

Chapter 1

Biological Agents and Bioterrorism

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Abstract For this very stimulating course, I want to share with you some of my studies and even some of my scientific and phylosophical considerations on biological agents living in the environment and their relations with humans, in the very wide concepts of ecological relationships, parasitism, immunological defenses and infectious disease mechanisms. All these concepts must be studied and considered in the event of criminal use of biological agents (bioterrorism) aimed at harming human populations in time and in geographical space.

Keywords Biological weapons • Toxins • Poisons • Parasitism • Immunology • Micro-biome • Fear as weapon

1.1 Definitions and History of Bioterrorism

Practice of bioterrorism goes back to very remote times of human existence and indeed to pre-historical conflicts between humans, when groups of people devised deliberately to use biological agents for conflict sustainment (damaging weapons) and for propagation of fear in the enemy populations.

Since the early times of combat between humans, some biological means have been used: for instance through the contamination of water wells in conflict areas, tainted with rotting animal remains, or through hunting-fighting arrowheads dipped in toxic plant extracts or venomous substances, before throwing them at the living target.

Usable biological agents for harm are in fact all the pathogens of biological nature, like microorganisms (bacteria, viruses, fungi, prions), toxins, animal and plant venoms, together with the related carriers (fomites, instruments) or vectors (insects, etc.).

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Terrorism is the use of violence to condition societies or governments in their political choices.

Bioterrorism is the use (or menace of use) of biological agents to enact terrorism events and induce generalized fear concerning negative consequences in target populations.

Here follow some examples of bioterrorism enacted in various ages:

- (a) use of poison darts/arrows (primitive populations): mostly for hunting, but also for battles against enemies.

From here derived many of the advances of toxicology, the science of toxic substances (the word “toxicology” derives from the greek words “toxon”=arch and “farmakon”=poison; toxicon farmakon=poison for arch hunting):

- (b) from such practice derives the knowledge we have of stricnin, curare, ouabain, aconite, other plant/animal poisons primarily devised for hunting and fighting.
- (c) impingement of darts in decomposing cadavers or putrefaction soil (or manure >> tetanus) before throwing at enemies (New Guinea, tribal combats; Sciites 400 b.C.)
- (d) last but not least, the use of fear that humans have of beasts. Here comes the example of Hannibal (from Carthago), leading the ships of Prusia I, king of Bithynia (West Turkey) in a battle of year 184 b.C. against Eumene II (Attalides, Pergamon); he won that naval battle because he managed to throw canisters full of reptiles at the enemy ships, causing terror and uncoordinated reactions leading to his victory. He therefore used fear as a weapon: snakes were not even harmful (not poisonous), but big was the surprise and reactions were out of control!
- (e) In recent history, we can see that First World War (also named the war of Chemistry) contributed to the development and use of Nervine gases, chemical weapons banned everywhere but still existing in some countries. Second World War (also named the war of Physics) led to the development and use of the atomic bomb.

ThePeace Treaties: *Geneva Protocol* ruled against chemical weapons (1925) and was followed later by additions concerning bacteriologic war. Not all the states however subscribed it. Most states anyway have banned, in time, chemical and bacteriological weapons by 1975.

Today, how scared should we be of biological and chemical terrorism? Well, since these are lethal and cheap weapons, they are of considerable concern, because they may be seen as the atomic bomb of poors and represent remarkable threats to peace in local conflicts and in terroristic attacks worldwide. We should all know more on the subject and do extensive prevention.

Albert Einstein once said “I do not know by what weapons the Third World War will be fought, but for sure the Fourth will be fought with stones”. Well, we do not want any more World Wars, for sure, period.

1.2 Microbes and Humans Interacting

Life began on Earth with unicellular beings: primitive bacteria and algae, ~3.5 billion years ago (Archean Age). Biological evolution of species produced today's forms of life, as we observe them. Millions of species co-exist and share the stage (biosphere).

Humans (we) pretend to have absolute priority, but ... share the stage in such a crowded environment on earth means to learn, respect, understand and prevent.

On this planet we have millions of different species of live beings, with variable proportions in the biosphere and in different ecosystems: they are all co-existing, interacting and competing for food and survival. This encompasses the very universal phenomena of competition and of parasitism.

One of the most recent and precise evaluations of the number of species existing on planet earth (2012), but still very approximate and provisional, (well illustrated and summarized in National Geographic, 2013) finds evidence of more than 5,000 species of mammals, 10,000 species of birds, 12,000 species of reptiles, 15,000 species of amphibians, 45,000 species of fish, 150,000 species of crustaceans, 200,000 species of mollusks, 600,000 species of arachnids, five million species of insects and many, many millions (unestimable indeed) species of bacteria, viruses and other microorganisms. Are we many on Earth? Is there enough work for the immune system of each living multicellular organism to distinguish "self" from possibly harmful "not self"?

Species of microorganisms ascertained as pathogenic for humans are indeed a very small fraction of the existing species: we come to know them better because we study the diseases connected with them, but we ignore a lot about the great number of other, presumably innocuous species.

In general, different species interacting may set a parasitic relationship, in which the larger animal (*the host*) may receive harm (food loss or disease) and the smaller one (*the parasite*) may get advantages (more food, protection). Both must preserve their identity and prevent contamination by foreign genetic material (immunologic surveillance, bilaterally). Some parasites can even live within the hosts (endoparasitism), like some bacteria and all viruses.

Three types of interactions may occur between a microorganism and a human host: (a) symbiotic relationship, in which the microorganism and the host both benefit; (b) commensal relationship, in which the microorganism gains but the host suffers no harm; and (c) a true parasitic relationship, in which the microorganism gains and the host is harmed.

Symbiosis offers frequently mutual advantages and remains very stable in time. Pathogens are a minimal part of existing microorganisms. We humans host some advantageous bacterial populations (intestine, surface germs on the skin, commensal germs on the mucosae): we indeed are also *made of the germs living in/on our body*. Indeed, only one cell out of ten in our body is a human cell: the

rest are bacteria (the so-called micro-biome). Among these, we count billions of bacteria in the intestines, useful for many functions (vitamin production, competition with pathogens, contribution to metabolism, etc.).

The balance between host and parasites depends on two basic forces, an aggressive force by the parasite depending on survival/proliferation/invasion capacity of the parasite itself and a defensive force by the host depending on the immune mechanisms (phagocytosis, cellular and humoral immune reactions). In this balancing of opposite forces the parasitic relationship is played by the contenders. If we have prevalence of parasite, we may have disease (and eventually death) of the host, but if we have prevalence of host defence we may have control (and eventually elimination) of parasites.

1.3 Main Diseases of Interest in the Field

In the light of recent concern and interest about the potential for biological terrorism (biofarware) there are several diseases and bacterial toxins that must be considered in particular, like anthrax [1, 2], smallpox [3, 4], plague [5], botulinum toxin [6], and tularemia [7]. A very detailed discussion of such diseases and other infectious diseases with similar risks in terms of bioterrorism goes beyond the scopes of this concise chapter, but some features of these and other infectious diseases representing important threats in the biofarware field will be mentioned.

In this respect, we may distinguish in time diseases which are:

1. old diseases which are disappearing and sometimes returning, like smallpox and polio virus infections (which are either extinct or close to be eradicated, thanks to planetary vaccination programs);
2. diseases still active at present times, like carbuncle (anthrax), plague, tularemia, tetanus, botulinum, TBC, etc.;
3. new diseases, which are appearing/spreading, like SARS (Severe Acute Respiratory Syndrome) and its more recent variety of MERS (Middle-East Respiratory Syndrome), infections by Ebola/Marburg viruses, hantavirus, filovirus, novel Flu virus strains, etc.

Now we will summarize the essential facts about some of these diseases. For a more complete medical reference to all of them, see for instance the Merck Manual of diagnosis and Therapy [8].

1.3.1 *Smallpox (Variola)*

Smallpox is a highly contagious disease (incubation 10–12 days) caused by the smallpox virus, an orthopoxvirus. It causes death in up to 30 % of infected subjects. Indigenous infection has been eradicated (last case, Ethiopia, 1990 – WHO). The main concern for outbreaks of smallpox is today from bioterrorism.

Smallpox is characterized by severe constitutional symptoms (fever, headache, extreme malaise) and a characteristic pustular rash. Treatment is supportive; prevention involves vaccination, which, because of its risks (eczema, encephalitis, etc.), is done selectively.

Pathogenesis of smallpox demonstrates that the virus is transmitted from person to person by direct contact or inhalation of droplet nuclei. Clothing and bed linens can also transmit infection. Most contagions are in the first 7–10 days after the skin rash appears. Once crusts form, infectivity declines. The virus invades the oropharyngeal and respiratory mucosa, multiplies in regional lymphnodes, causing viremia and localization in small blood vessels of the skin (rash) and rarely in CNS (encephalitis).

Officially, smallpox is dead on Earth. There are no longer cases detected in the world population since 1990, but can we destroy the samples of smallpox virus existing in some virology laboratories around the world? Certainly not [3], because we could no longer prepare vaccine doses without live virus samples to start from. And without vaccine, a small amount of wild virus could ignite a wide epidemic killing a large proportion of the human population, since the vaccination is no longer mandatory in any country and a large percentage of young populations have no longer been vaccinated after the early 1990s.

1.3.2 Poliomyelitis (Infantile Paralysis)

Poliomyelitis is an acute infection caused by a poliovirus. Manifestations include a nonspecific minor illness (abortive poliomyelitis), sometimes aseptic meningitis without paralysis (nonparalytic poliomyelitis) and, less often, flaccid weakness of various muscle groups (paralytic poliomyelitis). Diagnosis is clinical, although laboratory diagnosis is possible. Treatment is supportive. Vaccination is available, still mandatory in many countries, although soon legislations may change. Childhood vaccination produces immunity in 95 % of recipients. Declared cases worldwide have diminished remarkably, but some areas with particularly poor sanitary services or with conflicts preventing health services to operate are recording increased numbers of cases recently (Syria, 2013; China 2013).

Polioviruses have three serotypes. The virus enters the mouth via the fecal-oral route, then enters the lymphoid tissues of the GI tract. If not contained, infection may enter the CNS with significant damage in spinal cord and brain, specifically to nerves controlling motor and autonomic function (breathing). Spreading is through the enteric route. Vaccine is live, attenuated virus, able to immunize many contacts respect to the vaccinated subjects (community vaccination strategies; problems in nomad populations).

1.3.3 Anthrax (Carbuncle)

Anthrax is caused by *Bacillus anthracis*, toxin producing, encapsulated, aerobic or facultative anaerobic organisms. Anthrax, an often fatal disease of animals, is transmitted to humans by contact with infected animals or their products (wool sorter's disease).

In humans, infection typically occurs through the skin. Inhalation infection is less common; oropharyngeal, meningeal and GI infections are rare. For inhalation and GI infections, nonspecific local symptoms are typically followed in several days by severe systemic illness, shock and often death. Empyric treatment is with ciprofloxacin or doxycycline. A vaccine is available (antitoxin).

Pathogenesis of anthrax takes place since *Bacillus anthracis* readily forms spores when germs encounter dry environment -a condition unfavorable for growth. Spores resist destruction and can remain viable in soil, wool, and animal hair for decades. Spores germinate and multiply in favourable conditions (wet skin, tissue, blood) and can give human disease by contact (papules, black eschars, contagious also via fomites) ingestion (raw meat > fever, nausea, vomiting, diarrhea), and inhalation (flu-like illness, respiratory distress, cyanosis, shock, coma).

Of note is the anthrax bioterrorist attack through mailings (using spores in powder form) that took place in the USA in 2001 (US Postal Service, Washington DC), event that highly sensitized the public to the global theme of bioterroristic attacks.

1.3.4 Plague (*Pestis*, *Black Death*)

Plague is caused by *Yersinia pestis* (formerly named *Pasteurella pestis*). Short bacillus with hairpin shape, infects wild rodents and can infect humans via tick bites. Symptoms are either severe pneumonia or massive lymphadenopathy with high fever, often progressing to septicemia. Diagnosis is epidemiologic and clinical, confirmed by culture and serologic testing. Treatment is with streptomycin or doxycycline. Unfortunately, a vaccine is not available for plague.

1.3.5 Tularemia

Tularemia is a febrile disease caused by *Francisella tularensis*; it may resemble typhoid fever: symptoms are a primary local ulcerative lesion, regional lymphadenopathy, profound systemic symptoms, and, occasionally, atypical pneumonia. Diagnosis is primarily epidemiologic and clinical and supported by serologic tests. Treatment is with streptomycin, gentamycin and other antibiotics.

1.3.6 Tetanus

Tetanus is an acute poisoning from a neurotoxin produced by *Clostridium tetani*. Symptoms are intermittent tonic spasms of voluntary muscles. Spasm of the masseters accounts for the name “lockjaw” (trismus). Incubation requires 2–10 days. Diagnosis is clinical. Treatment with immune globulin and intensive support. Only

unbound toxin can be neutralized. A vaccine is available, with a good extent of preventive protection.

1.3.7 Botulism

Botulism is a neuromuscular poisoning due to *Clostridium botulinum* toxin. Botulism may occur without infection if toxin is ingested. Symptoms are symmetric cranial nerve palsies accompanied by a symmetric descending weakness and flaccid paralysis without sensory deficits. Diagnosis is clinical and by laboratory identification of toxin. Treatment is with antitoxin and support therapies.

1.3.8 Tuberculosis (TBC)

TBC is a chronic, progressive infection by *Mycobacterium tuberculosis*, often with a long period of latency following initial infection. It occurs most commonly in the lungs, with productive cough, chest pain and dyspnea. Diagnosis is most often by sputum culture and smear. TBC can involve any tissue (organ disease). Treatment is with multiple antimicrobial drugs. Forms of multiresistant TB bacteria are becoming more and more frequent.

1.3.9 SARS

Coronavirus infections in humans most frequently cause common cold symptoms; however in 2002, a relatively new coronavirus caused an outbreak of Severe Acute Respiratory Syndrome (SARS), which was much more severe than other coronavirus infections. SARS is an influenza-like disease leading to progressive respiratory insufficiency with significant mortality rate. First detected in China (Guandong, 2002), the SARS epidemic spread to more than 30 countries. In mid-July 2003, there were >8,000 cases with >800 deaths (10 % mortality).

Then the outbreak subsided and no new cases have been identified from 2004 to 2012. In 2012 a new similar epidemic (sustained by the virus nCoV, novel coronavirus) started in Middle East (Arabia), with an estimated mortality above 40 %. Later the nCoV epidemic has been named MERS (Middle East Respiratory Syndrome) and is being studied as a new zoonosis transmitted to humans from Dromedary camels. Studies are currently in progress, with great attention by the international sanitary authorities [9].

WHO in 2013 indeed alarmed many countries against the new SARS-like coronavirus responsible of MERS, that infected at the moment of this writing (December 2013; www.who.int/en) more than 160 persons (Arabia, Great Britain, France,

Germany, Tunisia, Italy, Abu Dhabi, United Arab Emirates, etc.) with reduced infective capacity as compared to SARS, but still highly lethal and communicable via close contacts (family members). The latest available numbers call for 163 ascertained diagnoses in humans, with 71 deaths (mortality, 43.5 %).

Updates can be found at the following web sites: www.who.int/en; www.cdc.gov and (recommendations for clinicians) emergency.cdc.gov, emphasizing the need to consider the novel (nCoV) coronavirus when treating patients with a severe respiratory illness who have recently traveled to the Arabian Peninsula (or close contacts of the travelers).

1.3.10 *Ebola/Marburg Diseases*

Marburg and Ebola are filoviruses that cause hemorrhage, multiple organ failure and high mortality rates. Diagnosis is with enzyme-linked immunosorbent assay, PCR or electron microscopy. Treatment is supportive. Strict isolation and quarantine measures are necessary to contain outbreaks. Incubation 5–10 days. Marburg virus has been identified in bats and in primates. Human to human transmission occurs via skin and mucous membranes contact (humans/primates).

Filoviruses can affect intestines (nausea, vomiting, diarrhea), respiratory tract (cough, pharyngitis), liver (jaundice), CNS (delirium, stupor, coma), and cause hemorrhagic phenomena (petechiae, frank bleeding) with high mortality rates (up to 90 % with Ebola virus). Survivors recover very slowly and may develop long lasting complications (hepatitis, uveitis, orchitis) with only supportive care available: no specific antivirals nor vaccines are available for filovirus infections.

1.3.11 *Hantavirus, Lassa Fever, etc.*

Bunyaviridae contain the genus Hantavirus (four serogroups, nine viruses) causing hemorrhagic fevers with renal and pulmonary consequences, starting with flu-like symptoms and evolving with severe renal and pulmonary consequences. Lethal in 10–15 % of cases.

Lassa fever is an often fatal arenavirus infection occurring mostly in Africa. It may involve multiple organs, except CNS. Treated with ribavirin. No vaccinations are available so far for hantavirus infections.

Outbreaks of such infections have been recorded in Nigeria, Liberia, central Africa, with some rare imported cases in the USA and in the United Kingdom.

The animal reservoir of such viruses is in wild African rats (*Mastomys natalensis*), frequently found in African houses. Direct human to human transmission is documented via urine, feces, saliva or blood. Mortality (up to 45 %) can be reduced

by prompt ribavirin treatment. Universal hygiene precautions, airborne isolation and surveillance of contacts are essential.

1.3.12 Influenza Virus, with New Strains Continuously Appearing

Last but not least ... we must mention now influenza! Flu viruses are in nature among the most rapidly changing (mutating) organisms through their ability to infect a variety of hosts: birds (migrating waterfowl -ducks-, stantial poultry -chickens-), mammals (pigs, felines) and humans. In South East Asia (mostly in China, but also in Viet-Nam, Laos, Thailand, etc.) it is very common to have mixed farms of pigs, poultry and ducks, attended by humans.

Every year, new strains appear in SE-Asia, favoured by the recyprocal passage between migrating birds (mostly fowl), pigs and chickens, with exposure of many humans in farms, markets, rooster fighting sports, and food preparation places.

A common say in China tells that “Anything with four legs (except chairs) and anything that flies (except airplanes), can be eaten”. With this phylosophy, there is generally a lot to be desired in food safety and in general hygienic prevention in such geographical areas.

After the avian flu H5N1 of 2005–2006, highly lethal but unable to give human to human contagion, new combinations of flu strains are expected and feared, with high lethality and high human to human transmissibility.

On this widely interesting theme for the world diffusion of new virus strains with pandemic potential, I wrote in 2010 together with the colleague virologist Aldo Lepidi a book entitled “Pandemics – virology, pathology and prevention of influenza” (Bollati Boringhieri publisher, Turin, Italy, 2010) [10].

In summary, we can see that a continuous surveillance is being devoted worldwide to the appearance of new strains of influenza viruses, in order to isolate as soon as possible potentially pandemic new strains and to prepare biological stocks suitable for massive vaccine preparations in due time to prevent the global spreading of potentially lethal new variants of the influenza viruses. Examples in time recall the cases of the highly lethal pandemics known as “Spanish flu” in 1917–1918 (in excess of 40 million deaths worldwide), “Asian flu” in 1956 (in excess of 100,000 deaths worldwide) and “Hong Kong flu” in 1978 (in excess of 700,000 deaths worldwide). The basic question is: when the new pandemic will strike ? Sometimes soon, as international experts say. The so called “Avian flu” came close to that, but sometimes in the future new mutations may emerge with the potential of being much worse.

In conclusion of this wide although rapid overview of the most frequent or alarming causes of microorganism-related human diseses with potential interest for bioterrorism, I hope to have provided sufficient matter for discussion and for further future diffusion of medical and microbiological culture that may be useful for prevention and the betterment of human social relationships and for peace promotion.

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