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Mycotoxins: A New Concern for Biosecurity?

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Introduction

Biological warfare and biological warfare agents

The potential spectrum of bioterrorism ranges from false alarms and use of agents by individuals or small groups against individuals or smaller subgroups of the population to state-sponsored terrorism that employs biological warfare agents (BWAs) and their diffusion systems that can produce mass victims.[1] BWAs are microorganisms like virus, bacteria, fungi, protozoa or toxins, that give rise to diseases in man, animals or plants, when deliberately released. These agents can cause large-scale mortality, incapacitate a large number of people in short time or have adverse effects on human health.[2] Depending on the agent, a lethal or incapacitating outcome may occur. In a military context, incapacitating agents may be more effective as the unit will not be able to carry out its mission and the victims consume little medical and evacuation resources.[3] BWAs strongly attract many terrorist groups due to different features. BWAs aerosols are invisible, silent, odorless and relatively easily dispersed.[4] BWAs are up to 2000 times cheaper than other weapons of mass destruction. Their production is relatively easy, using the common technology available for the production of certain antibiotics, vaccines, foods and beverages.[4,5] The consequences of using BWAs are many. They can quickly produce a mass effect that exceeds the services, and community health care system. Most civilians are susceptible to infections caused by these agents. They are associated with a high mortality rate. The resulting disease is usually difficult to diagnose and treat early, especially in areas where the disease is seldom seen.[6,7]

Fungi: main characteristics

Fungi are members of a large group of eukaryotic organisms classified as a distinct kingdom separate from plants, animals, protists and prokaryotes.[8] Fungi vary widely in size and shape, from unicellular, microscopic organisms to multicellular forms easily seen with the naked eye. Individual cells range from 1 to 30 µm. Internally, fungal cells are typical eucaryotic cells;[9] fungal cells wall contains chitin, unlike of walls of plant and bacteria cells.[10] Genetic studies have shown that fungi are more closely related to plants than animals.[11] Unlike animals, fungi are not able to ingest food or nutrients and cannot create for themselves nutrients like plants with photosynthesis.[8]

Diseases caused by fungi are collectively called mycoses.[12,13] They are divided into four general categories according to the tissue affected by the pathogen: superficial mycoses (limited to the hair and dead layers of the skin); cutaneous mycoses (affect only the skin, hair and nails); subcutaneous mycoses (affect the subcutaneous tissue below the skin) and systemic mycoses (infect the internal organs and may systematically infect the host).[12,13] Some fungi don't cause infectious diseases, but if ingested or inhaled they can produce secondary compounds that can damage, debilitate or kill the host. These substances are collectively called mycotoxins.

Mycotoxins

Mycotoxins are classified as secondary compounds of many filamentous fungi and have different adverse effects on humans, animals and crops.[14] Aflatoxins, ochratoxins, zearelenone, fumonisins and ergot alkaloids are the most important mycotoxins in the agro-economic sector; the contamination of foods with mycotoxins represents a serious concern.[14,15,16] All mycotoxins are low-molecular-weight natural products, produced as secondary metabolites.[17] These metabolites can have different toxicological and chemical nature, they are grouped together only because of their capability to cause disease and death in human beings and other vertebrates.[18]

The term mycotoxin was coined in 1962 in the aftermath of a strange veterinary disease just outside London (U.K.) during which approximately 100,000 turkey died. When this mysterious "*Turkey X disease*" was linked to a peanuts (groundnuts) contaminated with secondary metabolites from *Aspergillusflavus* (aflatoxins), it sensitized scientists to the possibility that other occult mold metabolites might be deadly.[15,16]

Currently, the mycotoxins identified are more than 300, and the scientific community is focusing its efforts on those that have shown a toxic/carcinogenic nature. Human exposure to mycotoxins may result from the consumption of plants contaminated by toxins, the deposit of mycotoxins and their metabolites in animal products such as meat, eggs and milk or exposure to air and dust containing toxins.[19] Human food can be contaminated with mycotoxins at various stages in the food chain and the most important genera of mycotoxigenic fungi are *Aspergillus*, *Alternaria*, *Claviceps*, *Fusarium*, *Penicillium* and *Stachybotrys*.[15,16] The principal classes of mycotoxins include a metabolite of *A. flavus*: aflatoxin B1 (AFB1).[15] In dairy cattle, one of principal problem is represented by the transformation of AFB1 and AFB2

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into hydroxylated metabolites, aflatoxin M1 and M2 (AFM1 and AFM2), which are found in milk, cheese and in other dairy products obtained from livestock that have ingested contaminated feed.[20] In addition, since the early 1990s, it has been found that aflatoxins, especially AFB1, may induce the development of liver and kidney cancer.[16]

Finally, mycotoxins have been and could still be used as biological weapons. There are evidence that between the early 1970s and '80s, mixed mycotoxin-based weapons were used in South-East Asia, Cambodia and Afghanistan.[21]

Mycotoxins outbreaks

Mycotoxins have caused serious problems for human health, livestock and farm crops. Like the aforementioned "*Turkey X Disease*" that struck England in the 1960s, there have been many cases of mycotoxicosis throughout history. Some of these have did not result in a strong impact on human health (Australia 1987, South Africa 1996-1997 and Brazil 1999) while others have caused large-scale effects.[22]

India 1974, 1985

In 1974, India population had a large increase in cases of hepatitis due to the presence of maize contaminated by *Aspergilullus flavus.*[23] The outbreak, which lasted for 2 months, was confined to the Western Indian tribal population belonging to Banswada district of Rajasthan and Panchmahals district of Gujarat.[23] Hepatitis was reported in 200 villages with 106 confirmed deaths.[23,24] Analyses of contaminated corn samples revealed contamination by *A. flavus*, in the range of 6.25-15.6 parts per million (ppm). Tandon et al. presumed that an epidemic of jaundice in North-Western India (1974) was also due to toxic hepatitis, which affected both humans and dogs.[25] In October 1985, egg production fell from 85 to 40% around Warangal, in Andhra Pradesh, as an impact of severe aflatoxicosis in poultry.[23] Bird mortality rate decreased sharply after the feed (maize and walnuts contaminated by aflatoxins) was changed.[23]

Ethiopia 1977 – 1978

The last recorded outbreak occurred in Ethiopia in 1977-1978, where 93 cases and 47 deaths were reported due to gangrenous egotism.[26] The severity of infection was observed on wild oats locally known as "*ginche*" with ergot infestation ranging from 10-55%; no ergot was observed on barley grains.[26] The consumption of the affected "*ginche*" mixed with barley and other grains resulted in the development gangrenous ergotism.[26]

Kenya 2004

In May 2004, the Center for Disease Control and Prevention (CDC) and the World Health Organizations (WHO) were invited by the Kenya Ministry of Health to participate in the investigation of an outbreak of jaundice with a high case-fatality rate (CFR), in the districts of Makueni and Kitui.[27,28] Preliminary studies on samples from the affected areas revealed high levels of aflatoxin, suggesting that the epidemic was due to poisoning by this mycotoxin. The contamination by aflatoxin occurred during storage of local maize under humidity conditions.[27] Aflatoxin poisoning will probably continue to be a public health problem until the local population will implement appropriate storage methods for maize.[27] As of July 20, a total of 317 cases had been reported, with 125 deaths (CFR = 39%).[27,28]

U.S.A. 2005

In 2005, more than 75 dogs died in the United States after consuming pet food contaminated with aflatoxins, and hundreds more experienced severe liver problems associated with the intoxication.[29] When the U.S Food and Drug Administration (FDA) posted a recall on December 20, 2005, nineteen different types of pet food produced in a single facility in Gaston, South Carolina, were removed from sale. Sixteen batches of pet food resulted contaminated with aflatoxins with levels greater than or equal to 20 parts per billion (ppb). The widespread panic that followed this tragic event motivated many pet food companies to set-up routine testing services for aflatoxins.[29]

Mycotoxins as bioweapons

The potential use of mycotoxins as BWAs

Mycotoxins can be used as BWAs;[15] there is evidence that, after the communist victories in Southeast Asia in 1975, the new regimes in Vietnam and Laos, launched military campaigns against the Hmong tribes (U.S. allied) in northern of Laos, using mycotoxins as BWAs.[30]

From 1974 to 1981, during the Cold War, mycotoxins were used by the Soviet Union and its allies in Afghanistan and Southeast Asia. Aerosol and droplet clouds were released by aircraft spray tanks, aircraft-launched rockets, Soviet handheld weapon (DH-10), and booby traps.[31] Trichothecenes (e.g. T-2) appear to have been used in some of these attacks.[32] The air attacks in Laos have been described as "yellow rain", a shower of sticky, yellow liquid that sounded like rain as it fell from the sky, or a yellow cloud of dust or smoke. In Laos, 50-81% of attacks involved material associated with a yellow pigment.[31] High-dose exposure caused symptoms such as mucous bleeding, tremors, seizures, blindness, and, in some cases, death. Further reports of similar experiences were mentioned by Khmer tribes in Cambodia in 1978 and by anti-Soviet resistance fighters in Afghanistan in 1979.[33]

Considerable evidence suggests that Iraqi scientists developed aflatoxins as part of their bioweapons program starting from 1974 and during all 1980s. Aflatoxins were extracted from cultures of toxigenic strains of *Aspergillus flavus* and *Aspergillus parasiticus* to produce over 2,300 liters of concentrated toxin. The majority of this aflatoxin filled warheads and the remaining was stockpiled.[15,34]

In 1981 the U.S. accused the Soviet Union of supplying their allies in Laos and Vietnam with T-2 mycotoxin. Soviet officials denied the use of such BWA, presenting alternative plausible natural causes to justify events and symptoms. Critics also questioned the reliability of the refugees' testimony and the integrity of laboratory analyses conducted on samples of the substance. To this day, the source of the yellow rain is not definitively verified.[31]

Pro and cons in the use of mycotoxins as BWAs

In the early 20th century, mycotoxins were investigated for possible military use, but were rejected because of the difficulty in weaponization. Mycotoxins have limitations for their manufacture process, difficult weaponization and dispersion in sufficient quantities as weapons.[35] However, the threat of a biological attack using toxins and particularly mycotoxins to contaminate food supplies remains a concern. To have an effective risk mitigation, mycological/chemical sampling and detection methodologies, as well as toxin inactivation and decontamination processes, need to be improved. Foods and water reserves should be classified in terms of vulnerability to attack, as well as the risks for people who may be contaminated.[36] Algorithms to differentiate natural from unnatural food contamination, and novel biomarkers in humans and animals, with particular relevance to metabolomics, need to be further developed.[35,36]

The exposure to few milligrams of T-2 toxin is potentially lethal. Unlike most biological toxins, T-2 mycotoxin is a potent active irritant and it is the only one that can be absorbed through intact skin causing systemic toxicity.[31] Clinical symptoms may appear within seconds of exposure. While larger amounts of T-2 toxin are required for a lethal dose than for other chemical warfare agents such as VX, its potent effect as a blistering agent is well noted. T-2 toxin can be delivered via food or water sources, as well as via droplets, aerosols, or smoke from various dispersal systems and exploding munitions. These properties make T-2 mycotoxin a potentially viable BWA.[31] Nevertheless, mass production of this compound may be impractical because of the quantity of growth medium required to produce significant amounts.[31]

Another example are the aflatoxins. Aflatoxins are relatively easy to produce and can be used to force enemy forces into protective gear, lowering their combat effectiveness. Aflatoxicosis is difficult to diagnose because of the broad range of relatively unspecific symptoms.[31]

Conclusions

As reported by several sources, mycotoxins were used in the past as BWAs.

The fungal toxins could be used as BWAs exploiting both their acute and incapacitating effects or the consequences of a long-term exposure. Moreover, the potential use of mycotoxins on the cattle and on the agriculture, could result in dramatic effects impacting on the economy and on the sustainability of a country.

Because of the uncertain and variable acute toxicity, their manufacture process, difficult weaponization and dispersion in sufficient quantities, and thanks to antidotes available for some mycotoxins, the use of these compounds as BWA is considered quite unlikely, also if cannot be excluded. It is for this reason that further research on the potential use of mycotoxins as BWAs should be conducted and potential mitigation measures should be studied and implemented.

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