gastrointestinal symptoms can be seen which include food poisoning- vomiting, diarrhea, intestinal cramping and nausea. Some gastrointestinal symptoms can be observed even after inhalation of toxin through aerosol. SEB is known to cause toxic shock syndrome as well as erythema. When the bacteria enters the host, it releases an exotoxin which is highly virulent [4] [12].It has super-antigen properties which leads to a strong inflammatory response along with stimulation of cytokine release and causes toxic shock syndrome. Since SEB is a super-antigen, release of large amount of cytokine causes inflammation which lead to Gastroenteritis. This toxin is not communicable. Severe exposure to toxin may be fatal [12].

MELIOIDOSIS

Burkholderia pseudomalleus is a gram negative bacteria which is known to cause an infectious disease called melioidosis.Various modes of transmission include contaminated drinking water, inhalation or entering of bacteria through a skin lesion from contaminated soil[4].It is contagious and can spread from one person to another. Infected needles can also be one of the ways to transmit this disease. This bacterium is known to cause two types of melioidosis, viz. acute melioidosis and chronicmelioidosis [13]. Melioidosis infection may be classified under four categories i.e. localized infection, pulmonary infection, bloodstream infection and disseminated infection. Melioidosis has variety of symptoms that is why it is termed as the great mimicker. These symptoms can be categorized into asymptomatic, chronic, acute and sub-acute infections. Symptoms of asymptomatic infection are most commonly seen in drug addicts and are fatal. Septicemic form of this infection causes dissemination of bacteria in blood and other organs which is fatal. Other symptoms of melioidosis are pulmonary infiltration, acute localized supportive infection, pulmonary infection, supportive infection and septicemic infection. Mortality is about 50% [3] [4] [13].

TULAREMIA

Francisella tularensis is a gram negative bacterium which is responsible for causing an infectious disease known as Tularemia. Other names for this disease are Pahvant Valley plague, deer fly fever and Ohara's fever. *Francisella tularensis* is a non-motile coccobacillus [14]. It is an intracellular bacterium i.e. it lives inside the host as a parasite. This infection attacks white blood cells, especially macrophages. The infection can spread by coming in contact with blood or tissue of infected animals and entering of bacteria through skin or mucous membranes [4][14].Various forms of tularemia on the basis of route of inoculation are ulceroglandular tularemia, gastrointestinal tularemia, typhoidal tularemia, pneumonic tularemia, oculoglandular tularemia, oropharyngeal tularemia and glandular tularemia. The symptoms of this infection are seen after 3-5 days.

Symptoms which are commonly seen in mammals are fever, lethargy, signs of septicemia, anorexia, reddening and inflammation of eyes and face. It is even fatal at times. In case of pneumonic tularemia or typhoidal tularemia rate of mortality is high about 50% in untreated cases. But this rate decrease to less than 10% after treatment [4].

CRIMEAN-CONGO HEMORRHAGIC FEVER

Nair virus is responsible for causing Crimean- Congo Hemorrhagic Fever. Transmission of this disease takes place through ticks. These ticks belong to genus *Hyalomma*. Infection in humans is caused via tick bites, crushing infected tick or killing a viremic livestock. It is rarely a contagious disease but it may spread through infectious blood and body fluids. Nairovirus is tick borne virus. It is a severe disease in infected humans. These ticks serve as both reservoir and vector for CCHF virus [4].Incubation period of this disease is about 5 to 6 days after exposure to infected blood and tissues whereas it is about 1 to 3 days in case of a tick bite. After infection, flu like symptoms may appear which include sudden onset of fever and chills. Severe headache, lumbar pain, nausea, delirium, joint pain, weakness and vomiting are also some of the symptoms observed. Extensive hemorrhage, coma and shock are the reasons which lead to fatal cases. Pain and swelling in liver can be seen. Some serious issues include disseminated intravascular coagulation, kidney failure, respiratory distress syndrome and shock. Mortality rate of CCHF is about 15-30% [4] [15].

EBOLA HEMORRHAGIC FEVER

It is the most virulent disease caused by a virus. It proves to be fatal in about 50-90% cases. It is one of the various Viral Hemorrhagic Fevers. *Filoviridae* is a virus which is responsible for causing Ebola HF. This virus is spread due to direct contact with infected blood, secretions, organs or semen [16].It may also spread through contaminated syringes and needles which turn out to be fatal. The five subspecies are as follows, Ebola virus (*Zaire Ebola virus*), Sudan virus (*Sudan Ebola virus*), Taï Forest virus (*Taï Forest Ebola virus*)/ *Côte d'Ivoire* Ebola virus, Bundibugyo virus (*Bundibugyo Ebola virus*), Reston virus (*Reston Ebola virus*). Incubation period of this disease is about 2 to 21 days. Initial symptoms include sudden onset of fever, muscles pain, weakness, stomach pain, sore throat, headache, diarrhea, vomiting, malfunctioning of kidney and liver, joint and muscles aches, internal and external bleeding, lack of appetite. Some patients may experience red eyes, hiccups, cough, chest pain and difficulty in breathing and swallowing [4] [16]

SMALLPOX

Smallpox was an infectious disease which was mainly caused by two types of viruses: *Variola major* and *Variola minor* .Small pox was limited to mouth, throat and blood vessels of skin. Maculopapular rash is caused due to infection in skin which in late stages leads to raised fluid filled blisters. *V. major* is more infectious than *V. minor*. *V. minor* was known to cause mild form of disease which was also termed as alastrim, cotton pox, milk pox, white pox and Cuban itch. Mortality rate of *V. major* was about 30-35% whereas in case of *V. minor* it was about 1% [4].In about 65-85% of survivors, *V. major* lead to prolonged impediments such as scar on face. There were other complications also but was seen in only 2-5% cases which included blindness due to corneal ulceration and scaring, limb deformities resulting from osteomyelitis and arthritis. This virus was an orthopoxvirus which was known to cause infections to humans only. It was declared eradicated in 1980[4] [17]. Inhalation of the virus present in air is the main mode of transmission. It can also spread by coming in contact with infected body fluids or contaminated objects such as clothing. Smallpox disease can be classified into four types, ordinary smallpox, modified smallpox,

malignant smallpox, and hemorrhagic smallpox. Incubation period of this disease is about 0-17 days. Late symptoms include skin eruptions known as exanthema (reddish spots) which appear on mouth, tongue, palate and throat which progresses with time [3]. They start appearing on forehead then spread to the face, proximal portions of extremities, trunk and then distal portion of extremities. Later these pustules get dry and form crusts which leave a depressed depigmented scar after healing [4] [18].

RIFT VALLEY FEVER

Rift Valley Fever is an acute viral disease which is caused by RVF virus. It is a mosquito borne disease. Infection in humans is caused through mosquito bite, contaminated blood, body fluids, organs and exposure to aerosols or droplets. This virus leads to abortion and death in domestic animals. RVF is not considered to be contagious. Mortality rate of this disease is about 50% in case of viral hemorrhagic fever. Fatal cases are less in RVF [4].Hemorrhagic fever, eye lesions and encephalitis can be seen as symptoms of RVF. This virus is responsible for causing various syndromes. Infected people show no or mild symptoms which include fever, myalgia, headache, weakness, back pain, dizziness, weight loss and liver abnormalities. In domestic animals vomiting, diarrhea, fever, respiratory distress, anorexia, lethargy and often death in young animals is seen. About 8 to 10% of people infected with RVFV develop much more severe symptoms which includeOcular disease, and Encephalitis and Hemorrhagic fever [4] [19].

VENEZUELAN EQUINE ENCEPHALITIS

Venezuelan equine encephalitis is a viral disease caused by Venezuelan equine encephalitis virus. This disease is also known as encephalomyelitis (VEE). It is usually abbreviated as VEE. This is a mosquito borne virus. Central nervous system disorders or death may be seen when equines acquire this infection. It can infect humans. Mosquitoes are responsible for spreading this disease by biting an infected animal and then feed on another animal or human. They are highly virulent to equines and may affect humans also. Infected equines are also responsible for spreading the disease as they develop large amount of virus in their circulatory system [4].There have eight types of VEE virus which have been associated with humans, but the main subtypes which have been responsible for major outbreaks are subtypes 1, variants A, B and C. VEE is not a contagious disease. Every infected person will definitely suffer illness and will show flu-like symptoms. These symptoms last for 24-72 hours. Mortality rate is less than 1% whereas in children it may reach 20% [4] [20].

TRICHOTHECENE MYCOTOXINS

Fungi produce more than 40 diverse compounds known as Trichothecene mycotoxins. They inhibit mitochondrial respiration and protein synthesis. Cell membrane structure and function is altered along with impairment in DNA synthesis [21].Consumption of contaminated food or inhalation of T-2 toxin or other toxins produced by secondary metabolites of fungi lead to production of toxic reactions known as mycotoxicoses. These mycotoxins have severe effect on humans and animals. Trichothecenes are strong inhibitors of protein synthesis. They are cytotoxic and immunosuppressive. According to studies, T-2 toxin can cause apostasies and programmed cell death in rodent and human cells. They can be absorbed via topical, oral and inhalation routes. Ingestion of these toxins lead to weight loss, vomiting, skin inflammation, bloody diarrhea, diffuse hemorrhage and sometimes death [4][20]. After being exposed to this toxin intravenously or via inhalation, symptoms can be seen within few hours. Lactic acidosis, arterial hypotension,

circulatory shock and reduced cardiac output are some of the symptoms which can be seen which finally leads to death within 12 hours. These toxins are responsible for causing Alimentary Toxic Aleukia (ATA) [4].

Q FEVER

Coxiella burnetti is an infectious zoonotic disease which causes Q fever. This bacterium infects animals as well as humans. It is rickettsia like organisms which does not have high virulence but is severely infectious. This infection transmits by coming in contact with infected animals or their body fluids such as milk, urine, vaginal mucus or semen or by inhalation of spore like small cell variant. The most common symptoms are mild flu like symptoms [4].Late symptoms of this disease include atypical pneumonia which can lead to Acute Respiratory Distress Syndrome (ARDS). ARDS is a deadly syndrome. Sometimes, Q fever may cause hepatitis which does not show any symptoms but it may start showing symptoms along with malaise, fever, pain in upper right portion of abdomen, enlargement of liver. Inflammation in endocarditic i.e. the inner lining of heart is a symptom of chronic Q fever. There is about 10% fall in the mortality rate if proper treatment is given [22].

BOTULINUM TOXIN

Clostridium botulinum produces a neurotoxin and protein which causes serious paralytic disease known as botulism. This is a rare disease. Some strains of *Clostridium buryricum* and *Clostridium baratii* also produce toxins which causes botulism. This bacterium produces seven different types of botulism toxins which are represented by letters A to G. A, B, E and F are known to cause this disease in humans [23].The toxin binds to presynaptic membrane of neurons which blocks neurotransmission and prevent release of acetylcholine. Botulism is divided into five types, food borne botulism, infant botulism, wound botulism, inhalation botulism and waterborne botulism. The most common symptoms of botulism are double vision, slurred speech, drooping eyelids, blurred vision, dry mouth, and difficulty in swallowing and muscle weakness [24]. Symptoms generally appear in 18-36 hours in case of food borne botulism but they can be seen as early as 6 hours and as late as 10 days [25][26]. Sudden respiratory failure can be observed. Flaccid muscle weakness of palate, larynx, tongue, respiratory muscles and extremities are also observed. Mortality rate is about 60% [3] [4].

SAXITOXIN

Saxitoxin is a neurotoxin. They are mainly produced by dinoflagellates. Paralytic shellfish poisoning (PSP) is due to intoxication in humans. PSP is a severe and life threatening disease which needs immediate treatment. Humans get infected by this toxin due to ingestion of a fish containing this toxin in its tissues [4]. Gastrointestinal and neurological symptoms can be observed in case of severe poisoning. Saxitoxin acts as a selective sodium channel blocker. It is one of the most dangerous natural toxin which acts on voltage gated sodium channels of nerve cells. This action of toxin leads to abnormal cellular function and causes paralysis. Symptoms can appear in 10 to 60 minutes after exposure but the onset of symptoms may be delayed depending upon the dosage. Early symptoms include numbness or tingling of lips, tongue, fingertips, neck and extremities, general muscular incoordination, nausea, vomiting in some cases, difficulty in swallowing, sense of throat constriction, speech coherence or complete loss of speech along with brain dysfunction. Respiratory distress and flaccid muscular paralysis are some of the late symptoms. Respiratory

paralysis may lead to death. Cardiac conduction defect may also appear. Mortality rate of this poisoning is about 8-10% [4] [27].

RICIN

Ricin is a glycoprotein toxin which is derived from seed of castor plant (*Ricinus communis*). A compound known as lecithin produced in this plant which makes it highly toxic. It is a type II ribosome-inactivating protein [RIP] [28]. Less than 500 micrograms of ricin can be fatal for an adult whether it is inhaled or injected. It could spread through exposure to contaminated food, water and air. It is not a contagious poisoning. It can only spread if a person is exposed to ricin containing clothes or other materials [4].Appearance of symptoms depends on the route of exposure and the dosage of the poison. In case of inhalation, fever, nausea, cough, tightness in chest, heavy sweating, cyanosis, low blood pressure and respiratory failure which may finally lead to death can be seen, in case of ingestion blood in urine, internal bleeding may start leading to liver, spleen and kidney failure which may cause death. In case of skin or eye exposure pain and redness in eyes and skin can be seen. Depending upon the route of exposure and the dosage of ricin received, death may occur within 36 to 72 hours of exposure [29].

Table No. 1:

SUMMARISED TABLE OF DISEASES USED AS BIOWEAPON					
S. NO	**DISEASE**	**CAUSATIVE AGENT**	**CLASSIFICATION**	**INCUBATION PERIOD**	**EARLY SYMPTOMS**
1.	**Anthrax**	*Bacillus anthracis*	• Cutaneous anthrax (transmitted through skin)	1 to 7 days	Skin lesions
			• Inhalation anthrax (transmitted through inhalation)	1 to 6 days	• Fatigue • Malaise • Fever • Non- productive cough • Mild chest discomfort
			• Gastrointestinal anthrax (transmitted through ingestion)	About 7 days	• Nausea • Anorexia • Vomiting • Fever • Abdominal pain • Bloody diarrhea • Red face and eyes • Sore throat • Pain in swallowing

SUMMARISED TABLE OF DISEASES USED AS BIOWEAPON					
					• Swelling in neck and neck glands • Hoarseness
2.	**Plague**	*Yersinia pestis*	• Bubonic plague (transmitted through flea or ingestion of contaminated food)	2 to 10 days	• Malaise • High fever • Tenderness of lymph nodes
			• Pneumonic plague (transmitted through inhalation)	2 to 4 days	• Malaise • Vomiting of blood • Weakness • Headache • Fever • Cough with bloody sputum • Toxemia
3.	**Brucellosis**	*Brucella species*	• *Brucella melitensis*	3 to 4 weeks	• Ovine Brucellosis • Malta fever

SUMMARISED TABLE OF DISEASES USED AS BIOWEAPON		
		• Severe inflammation of epididymis • Formation of fibrinous adhesions Spermatocoeles
	• *Brucella abortus*	• Contagious abortion • Undulant fever • Premature calving
	• *Brucella suis*	• Swine Brucellosis • Inflammatory lesions in reproductive organs. • Abortion • Lameness • Permanent sterility • Spondylitis • Posterior paralysis • Abscess formation
	• *Brucella canis*	• Epididymitis in male dogs • Orchitis in male dogs • Abortions in female dogs • Placentitis in female dogs • Endometritis in female dogs

SUMMARISED TABLE OF DISEASES USED AS BIOWEAPON				
				• Impotency • Inflammation of testes
		General symptoms caused due to Brucella species		• Fever with chills • Headache • Fatigue • Sweats • Myalgia • Anorexia • Arthralgia • Malaise • Depression • Weight loss
4.	**Cholera**	*Vibrio cholerae*	1 to 5 days	• Diarrhea • Vomiting of clear fluid • Lethargy • Headache • abdominal cramps • sunken eyes • Dry mouth • Cold clammy skin • Wrinkled hands and feet Blood pressure drops

SUMMARISED TABLE OF DISEASES USED AS BIOWEAPON					
					• Pulse raises Passage of urine decrease with time
5.	**Clostridium Perfringens Toxin**	*Clostridium perfringens*	• Type A (Produces alpha toxin and sometimes beta toxin)	Few hours	**In animals:** • Haemorrhagic bowel syndrome (HBS) • Bloody gut **In humans:** • Mild food poisoning
			• Type B (produces alpha, beta and epsilon toxin)		**In animals:** • Necrosis • Inflammation in small intestine. **In humans:** • Rarely infects
			• Type C (produces beta toxin)		**In animals:** • Inflammation of small intestine • Dehydration • Weakness • Diarrhea **In humans:** • Necrosis of intestines • Septicemia
			• Type D		**In animals:** • Affects small intestine

SUMMARISED TABLE OF DISEASES USED AS BIOWEAPON			
			• Enterotoxaemia • Swelling in kidneys • Brain and lung edema **In humans:** • Rarely seen
		(produces alpha and epsilon toxin)	
		• Type E (produces alpha and iota toxins)	**In animals:** • Hemorrhagic enteritis • Enterotoxaemia **In humans:** • Rarely seen
	With respect to the mode of exposure	1 to 6 days	• Redness • Burning skin pain • Sloughing of large areas of skin • Tenderness • Blistering • Progression to skin death with leathery blackening. • Nasal itching • Pain • Epistaxis • Runny nose • Sneezing • Pain • Blood tinged sputum and saliva

SUMMARISED TABLE OF DISEASES USED AS BIOWEAPON

				• Difficulty in breathing • Coughing • Wheezing • Nausea • Vomiting • Loss of appetite • Crampy abdominal pain • Bloody diarrhea • Eye pain • Tearing • Blurred vision • Foreign body sensation • Redness • Systemic toxicity
6.	**Staphylococcal Enterotoxin B**	*Staphylococcus aureus*	4 to 10 hours	• High fever Headache • Chills • Myalgia • Varying degrees of prostration • Fever • Chest pain Dyspnea • Non- productive cough • Food poisoning • Vomiting • Diarrhea • Intestinal cramping

SUMMARISED TABLE OF DISEASES USED AS BIOWEAPON					
					• Nausea
7.	**Meliodiosis**	*Burkholderia pseudomallei*	• Acute meliodiosis	1 to 21 days	• Fever • Pain • Cough or pleuritic chest pain • Indicative of pneumonia • Bone and joint pain indicative of osteomyelitis or septic arthritis or cellulitis • Intra-abdominal infection
			• Chronic meliodiosis	Around 2 months	• Chronic skin infections • Chronic lung nodules • Pneumonia
			• Localized infection		• Ulcer • Nodule • Skin abscess • Abrasion • Fever • Muscle aches
			• Pulmonary infection		• High fever • Anorexia • Muscle soreness • Headache • Chest pain

SUMMARISED TABLE OF DISEASES USED AS BIOWEAPON					
					• Productive or non-productive cough with sputum • Cavitary lesions
			• Bloodstream infection		• Headache • Fever • Respiratory distress • Joint pain • Muscle tenderness • Abdominal discomfort • Disorientation
			• Disseminated infection		• Fever • Stomach or chest pain • Weight loss • Headache • Muscle and joint pain • Seizure.
8.	**Tularemia**	*Francisella tularensis*	• Ulceroglandular tularemia	2 to 10 days	• Skin ulcer • Fever • Chills • Lymphadenopathy • Headache • Malaise.

SUMMARISED TABLE OF DISEASES USED AS BIOWEAPON					
			• Gastrointestinal tularemia		• Abdominal pain • Fever • Diarrhea • Nausea • Vomiting
			• Typhoidal tularemia		• Ingestion • Fever • Weight loss Prostration
			• Pneumonic tularemia		• Fever • Headache • Substernal • Discomfort Malaise • Non-productive cough.
			• Oculoglandular tularemia		• Rarely seen
			• Oropharyngeal tularemia		• Rare
			• Glandular tularemia		• similar to ulceroglandular tularemia • no skin ulcer • Rare
9.	**Crimean-Congo Hemorrhagic Fever**	*Nairo virus*		5 to 6 days	• Sudden onset of fever • Chills

SUMMARISED TABLE OF DISEASES USED AS BIOWEAPON

					• Severe headache • Lumbar pain • Nausea • Delirium • Joint pain • Weakness • Vomiting
10.	**Ebola Hemorrhagic Fever**	*Filoviridae*	• Ebola virus (*Zaire Ebola virus)*	2 to 21 days	• Sudden onset of fever • Muscle pain • Weakness • Stomach pain • Sore throat • Headache • Diarrhea • Vomiting • Malfunctioning of kidney and liver • Joint and muscles aches • Rash • Internal and external bleeding

			• Sudan virus (*Sudan Ebola virus)*		
			• Taï Forest virus (*Taï Forest Ebola virus)/ Côte d'Ivoire Ebola virus*		• Lack of appetite
			• Bundibugyo virus (*Bundibugyo Ebola virus*)		
			• Reston virus (*Reston Ebola virus)*		
11.	**Small pox**	• *Variola major* • *Variola minor*	• Ordinary smallpox	0 to 17 days	• Macules turn into raised papules. • Papules turn into vesicles. • Vesicles are filled with opalescent fluid. • Pustulesare opaque and turbid • Pustules filled with tissue debris • Pustules are tight, round, sharply raised and firm in the skin

SUMMARISED TABLE OF DISEASES USED AS BIOWEAPON

	• Modified smallpox	• Eruption of rash • Skin lesions do not show properties like typical smallpox
	• Malignant smallpox	• Lesions remain flat • Lesions turn in vesicles
	• Hemorrhagic smallpox	• Extensive bleedingin the skin, mucous membrane and gastrointestinal tract. • Small pox blisters do not develop • Skin appears black and charred.

SUMMARISED TABLE OF DISEASES USED AS BIOWEAPON

12.	**Rift valley fever**	*Rift valley fever virus*	2 to 6 days	• Fever • Conjunctival injection • Abdominal tenderness • Petechiae • Viral hemorrhagic fever syndrome • Myalgia • Headache, weakness • Back pain • Dizziness • Weight loss • Liver abnormalities.
13.	**Venezuelan Equine Encephalitis**	*Venezuelan equine encephalitis virus*	2 to 6 days	• Malaise • Fever • Rigors • Photophobia • Severe headache • Myalgia in legs and lumbosacral area • Nausea • Sore throat • Vomiting • Diarrhea
14.	**Trichothecene mycotoxins**	Produced by fungus	Few hours	• Weight loss

SUMMARISED TABLE OF DISEASES USED AS BIOWEAPON

- Vomiting
- Skin inflammation
- Bloody diarrhea

SUMMARISED TABLE OF DISEASES USED AS BIOWEAPON

15.	**Q fever**	*Coxiella burnetti*		9 to 40 days	• Fever • Profuse respiration • Malaise • Muscle pain • Joint pain • Severe headache • Loss of appetite • Dry cough • Chills • Pleuritic pain • Upper respiratory problems • Confusion • Gastrointestinal problems such as nausea, diarrhea and vomiting
16.	**Botulinum toxin**	*Clostridium botulinum*	• Foodborne botulism • Infant botulism • Wound botulism • Inhalation botulism	0 to 10 days	• Double vision • Slurred speech • Drooping eyelids Blurred vision • Dry mouth • Difficulty in swallowing

SUMMARISED TABLE OF DISEASES USED AS BIOWEAPON

				• Muscle weakness • Lethargy • Low appetite • Constipation • Weak cry • Poor muscle tone • Muscle paralysis • Ptosis • Lassitude • Dizziness • Diminished salivation • Extreme dryness in mouth leading to sore throat • Urinary retention • Generalized weakness • Blurred vision due to effect on cranial nerves • Diplopia • Photophobia
		• Waterborne botulism		
17.	**Saxitoxin**	• Dinoflagellates	Few hours	

SUMMARISED TABLE OF DISEASES USED AS BIOWEAPON

					• Paralytic shellfish poisoning • Numbness or tingling of lips, tongue, fingertips, neck and extremities • General muscular incoordination • Nausea • Vomiting • Difficulty in swallowing • Sense of throat constriction, Speech coherence or complete loss of speech • Brain dysfunction
		• Paralytic shellfish • *Saxidomus giganteus*			
18.	**RICIN**	*Ricinus communis*		Few hours	• Fluid in lungs • Fever • Nausea • Cough • Tightness in chest • Heavy sweating • Cyanosis • Low blood pressure • Respiratory failure • Bloody vomiting • Diarrhea • Low blood pressure

SUMMARISED TABLE OF DISEASES USED AS BIOWEAPON

- Dehydration
- Seizures
- Blood in urine
- Pain and redness in eyes and skin

DETECTION OF BIOWARFARE AGENTS

Detection of biological agents is considered to be high priority in various fields and programs such as defense, intelligence etc. For effective and active defense against the use and development of these agents various technologies and techniques are being practiced. Microbial forensics is a new discipline developed by combining microbiology and forensic applications [30].It is a scientific field which deals with investigation of the evidence collected from a bioterrorist attack, release of microorganism etc. Several advanced techniques are being used for detection of these agents [31]. Some of the detection techniques which are used include:

BIOCHEMICAL TEST BASED ASSAYS

It is a conventional identification method to determine various biowarfare agents. Some of the biological warfare agents like *Ba. anthracis, Y. pestis*, *Burkholderia* sp. and *Brucella* sp. can be determined using this technique. Nowadays, commercial automated biochemical test platforms are available which are frequently used for bacterial diseases that are clinically important. These systems have both visual observation and reader based formats for analysis of results and a database for comparison purposes. The major drawbacks of these systems are that they need pure culture and also require trained personnel to perform and analyze. These system converts cellular fatty acids from pure culture of bacteria to fatty acid methyl esters and for separation and identification it uses gas chromatography.Use of several steps like harvesting, saponification, methylation, extraction and washing before analysis is involved in this method whereas chromatographic technique helps in storage and database based retrieval for easy computing and analysis.[3].

BIOLUMINESCENCE BASED DETECTION

The enzyme, luciferin and luciferase and their interaction with ATP is monitored in Bioluminescence. The basic principle used here is that the microbial load in air, water and in other environmental samples is equal to the amount of ATP found in bacteria and viruses. It uses a micro-luminometer to read the sample's bioluminescence. These systems are sensitive to ATP contamination from non-microbial sources. As all of them detect only ATP, one cannot point towards biowarfare agents as the results are specific. These systems can be used for real time air monitoring and any unusual raise in the microbial load in the environment will trigger the alarm. These systems are cost effective [3].

ANTIGEN AND ANTIBODY BASED DETECTION

For detection of various bacterial and viral biowarfare agents, antigen and antibody based immunoassays are under being developed. Detection of anthrax, plague, botulism, brucellosis, glanders and meliodiosis is currently done using antigens or antibodies based on ELISA. The efficiency of detection depends on the quality of the antigen- antibody complex formed and the ability of the detection method to detect the complex.The major drawback of ELISA based systems is that they are time consuming as they can detect only one agent at a time and sequential assays need to be performed to detect more than one agent. There are many modified ELISA techniques which are used these days such as fluorescent, chemiluminescent, electrochemiluminescent, biosensors, flow cytometry, microarray,sandwich antigen capture assay format and lateral flow system. Lateral flow system is the latest technique in which it used disposable cartridges for detection process. A visual line or dot is produced due to the presence of colloidal gold or micro particle coated detector antibodies which forms antigen- antibody complex.These tests are rapid and easy but they are not much sensitive as they produce high false positive results. It can be used as presumptive test. Different agencies have developed lateral flows for all biowarfare agents but its use for detection and comparative evaluation is not yet reported.A procedural control is incorporated that acts as a negative control. These tests are rapid and easy but they are not much sensitive as they produce high false positive results. It can be used as presumptive test [3].

FLUORESCENT LIPOSOMES DETECTION

This type of detection is done in 2 phases through which biowarfare agents such as botulism toxin, staphylococcal enterotoxin, Saxitoxin etc. can be detected. To detect toxins in aqueous samples polymerized liposomes or antibody conjugates are used that will be integrated into portable fluorescence detection device. Multiple biological warfare toxins can be tested easily and rapidly. Due to binding of antibodies the liposomes become fluorescent. These liposomes can be articulated with different antibodies targeting different toxin targets. They can be presented in a multiplex format for comparable screening. This technique is portable, easy to use and rapid which can be used by both combat support units and first responders for early warning of attacks with biowarfare agents and post attack forensic purposes. [3].

NUCLEIC ACID BASED DETECTION

The most researched and developed detection systems used for biowarfare agents are nucleic acids, DNA, RNA based systems. There are two types of PCR. Standard and Q-PCR.In standard PCR, amplification of specific region of genome is done and then it is checked on electrophoresis for amplification of correct size of product. In Q-PCR, combination of PCR amplification and detection based on reported fluorescence dyes is done. The specific Q-PCR assays based on probes are used

for detection of all agents in biowarfare detection. This method is most preferred method of detection as it is compact, fast and sensitive. The major drawbacks related to nucleic acid based assays are variation in nucleic acid based amplification techniques, availability and quality of starting material, inhibitory substances present in the matrices, sensitivity and specificity based issues.[3].

SENSOR BASED DETECTION SYSTEMS

This type of system has been developed for biological, immunological and nucleic acid based detection systems. Transformation of response into an analyzable signal through transducer is the basic principle of these systems. Growing bacteria or fungi (commonly used biowarfare agents) produces volatile organic compounds. Such compounds are extracted from the biowarfare agent. These are also called as bio-elements. This extracted substance/ bio-element is the response which is converted into electrical signal by transducer. These systems which integrate biological component with a physical transducer are known as biosensors. The products that are formed due to an enzymatic activity associated with microbial metabolism are monitored based on conducting polymers, in electrochemical systems. Growing bacteria or fungi produces volatile organic compounds which are detected by using this technique. This technique helps in detecting many toxins that are highly specific and are produced by growing bacteria [3].

Bio-element (biological response)

Transducer (converts response into electrical signal)

Electrical signal detected and amplified

Processor processes it

Results are displayed

NANOPARTICLES BASED DETECTION

Various chemicals and biosensors can be developed using nanoparticles as these particles have unique properties. These particles can be used to develop biosensors with desirable features like increased sensitivity and lower detection limits. For detection of various biological substances sugar is coated on gold nanoparticles. The target substance such as Ricin, when mixed with a weak solution, it attaches to the sugar and leads to alteration of its properties and color change. Carbon dots are used for labelling target substances. When carbon dots are attached to spores of bacterium, it is found to glow upon illumination when visualized under confocal microscope. Some nanoparticles made up of silver atoms and shaped as Nano prisms appear red on exposure to light. Such nanoparticles are used for detection of biowarfare agents such anthrax. These are used as labels which glow when target DNA are present. Nanoparticles are of great importance in detection of biowarfare agents. [30]

USE OF GOLD NANOPARTICLES FOR DETECTION OF ANTHRAX

Anthrax lethal factor is one of the most dangerous biological warfare weapon which is a protease derived from *Bacillus anthracis*. Rapid detection of anthrax lethal factor is very important. For detecting anthrax lethal factor colorimetric sensors are used. Gold nanoparticles are functionally modified and then these modified GNPs are used as sensor probes. Visual detection of lethal factor can be done observing the color change in the solution containing gold nanoparticles. MAPK-kinases consist of amino terminal trail which is cleaved in the presence of lethal factor. It interrupts

the protein signal pathway. The best substrates which can be used for cleavage by lethal factor are those peptides which contains C- and N- terminal derivatives of cysteine. Lethal factor is incubated with peptide containing pair of cysteine derivative in presence of GNPS. Due to presence of lethal factor, the peptide cleaves into two fragments. Each fragment contains a single cysteine. This cleavage prevents aggregation of gold nanoparticles. Cleavage of peptide and absence of aggregation of GNPs indicate that lethal protease is present. In absence of lethal protease, no cleavage takes place which results in aggregation of gold nanoparticles. Due to aggregation, the color of the solution containing gold nanoparticles changes from red to blue (purple). This colorimetric sensor acts as a turn off sensor which shows color change in the absence of lethal factor. Inspite of its low sensitivity in comparison to ELISA and PCR, it serves as a rapid method of detecting presence of a toxin. This technique of detection of a toxin is important for defense and security purpose. [31]

ELECTROCHEMICAL BIOSENSORS

These biosensors are commonly used for detection of biowarfare agents. They are used with electrochemical transducer. These transducers are based on bio-interaction process. Electrochemical signal is used in this method to generate electrons which are detected by electrochemical detector. They are further divided into conductometric, potentiometric, and amperometric biosensors according to the electrochemical properties to be measured. Conductometric biosensors measure the changes in the conductance of the biological component arising between a pair of metal electrodes. Microbial metabolism usually results in an increase in both conductance and capacitance, causing a decrease in impedence. This technique has been used for detecting the concentration and physiological state of bacteria. Potentiometric measurements involve determination of the potential difference between an indicator and a reference electrode. They function under equilibrium conditions and monitor the accumulation of charge, at zero current, created by selective binding at the electrode surface. Antigen immobilized on the electrode surface interacts with enzyme labeled antibody, resulting in attachment of the enzyme to the electrode surface. Therefore, the formation of antigen labeled antibody complex on the electrode surface is accompanied by an electrode potential shift. The presence of free antigen in the solution leads to competition in the binding of labeled antibodies (conjugate) with free and immobilized antigen. The competition results in a decrease of the rate of potential shift. The decrease in rate is proportional to free antigen concentration in the solution. Amperometry is based on the measurement of the current resulting from the electrochemical oxidation or reduction of an electroactive species at a constant applied potential. Amperometric biosensors have the advantage of being greatly sensitive, rapid, and inexpensive. The advantage of linear concentration dependence of amperometry makes it well suited for bacterial assay. [32]

CONCLUSION

Since long biological agents are being used as a potential weapon. There are various types of biological agents which are used as weapons which include parasites, yeasts and fungi, bacteria, rickettsia and Chlamydia, viruses, prions and toxins. All the diseases related to these agents show various symptoms and according to their pathogenicity and virulence they are used as bioweapon. Anthrax and Ricin are the most used bioweapons due to their high level of toxicity and easy availability. There are various detection techniques which are used to detect these bioweapons. Microbial forensics is an emerging field which deals with various microorganisms which are used as bioweapon and their detection. An ideal detection system is required for proper detection of these biological warfare agents. Various detection techniques are used these days such as Biochemical assay based detection, Bioluminescence based detection, Antigen and antibody based detection, Fluorescent liposomes based detection, Nucleic acid based assay and Sensor based detection systems including Nanoparticle based systems and Electrochemical Biosensors. These detection techniques use various ways and matrices for detection. Most preferred detection technique is Nucleic Acid Based Detection and Sensor based detection techniquesas it is easy, rapid and gives accurate results. Many new techniques are under development for detection of biological warfare agents.

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