MODERN SURVIVAL
The Magazine of Freedom and Independent Thought
Volume 1, No. 2  October/November 2001

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Essentials of Biological Warfare

Given the proper biological conditions, biological agents can cause disease of epidemic proportion (plague and cholera, for example), can cause disease as a result of occupational exposure (anthrax and Woolsorter’s disease), or simply can be endemic in certain parts of the world, as anthrax is in Asia and the Middle East

By Dr. John R. Pettinato

Photos from the US Centers for Disease Control

So --- the unthinkable happened.

Be prepared. It will probably happen again.

The next time, expect a little more finesse, perhaps an invisible threat without odor, color, or significant distinguishing features.

Think about the potency of such a weapon, one that can be delivered by common, unobtrusive equipment, something that you may not glance twice at. Something you see every day, perhaps like that airplane you hear flying overhead as you read this article.

Too late. You don’t have time to get your gas mask.

Do you prefer a more sophisticated approach to terrorism? Okay. You’re sitting next to a young man on a crowded subway in Boston who has what seems to be a cold or the flu. He’s constantly sniffling and wiping his runny nose and although you’re worried you’ll get sick your only consolation is he hasn’t sneezed.

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Then he does, right in your face. You're okay for a week or so, but then you get sick.

A few days later, you get a rash that eventually covers your entire body. Before it goes away, the rash becomes oozing, pus-filled blisters.

Congratulations. You have smallpox. You probably won't die, but the next several weeks aren't going to be very pleasant.

To put the reality of biological agents into perspective, or rather if you think terrorists are unlikely to use biological weapons, consider the following:

In 1984, the Rajneeshee religious cult contaminated the salad bars of 10 Oregon restaurants with Salmonella typhimurium, resulting in 751 cases of Salmonella gastroenteritis.

The method of respiratory isolation needed to prevent transmission of smallpox.

The Aum Shinrikyo cult was clearly interested in the development of biological weapons for several years prior to their 1995 sarin attack of the Tokyo subway system. In 1992, the group sent members to Zaire to obtain Ebola virus for weapons development and there is evidence the cult had attempted three unsuccessful biological attacks in Japan using Bacillus anthracis (the agent responsible for anthrax) and botulinum toxin.

The potential horror associated with the use of biological weapons was recognized early in the last century, and as is the case with conventional weapons, our political leaders have attempted to control the proliferation and use of these weapons.

The Hague International Peace Conferences of 1899 and 1907 called for the prohibition of the use of poison during war. In 1925, the Geneva Protocol further prohibited the use of asphyxiating, poisonous, or other gases in war and also extended this ban to include the use of bacteriological weapons. However, an important point to note is the Geneva Protocol did not ban possession of agents that could be used as chemical or biological weapons, but only prohibited the use of such agents as weapons of war.
During the years 1943 to 1969, the United States produced 10 biological warfare agents that could be effective against humans (anthrax, botulinum toxin, Staphylococcal enterotoxin, among others) or food crops (wheat and rye stem rust, and rice blast). In 1969, an executive order from President Nixon renounced the use of biological weapons and ordered the US Department of Defense to destroy all existing stocks of biological agents and weapons. An extension of the executive order to include toxin agents was added in 1970. Defensive research and development (especially of vaccines) continues to this day.

The resolve of the United States to end the development of offensive biological weapons was endorsed by Canada, Sweden, and the UK. This unilateral agreement led to the Biological and Toxin Weapons Convention (BWC), which was opened for signature in 1972 and went into force in 1975. Signed by 158 nations (but ratified only by 140), this treaty prohibits the development and use of biological agents and the means of their delivery for hostile purposes or in armed conflict. Also prohibited is the exploitation of future biotechnological developments for other than peaceful use.

Although a valuable starting point to biological weapons control, the most significant deficiency of the BWC is that it lacks verification and implementation provisions. Both the former Soviet Union and Iraq continued to proliferate biological weapons after signing the BWC, and there is concern in the international community that such programs still exist.

An article in the New York Times on the web (February 1, 2000) cites intelligence data showing that in the previous year or two, Iraq rebuilt some of the military and industrial complexes that were destroyed by United States and British air strikes in 1998. Finally, current intelligence estimates suggest that since the BWC went into force in 1975, the number of nations with active offensive biological warfare programs has increased to approximately a dozen.
Biological Warfare Agents --- By definition, biological warfare is the intentional use of naturally occurring biologic agents (bacteria, viruses, and toxins) to cause incapacitation or death in humans and animals, and in the case of food crops, destruction or decimation of that particular crop. Given the proper biological conditions (that is, in nature and not for weapons purposes), these agents can cause disease of epidemic proportion (plague and cholera, for example), can cause disease as a result of occupational exposure (anthrax and Woolsorter’s disease), or simply can be endemic in certain parts of the world (as anthrax is in Asia and the Middle East).

Twelve different agents appear repeatedly in the biological warfare literature and are deemed the most likely to be used as weapons. Although many can cause disease and/or death via different routes of exposure (skin, gastrointestinal), the most common route of exposure for weapon purposes is via inhalation. This method of infection/weaponization is an attractive one for several reasons I will elaborate below.

Why bioterrorism? --- It’s best not to delve too deeply into the mind of a terrorist (you might get trapped and never get out) but the following are plausible explanations for choosing to use biological weapons.

First, compared to conventional warfare, the creation of weapons for use in biological warfare is relatively inexpensive. One study estimates the cost of civilian casualties is $2,000 per square kilometer for conventional weapons, $800 with nuclear weapons, $600 with nerve-gas agents, and $1 with biological weapons (please see The Threat of Deliberate Disease in the 21st Century at www.brad.ac.uk).

Costs for producing “the poor man’s atom bomb” are being further reduced by the rapid advances we’ve seen in biotechnology and microbiology. It is also possible to use the same technology to create strains of bacteria and viruses that are more virulent and/or resistant to traditional treatment modalities.

Second, the equipment and technology used for legitimate purposes (pharmaceutical and biologic research, for instance) can also be used in the production of biological weapons. This “dual-use” phenomenon reduces costs as noted above but also makes determination of
prohibited use very difficult, at best. Obtaining cultures and samples of these agents as well as finding employees with the necessary training is also relatively simple and if inspected for verification purposes, the host nation can revert the personnel and equipment back to an accepted use.

Third, if atmospheric conditions are favorable (low wind speeds and inversion conditions), it is possible to spray an inhalational agent upwind of the intended target and thus significantly increase the number of casualties. Again, this can be accomplished by the modification of industrial equipment (changing spray nozzles to generate smaller particle size, usually in the 5 to 10 micron range for inhalational agents) such as crop-dusting aircraft or by adding spraying equipment to other line source dispersal methods; boats or trucks, for example. Traditional explosive munitions can be used as well but the explosion tends to destroy a portion of the biologic agent within.

Finally, inhalational agents used as described above are odorless, colorless, and tasteless -- can you tell when you have first caught the virus that causes the common cold? This makes detection extremely difficult, if not impossible. Use of equipment familiar to the target population would not likely arouse undue suspicion and permit essentially unchallenged dispersal of the deadly agent.

Our innate fear of illness caused by an unknown entity adds a powerful and horrific psychological element to a biological weapons attack and literally puts the “terror” into terrorism.

**Three Most Likely Bio-Terror Agents ---** In this article I will present the biology and medical aspects of three agents that could be used as biological weapons: anthrax, smallpox, and botulinum toxin; anthrax and botulinum toxin because they have been weaponized already and smallpox because its virulence and high level of transmissability may make it the ideal biological warfare agent.

**Anthrax ---** The clinical disease anthrax is caused by the bacterium Bacillus Anthracis, a rod-shaped, unicellular, spore-forming organism. These spores are resistant to many adverse environmental conditions (drought, heat, even direct sunlight, to some extent) and are the usual infective form.

Anthrax is an infectious disease shared in nature by man and animals (a zoonosis), with cattle, sheep, and horses being the most common hosts. Handling the contaminated hair, wool, hides, blood, or excreta from infected animals is the most common way to contract the disease and transmission occurs through scratches or abrasions of the skin, wounds, inhalation of spores, via flies, or by eating contaminated meat.

Three types of anthrax are seen in humans: cutaneous (skin), inhalational, and gastrointestinal. The gastrointestinal form of the disease occurs after eating the meat of an infected animal and I will not further discuss this sub-type except to note that a case of gastrointestinal anthrax has never been reported in this country.

The most common type of anthrax world-wide is the cutaneous form, which usually occurs in those working with infected livestock. A painless papule (a small elevation of the skin) heralds disease onset and usually develops 3 to 10 days after inoculation of the spores. The upper extremities, neck, and face are the areas most frequently involved. Several days later, a vesicle (a small, circumscribed elevation of the skin containing serum) or ring of vesicles develops and the original lesion enlarges to 4 to 6 centimeters. The base of the vesicle bleeds and may discharge clear fluid. The lesion ulcerates, and a central eschar (a thick, coaglated crust --- in this case, scab) forms and may last for up to 3 weeks. Healing usually results in scar formation that can be extensive enough to require plastic surgery to repair.
20 percent mortality may be seen in untreated cutaneous cases.

Inhalational anthrax usually begins 3 to 5 days after inhaling the spores and has a gradual and nonspecific onset. The first symptoms are usually fever, malaise and fatigue. These can be associated with a cough and chest discomfort. There may be a period of improvement (from several hours to several days), followed very quickly by severe respiratory distress with dyspnea (shortness of breath), diaphoresis (sweating), stridor (high-pitched, noisy respiration), and cyanosis (dark blue or purple coloration of the skin). Shock and death usually follow 24 to 36 hours after the onset of respiratory distress.

Examination of the patient does not reveal any specific physical findings. The chest x-ray may show a widened mediastinum (the median partition of the thoracic cavity) but is not otherwise abnormal. Bacillus anthracis can be cultured from the blood, but often not until late in the course of the disease. Studies in rhesus monkeys have shown that the organism and toxin appear in the blood 2 to 3 days after symptom onset and there is a test available at certain specialized laboratories to rapidly detect presence of the toxin.

Treatment is not usually effective if begun after symptom onset. Penicillin (2 million units intravenously every 2 hours for 5 to 7 days) has been the treatment of choice, but other options include ciprofloxacin, erythromycin, and tetracycline. If exposure has occurred or is imminent, prophylactic treatment with ciprofloxacin (500 mg by mouth twice a day) or doxycycline (100 mg by mouth twice a day) for 4 weeks is advised, followed by vaccination (three doses) in those who did not receive the initial series and a booster for those who received less than three doses of the vaccine.

As noted, a vaccine is available and consists of six doses at 0, 2, and 4 weeks, then 6, 12, and 18 months, followed by yearly boosters. Limited human data show that protection against cutaneous anthrax is afforded after 3 doses of vaccine and animal studies suggest protection against inhalational anthrax occurs after 2 doses.

Human-to-human transmission of anthrax does not occur. Precautions should be taken if handling contaminated objects and instruments, which should be decontaminated with a sporicidal agent, i.e., chlorine or iodine. Detailed instructions for decontamination using formaldehyde are available on the World Health Organization website.
Smallpox --- Smallpox is caused by the variola virus, a member of the genus Orthopoxvirus. This highly contagious disease in its naturally occurring form has been eradicated; the last case was seen in Somalia in 1977. Nonetheless, the virus is still considered an agent that could make an effective weapon for three reasons: 1) the aerosol is highly infectious; 2) the ease of large-scale production; and 3) the increasingly large number of Orthopoxvirus-naive people who have never been vaccinated. Two different strains of the virus (variola major and minor) caused two distinct clinical syndromes, but I will only discuss variola major at this time.

The incubation period for smallpox is about 12 days. The infection is transmitted via the aerosol route through droplet nuclei (large amounts of the virus are shed in nasal and oropharyngeal secretions), dust, and fomites (a substance, such as clothing or bedding, capable of absorbing and transmitting a disease). The clinical manifestations begin acutely and malaise, fever, vomiting, rigors, and headache predominate. Two to 3 days later, an enanthem (a mucous membrane eruption) appears along with a discrete rash involving the face, hands, and forearms.

The rash then erupts on the lower extremities, and spreads quickly to the trunk over the next seven or so days. Lesions rapidly progress from macules (a small, discolored spot on the skin), to papules (a small, solid elevation of the skin), and eventually to pustular vesicles (a pus-containing blister). An important diagnostic feature is that the lesions are more abundant on the extremities and face. From 8 to 14 days after onset, the pustules form scabs which leave depressed depigmented scars upon healing. The virus can be recovered from the scabs throughout convalescence. Therefore, patients should be isolated and considered infectious until the scabs separate.

The diagnosis can usually be made on clinical grounds but the characteristic virions can be seen with light microscopy (usually obtained from vesicular scrapings) and polymerase chain reaction techniques may eventually allow pathologists to distinguish the variola virus from other Orthopoxviruses (monkeypox and cowpox, for example). Other conditions that can appear similar to smallpox include chickenpox, allergic contact dermatitis, and erythema multiforme (red patches on the skin).
A confirmed case of smallpox should be considered an international emergency and be reported to the appropriate public health authorities. Quarantine with respiratory isolation for 17 days is mandatory for all those who have had contact with the index case, especially the unvaccinated. Immediate vaccination or revaccination is required for all individuals exposed to a clinical case of smallpox or to weaponized variola virus.

A new smallpox vaccine (vaccinia virus) is under development but the first doses won’t be available until 2004 (there is a limited stockpile of vaccine --- but who will get those precious doses?). This new version of the vaccine is well-tolerated, with a low-grade fever and swelling of the axillary lymph nodes being the most common side effects.

Treatment is otherwise supportive. Vaccinia-immune globulin is available and felt to be of prophylactic value if given with vaccination and within the first week of smallpox or weaponized variola exposure. Methisazone is an antiviral drug that was used prophylactically for smallpox exposure but it is no longer available.

**Botulinum toxin ---** Botulinum toxin (Botox) is produced by the anaerobic bacterium Clostridium botulinum and is considered to be the most potent toxin known ---100,000 times more toxic than sarin with a lethal dose of 0.001 microgram per kilogram of body weight. There are actually seven distinct toxins, A through G, that are produced by different strains of the clostridial bacillus. These toxic proteins are neuroparalytic and act by blocking the release of acetylcholine at the nerve-muscle (neuromuscular) junction, thus effectively blocking neurotransmission and causing profound muscle weakness and/or paralysis.

The clinical disease is called botulism and although foodborne botulism is the most common type, wound and infant botulism can also be seen. Foodborne botulism is usually a result of eating improperly canned foods, wound botulism is caused by contamination of an injury that has broken the skin, and the infant form is due to colonization of the digestive tract. For weapon purposes, the inhalational route would be used, and the clinical outcome would be essentially the same as if the oral route was used (the onset of symptoms after inhalation could be longer than for foodborne transmission).

The onset of symptoms could begin as early as 24 to 36 hours or up to several days after exposure. Initial symptoms include blurred vision, double vision, droopy eyelids, and ocular sensitivity to light. Slurred speech, difficulty swallowing, and hoarseness could also be seen early on in the disease course. Skeletal muscle paralysis follows, which could end abruptly in respiratory failure. Progression from onset of symptoms to respiratory failure has occurred in as little as 24 hours in some cases of foodborne botulism. Levels of awareness and cognition are not affected.

Other diseases that have a similar presentation include Guillain-Barre syndrome, myasthenia gravis, and tick paralysis. However, none of these would be seen in an epidemic, as inhalational botulism might. Exposure to a nerve agent can also be clinically similar to botulism, but nerve agent poisoning causes small (miotic) pupils and copious secretions, whereas patients with botulism will have decreased secretions and will be “dry” and parched. Laboratory testing is generally not helpful in making the diagnosis.

The medical management is supportive --- patients who develop respiratory compromise will need artificial respiration (mechanical ventilation). Patients will also need extensive nursing care for the duration of the illness and throughout recovery, which could take weeks or months. Mortality had been up to 60 percent in the 1950s but with aggressive medical care and mechanical ventilation, present day mortality is less than 5 percent.

Botulinum antitoxin (equine) has been used in foodborne cases of botulism (presumably,
there is circulating toxin in the bloodstream) and is considered to be clinically helpful. Animal studies have shown that if the antitoxin is given prior to the onset of symptoms in patients with aerosol exposure, it can improve outcome in these patients as well.

The Centers for Disease Control has a trivalent equine antitoxin. The risks of using a horse serum product include serum sickness and anaphylaxis (a severe allergic reaction resulting in shock and perhaps death). Thus, patients must be tested for horse serum sensitivity prior to administration of the antitoxin. It should be clear that use of this antitoxin would be cumbersome at best for nearly all patients except those seen at medical centers that have experience in handling patients with this disease.

There is a vaccine (a pentavalent toxoid of toxin types A,B,C, and D) that has been given to volunteers and occupationally at-risk workers but availability at this time is only under Investigational New Drug (IND) status.

For the average person in a survival scenario (assuming mechanical ventilation is available), supportive care for those with botulism is the only realistic treatment available at this time.

So --- the unthinkable happened. Crop-dusting has been banned, gas masks are literally flying off the shelves, weapon and ammunition sales have skyrocketed, pharmacies are scrambling to stock ciprofloxacin, and the United States has begun the campaign to eradicate terrorism. Good luck to our men and women in uniform and especially to my friends and colleagues at Fort Bragg.

There has been a good deal of debate about the possibility of an attack against the United States using biological agents. Many of the pundits think it unlikely, but if that’s true, I wonder why our political leaders need those gas masks.

The war has really started. Be prepared. I