

# Symposio sobre bioterrorismo, grandes amenazas epidémicas y bioseguridad: sesión III (parásitos y bioterrorismo y ciencia forense)

Aula 13F, Facultad de Farmacia  
Martes 20 de Julio: 15.00-17.00

## 180.- The role of parasites as potential bioterrorism agents

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The use of biologic agents for purposes of bioterrorism is very much in the news today. There are literally hundreds of papers in the scientific literature that chronicle the potential use of a wide range of biological agents in causing not only human disease but can be used for causing diseases in food animals. The vast majority of these agents include many examples of bacteria (eg, anthrax, plague, et al) and viruses (eg, smallpox, filoviruses, arenaviruses, et al.). However, the use of parasites as agents of bioterrorism has not received nearly so much attention.

Since parasites cannot be as readily propagated in the laboratory nor can they be utilized as aerosols as can many bacterial and viral agents, the use of these organisms revolves principally around their introduction into the water and food supply. To be introduced into the water supply would generally require large quantities of organisms which are either immediately infective when obtained from animal or human hosts or would require considerable periods of time for external incubation to the infective stage before their usage. Parasites can also be evaluated for bioterrorism use on the basis of the rapidity in which they can produce disease and/or pathology in the human host.

Protozoan organisms which can be obtained in large numbers from animal or human hosts include *Cryptosporidium spp.*, and *Cyclospora cayetanensis*. Both of these can produce severe and prolonged diarrhea in humans but are unlikely to produce fatal disease without special circumstances (eg, as opportunistic infections). Although frequent mention of *Giardia* infections occurs in the literature, the problem of collecting large numbers of organisms poses difficulties for bioterrorism use on a large scale. The use of such organisms as *Entamoeba histolytica*, *Balantidium coli*, and *Toxoplasma gondii* in bioterrorism is probably compromised for a number of different reasons. None of the

serious blood-borne protozoan infections (ie, malaria, trypanosomiasis) presently can be used on a mass scale.

There are some helminthic parasites which offer opportunities for collection in large numbers and subsequent dissemination into food or water. Roundworm (nematode) parasites that could be utilized as bioterrorism possibilities because of their large size, extreme fecundity, and ready availability in animal hosts include a variety of ascaridoids.

*Ascaris lumbricoides* from humans and pigs, has already been used to intentionally produce serious disease in a small number of people. Related zoonotic ascarid parasites that can produce visceral, neural and ocular larva migrans in humans include *Toxocara canis* and *Baylisascaris procyonis*. These parasites are large, females harbor and produce large numbers of eggs in their uteri, and they can be readily collected directly from dogs and raccoons in large numbers. Although the eggs of these nematodes require incubation periods of 2-4 weeks to become infective, this can be readily accomplished in the laboratory and the eggs can retain their infectivity in dilute formalin solutions for many months and often much longer. Other helminth species which produce immediately infective eggs that can produce severe human disease include *Taenia solium* (causing cysticercosis) and *Echinococcus spp.* (causing hydatid disease). The pathologic manifestations of these cestode parasites are considerably delayed in comparison to the roundworm infections mentioned above.

In conclusion, the utilization of parasites as effective bioterrorism agents is less than with bacteria or viruses but still could contribute to the installation of fear in human populations concerned with their food and water supplies.

## 181.- Long-term bioterrorism: potential use of *Echinococcus* species

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The possible use by regular armies of pathogenic microorganism mainly bacteria, viruses and fungi, as potential biological weapons (BW), has been a long time focus of concern. Over the last years, the international terrorism increase has raised up the possibility of the use of BW to attack civilian populations, causing adverse health effects, panic and insecurity among them.

In general, the potential or already used BW include the use of any human pathogenic microorganism which may be fast and easily disseminated in the environment, mainly airborne, and also by contaminating the water supplies. These mass-casualty agents may cause invalidating, acute or lethal diseases. In this context, the possible use of long-term BW by international terrorist groups is considered of high risk by the policymakers worldwide.

These long-term BW, whose effects will not be showed immediately after the attack but through the development of subacute and/or chronic diseases, are of high potency and easy delivery, and the following characteristics would be considered:

- a) An attack with long-term BW would allow terrorist groups to disseminate the agents in the environment and in civilian populations with enough time to leave the contaminated areas or even the attacked country, before the first symptoms of the disease would appear.
- b) Terrorists could inform of the performed BW-attack during the asymptomatic period or once the first clinical cases had been declared.
- c) An attack with BW by contamination of food or supply water notified to the population during the incubation period (mainly if it was a long one), would raise a great amount of insecurity among consumers, with the subsequent negative economic effect on the country affected.
- d) This psychological insecurity would drive citizens to massively assist to Medical Centres, overburding and blocking their services.
- e) It would be necessary to implement long-term control and diagnostic schedules to detect any suspected slow-development symptomatic disease associated with BW; protocolized long-term diagnostic methodologies together with extended follow-ups would be required. These actions would greatly increase Public Health System expenses.
- f) If long-term monitorized medical treatment or surgery would be required, incurred health care expenses will also increase considerably.
- g) Besides, non-lethal chronic development diseases would cause main socio-economical after-effects: long-term sick-leaves and even definitive invalidations. These facts would generate a long-term strong expenses increase thus affecting the social prevision system of the country attacked.
- h) If the disseminated microorganisms or parasites endemically establish in the attacked country, they may become focus or reservoirs of the disease, with the long-term consequences involved.

There are, therefore, many facts, which approach subacute or chronic-development diseases as may constitute threatening long-term bioterrorism weapons.

Among the extensive list of parasites which may affect humans, cestodes of the genus *Echinococcus* present many of the mentioned long-term bioterrorism characteristics, together with the fact they are zoonotic parasites of easy establishment among the wild and home fauna in many countries. Therefore, they could constitute native reservoirs of the disease.

*Echinococcus granulosus*, the taenid that causes the cystic echinococcosis or unilocular hydatid disease in humans as well as in sheep, cattle, pigs, horses, camels and other farm animals, present several characteristics that would allow it to be used as BW agent:

- a) Human and other intermediate host infections are caused by the microscopic eggs of the parasite, which easily survive in the environment, even under freezing conditions.
- b) The larval stage (metacestode) has a low degree of host specificity as well as a great reproductive potential.
- c) Parasite eggs from natural or experimentally infected dogs are quite easy to obtain, deliver and disseminate, especially those of endemic diseases established in the countries where terrorists are native.
- d) Contamination of vegetables, fruits and other raw food is easily performed, either where originally grown or during the food chain processes.
- e) The disease development is progressive and very slow (generally it takes years); in fact, early stages of the disease (when treatment results are optimum) are often asymptomatic and thus very difficult to detect.
- f) Diagnosis requires the use of radiologic, ultrasonographic and serological methods, which are all together of high cost.
- g) Treatment could be pharmaceutical early in the course of the disease and in specific clinical cases, but generally surgery is required.
- h) Long-term sick-leaves constitute great socio-economic costs.
- i) Parasite establishment in the attacked country is possible, specially when extensive cattle raising is performed lacking strict veterinary controls over all cattle slaughtering done at the slaughterhouses, or when the slaughtering is performed under non-veterinary controls at unauthorized places.

As to *Echinococcus multilocularis*, causative agent of the alveolar echinococcosis (AE), some of its biological and development characteristics are:

- a) The eggs of *E. multilocularis* may be obtained from naturally infected hosts (foxes), experimentally infected ones (foxes and dogs) and from a combined *in vivo* and *in vitro* obtained samples.
- b) These eggs have a good viability in the environment.
- c) The development of the metacestode in the intermediate hosts, including humans, is relatively fast.
- d) The AE is mainly a liver disease, where the proliferation of vesicles continues indefinitely, producing a serious damage. The mortality rate in untreated or inadequately treated patients is high.
- e) As the parasite lesion is similar to a malignant tumor, the early diagnosis is important for a successful treatment. This diagnosis of AE is expensive since it requires ultrasonography, computed tomography, magnetic resonance imaging and serology.
- f) Treatment of AE includes surgery and chemotherapy, and in certain cases long-term chemotherapy with monitoring. In very sever hilus extension of the disease, liver transplantation was practiced.
- g) The disease causes long-term sick-leaves, and occasionally definite ones.
- h) The disease could be endemically introduced in the target country by infecting its native fox and wild rodents populations.

In conclusion, all the *E. granulosus* and *E. multilocularis* mentioned characteristics show that these parasites may be considered as potential long-term bioterrorism weapons.

## 182.- Parasitology proficiency testing: origins, objectives and problems

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It has been some 30 years since the first published paper appeared in the US literature which discussed the parasitology proficiency testing program within the Quality Evaluation Program of the College of American Pathologists (CAP). Since that time, the number of individual States and organizations offering parasitology proficiency testing programs broadened while at the same time the governmental organization, the Centers for Disease Control (CDC), discontinued their testing program in 1986 following 13 years of activity. The development of externally offered quality control programs originated as a basis for laboratories to voluntarily successfully participate in testing programs as a requirement for accreditation and licensure. Proficiency testing programs actually were first developed in the 1940s and subsequently in 1967, because of congressional concerns about laboratory performance, the Clinical Laboratories Improvement Act was passed which mandated external testing programs. This resulted in laboratories needing to participate in proficiency testing programs to meet federal or state licensing requirements.

Most testing programs must send out three shipments or challenge cycles per year depending on the survey and the specimens sent. All State or organization-based testing programs send out parasitology challenge specimens. In the CAP Program there are three surveys that involve parasitology: 1) blood parasite survey that covers malaria, *Babesia*, trypanosomes and microfilariae; paired thick and thin Giemsa-stained films are sent out with a total of 10 sent in each shipment three times per year; 2) parasitology survey that covers both blood parasites

and intestinal parasites; each shipment comprises two Giemsa-stained blood smears, two unstained PVA preserved fecal smears, and two formalin-preserved fecal suspensions for a total of six specimens sent out three times per year; 3) survey covering just intestinal parasites comprised of two formalin-preserved fecal suspensions on which *Giardia* and *Cryptosporidium* immunoassays can be performed though not required.

Problems associated with such testing programs often revolve around finding sufficient quantities of suitable parasite specimens for distribution to large numbers of participating laboratories. Collection of specimens from patients is frequently hampered by informed consent regulations, the provision by patients of relatively small quantities of specimens for diagnosis, and the lack of knowledge by laboratory personnel to collect large quantities of fecal material when available, or the preparation of large numbers of blood slides containing parasites. Improvement in diagnostic testing depends on having competent personnel who know both parasite identification and the procedures to be employed to be successful. Many laboratories have infrequent opportunities to see positive specimens and often the personnel assigned to the parasitology diagnostic area are not highly proficient. Employment of reference atlases or books and the availability of parasitic specimen material for reference purposes can improve laboratory diagnostic proficiency. Testing programs can alleviate some of these needs but the quantity of material sent out precludes the opportunity to develop adequate supplies of reference specimens for future use in training other individuals.

## 183.- Biological agents and forensic science

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The recent sprout of terrorist activities in various parts of the world has aroused tremendous scepticism in the minds of the public (national and international) about the safety of our future generations. The terrorist attacks at Tokyo suburbs in 1995 and the anthrax scare in US have spread horror through out the globe and affected the psyche of an average individual. The use of weapons of mass destruction usually considered as warfare agents has crossed the boundary and the accessibility and the technological know how of these agents pose a major threat to countries that are facing terrorist activity & proxy wars. The law and order being a state subject, safety of the area is highly under pressure and the local police officers are to carry out, manage such type of incidents with other government functionaries. As the preventive aspects of any crime, has its own limitation there is a line of risk in presuming the same. Forensic scientists are the first along with the police to enter the scene in case of accidental release or in theft of such materials to prove that these are biological agents or not. The first known use of the biological agent by organized group was the famous Bulgarian journalist incident. The use of ricin in micro platinum capsule to evade the medical autopsy by way of an accident was considered as an isolated incident and the full investigation in the background of such a criminal activity could not be proved. "Prevention is better than cure"

is the main principal in this type of attack. Disease producing organisms are classified as biological agents, they are of living organisms or the products derived from them (endotoxins or exotoxins) that cause disease or harm humans. Prevention of exposure to these agents is usually accomplished by using gas masks and protective suitings. To aid the treatment of such agents the causative agent should be conclusively identified, based on signs and symptoms, spot chemical tests to carry out treatment. The army has such facility as prophylaxis, but the civilian and local police awareness in this subject is very scanty. The protection and prevention of the personnel handling/involved in such activities should be well taken care by basic training in agents, their protective suits, gas masks, collection kits all should be kept stand by and the decontamination of the area is more important for declaring safe the area and not to spread or disperse and affect the other areas. These will be the first line of defense in attack to crack the leak, exposure or contaminated area to spot the area and draw a outer boundary as safe for movement. The latency period between infection and onset of signs and symptoms may take days to weeks to appear. The odorless, tasteless and not responsive to body's sensory function complicates the scene with such microscopic particles as biological warfare agents and confuses the public of natural disease signs and symptoms.