Diversas manifestaciones toxicológicas debidas a diferentes espectros de toxinas fúngicas en dos micotoxicosis causadas por Aspergillus spp. en Israel

Resumen.
Se presentan dos micotoxicosis causadas por Aspergillus y reportadas en Israel. El síndrome letal neurotóxico causado por las toxinas de A. clavatus en borregos en Israel tiene diferentes características de las toxicosis de Inglaterra, Sudáfrica y Australia (con la toxicosis de Sudáfrica hay similaridades). La Ocratoxina es una toxina nefrotóxica característica, mientras que la toxicosis inducida por ocratoxina en aves en Israel se manifestó por afectar gansos con hepatotoxicidad severa y daños renales ligeros, mientras los pollos de engorda del mismo incidente mostraron ascitis severa, hepatotoxicidad y no tuvieron lesiones renales. Estos descubrimientos se pueden explicar al encontrar otros aislamientos de A. ochraceus que produjeron ocratoxina y causaron mortalidad, mientras que otras dos líneas no produjeron ocratoxina, y aún así causaron mortalidad, caracterizándose por hepatopatía y ascitis. Estos criterios se deben tomar en cuenta por los toxicólogos clínicos, quienes al no encontrar micotoxinas en el alimento de animales o el hombre, encuentran la micotoxicidad difícil de detectar por la incertidumbre de las manifestaciones de clínica básicas o casos reportados como “típicos”.

Palabras clave: Aspergillus clavatus toxicosis, manifestaciones clínicas, diagnóstico, Mycotoxicosis, ocratoxicosis.

Abstract. This hypothesis is illustrated by 2 Aspergillus mycotoxicoses recorded in Israel. The lethal neurotoxic syndrome caused by A. clavatus toxins in sheep in Israel had different characterizations from toxicoses in England, South Africa and Australia, with quite dissimilar manifestations between the reported cases. Ochratoxin is a characteristic nephrotoxin, whereas an ochratoxin-induced toxicosis in poultry in Israel was manifested in affected geese by severe hepatotoxicity with only mild kidney damage, whereas broilers in the same incident showed severe ascites, hepatotoxicity and no kidney lesions. These findings may be explained by the finding of others that isolates of A. ochraceus produced ochratoxin and caused mortality, whereas 2 other strains did not produce ochratoxin, and yet still caused mortality, characterized by hepatopathia y ascites. Considering these facts, clinical toxicologists may therefore find diagnosis of mycotoxicoses to be fraught with difficulty, due to the uncertainty of basing clinical manifestations on “typical” previously reported cases.

Key words: Aspergillus clavatus mycotoxicosis, clinical manifestations, diagnosis, Mycotoxicoses, ocratoxicosis.

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Autor para correspondencia: Alan Shlosberg
alans@moag.gov.il
Introduction

It is well known that many fungal species produce secondary compounds - toxins to repel, injure or kill biological organisms that have the potential to harm the fungi. These toxins are often classified as mycotoxins if that activity can harm man or higher animals, or as antibiotics if that activity is mainly manifested in lower organisms. Several important principles relating to mycotoxins that are less well appreciated by clinicians may make recognition of mycotoxicoses in animals or man to be fraught with difficulty. Mycotoxicogenic fungi produce these secondary compounds against potentially harmful enemies and competitors, and so usually produce several toxins simultaneously in their broad-based arsenal. For example, Aspergillus clavatus is a commonly encountered fungus in the environment, producing a number of mycotoxins including patulin, kojic acid, cytochalasins and tremorgens [8, 45]. Fusarium spp in particular may produce many different toxins, dependent on their genetic potential and environmental conditions [27], but this has not been thoroughly examined in food and feedstuffs.

However, this was well demonstrated in one small-scale survey conducted in Germany [32] for 9 fusarotoxins (out of >100 known such toxins) examined in wheat from 79 farms. Of 85 samples, 94% contained 2-6 toxins, showing that multiple fusarotoxin presence in grains could be the rule rather than the exception. Some of these toxins may be toxic to vertebrates, but many are unknown, difficult to analyze, improperly characterized, or of unknown vertebrate toxicity [1, 27]. In addition to the trichothecene group, Fusarium spp. also may produce the well-described toxins zearalenone, moniliformin, fumonisins and other toxins and antibiotics [31]. Some of these have been shown to be toxic to poultry too, the animal species most thoroughly examined. Cereal grains may concomitantly be infested with toxigenic strains of both Aspergillus or Penicillium spp., each with their own spectrum of toxins. It should also be taken into account that the vast majority of diagnostic laboratories will routinely analyze for no more than 2-6 of the most commonly recorded mycotoxins (aflatoxin, ochratoxin, zearalenone, fumonisins, T-2 toxin, deoxynivalenol) and even if more comprehensive examinations could be made, the very high cost to the farmer of all these analyses would practically preclude many such feed examinations being so conducted. In addition, most of the clinical manifestations of the more common grain mycotoxins in ruminants and poultry are non-specific, and therefore the likelihood of sending suspect mycotoxin-contaminated feeds for analysis is not great. Moreover, levels of mycotoxins inducing deleterious effects in farm animals are usually at the μg/kg level, so inducing sub-acute or chronic toxicoses. By the time such manifestations are noticed, the offending contaminated feed will almost certainly have been totally consumed, not leaving any sample available for analyses [17]. Therefore such multiple mycotoxin contamination of feed will virtually never be detected.

There may also be interactions between the toxic effects of the toxins, often as a synergism. Potential additive and synergistic deleterious effects of these mycotoxin co-contaminants of cereal grains have not been comprehensively examined in farm animals. This is due to the countless number of possible combinations of toxin and dosage interactions and the feasible number of experimental trials to determine interactions, obviously limited by the considerable expense and workload necessary to conduct such studies. These limitations preclude publication of scientific guidelines on probable interactions. The studies that have been conducted have been virtually all been conducted in fowl. These specific works with trichothecenes have shown that synergism is expressed, between T-2 and deoxynivalenol [24], T-2 and fumonisins B1 [23], T-2 and aflatoxin [20], or with mycotoxins used as drugs such as ionophore coccidiostats [44] or with bacterial toxins [47].

All these facts may bring about a state whereby a certain spectrum (an appreciation of the number of toxins, their toxic properties, their concentrations, and interactions with other co-existing compounds) of toxins may culminate in different clinical manifestations than other spectra produced by the same fungal species. This hypothesis is demonstrated by 2 mycotoxicoses recorded in Israel, a chronic Aspergillus clavatus toxicosis in sheep, and a sub-acute Aspergillus spp. toxicosis in poultry.

Exposure to aspergillus clavatus

A flock of 168 mixed-breed sheep suffered this unique clinical syndrome [39]. The sheep were fed a balanced diet partially comprising a calculated 2.5 kg of sprouted barley grains, which was the waste product from a factory making malt extract; the grains were brought daily and freshly fed. This feeding regime had been successfully practiced for over 2 years with no nutritional or disease problems. At first, one ewe was found dead and on the next day 2 more ewes were recumbent and died within a few hours. Five days later, 10 ewes and 2 rams were severely affected, and it soon became apparent that a severe, distinct, clinical syndrome was emerging. The sheep initially showed mild ataxia, with swaying of the hind limbs. The more severely affected animals were all recumbent, at first in a sternal position and at a later stage of paralysis in a lateral position. Their demeanor was normal, but in all recumbent sheep a marked muscular tremor was evident. This was most obvious in the muscle beds of the barley grains. In the many necropsies performed, nothing consistent or characteristic was found to typify the hind limbs. In the initial cases (recumbent sheep) were recorded each day and 50% of the flock died within 17 days. Around this time, the clinical syndrome changed a little, in that much of the flock could be seen to exhibit lameness and gait abnormalities. The hind limb abnormalities were mainly manifested by unsure and abnormal limb placement, swaying, stiffness and occasional leg knuckling. Forelimb abnormalities comprised a shifting and lifting of the feet, as though in pain, although palpatation did not reveal any focus of pain, and a lifting of the feet onto raised objects, which in many instances was a recumbent sheep. Gaits abnormalities included stiffness, stumbling, and weakness. At this stage, recumbent animals showed less tremor than before and died in a shorter time than in the initial cases. Although signs of central nervous system involvement such as nystagmus and hypothermia were not apparent, several sheep when their vulva was touched developed a transient state of severe tremor, dorsal opisthotonus and mouth movements previously described (in cases of the prion disease scrapie) as a nibbling response [33]. All animals throughout the toxicosis ate well and maintained excellent body weight. After about 30 days, the clinical syndrome had evolved further, with most of the remaining animals showing lameness, and in addition many animals repeatedly pawing or tapping the ground with a foreleg, often held extended in front of them. Recumbent animals barely showed tremor, and died within a few hours. By 6 months, 96% of the 168 adult sheep had died. Infectious and toxicological causes of this highly lethal and very characteristic syndrome were excluded. Rich Aspergillus clavatus growth was found in the germinating beds of the barley grains. In the many necropsies performed, nothing consistent or characteristic was found to typify the syndrome. Histology of organs also did not reveal characteristic lesions, apart from examinations of the nervous system (frontal lobe, occipital lobe, hippocampus, thalamus, cerebellar peduncles, sagittal sections through the cerebellar vermis, medulla). Swollen neurones with central...
A mycotoxic syndrome caused serious losses in domestic poultry [40]. A flock of 16 week-old geese being force-fed for fatty liver (paté) production exhibited pale beaks, weakness and mortality, which within a few days of onset became numerically severe and eventually reached 30%. Most of the surviving birds at slaughter showed small livers weighing about 400g, compared to a normal weight of 600-900g in such birds. In addition, many birds had ascitic fluid in the abdomen, which together with a bluish tinge afforded the birds contained the mycotoxin ochratoxin A (OA) at abnormally high concentrations (for corn in Israel). Of 15 samples analysed from infected farms, 6 were positive for ochratoxin at concentrations of from 110 - 930 µg/kg. The analyses revealed a markedly heterogeneous distribution of OA, as illustrated by 3 samples from one farm feed-bin that contained <20, 110 and 410 µg/kg OA. A feeding trial conducted in Israel with suspect feeds duplicated the field cases [40], which is unusual for poultry mycotoxiceses [17]. OA is regarded as being a marked nephrotoxin, with associated immunosuppressive, teratogenic and carcinogenic effects in diverse species [5, 11, 14].

**Discussion**

The ramifications of this hypothesis, exemplified herein, and that appears to have been at least partially validated, are important to animal and human health.

<table>
<thead>
<tr>
<th>Manifestation</th>
<th>Israel</th>
<th>South Africa</th>
<th>England</th>
<th>Australia</th>
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<tr>
<td>Latency</td>
<td>+++</td>
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<tr>
<td>High mortality</td>
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<td>Recombency</td>
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<tr>
<td>Lameness</td>
<td>+++</td>
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<tr>
<td>Tremor</td>
<td>++</td>
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<td>-</td>
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<tr>
<td>Abnormal gait</td>
<td>++</td>
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<td>-</td>
<td>+</td>
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<tr>
<td>Chewing</td>
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<tr>
<td>Hyperesthesia</td>
<td>-</td>
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<td>Loss of weight</td>
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**Aspergillus clavatus** is a commonly encountered fungus in the environment, producing a number of mycotoxins including patulin, kojic acid, cytochalasins and tremorgen [8, 16, 45]. *A. clavatus* belongs to the *Aspergillus* section clavati, together with 6 other species, the taxonomic position of isolates indicating that patulin- and ribotoxin-producing abilities were lost several times during evolution of the *Aspergillus* section clavati [45]. This evolutionary process supports the hypothesis of different strains or isolates capable of producing different spectra of mycotoxins. It should be noted that none of the known toxins produced by *A. clavatus* have been incriminated in any degree in causing the neurotoxic syndromes seen in sheep or cattle [21, 22].

The two most characteristic facets of the syndrome seen in Israel in sheep were the high mortality rate of 99% and the marked latency in many animals, several dying 6 months after the initial mortality (which was days after cessation of the suspect exposure). These two unique manifestations were not recorded together in any of the cases in sheep from South Africa, England, or Australia. High mortality was only seen in South Africa, but not in England or Australia. The latency phenomenon was not seen anywhere (Table 1). Lameness was also pronounced only in the Israeli case. On the other hand, hyperesthesia was found in South Africa and England, but not at all in Israel, and loss of weight was typical of all 3 cases, whereas Israeli sheep had excellent body weight even on day of death. Tremor, abnormal gait and recumbency were well marked in all 4 cases. The reason for the mortality is not easily explained, as no severe histopathological lesions were found, and the degree and nature of the lesions in the nervous system were not consistent with the terminal manifestations leading to death. Such long latency periods between toxicant exposure and mortality are typical of toxics by another toxin group, the plant pyrrolizidine alkaloids [38], these toxics being severe hepatotoxins. However, the terminal clinical syndrome induced by these alkaloids is that of an hepatonecephalopathy, that was not found in any of the *A. clavatus* toxicoiseses. This makes this particular non-hepatotoxic syndrome to be once again unique in veterinary science. As it is not known which animals had light or severe exposure to the putative toxins, it is interesting to surmise that in the almost certain heterogeneous distribution of mycotoxins in feeds, and particularly in the beds of germinating seeds, some sheep may have ingested a heavy toxin load and died earlier, whereas sheep having a lighter toxin exposure might have succumbed much later. However, it was concluded [29] that prolonged ingestion of small amounts of neurotoxic compounds are required to induce the syndrome.

Most literature sources emphasize the nephrotoxicity of OA, with hepatotoxicity being a minor effect [3, 7, 10, 34]. In the field case in Israel, the predominant lesion in geese and fowl was hepatic necrosis, with nephropathy being seen only in a few geese. In acute toxicoiseses with pure OA in chicks [34], the main post-mortem finding was visceral goit and acute nephrosis. Hepatic lesions were less frequent [34], and varied in severity, with some birds having necrotic liver foci. Another work with pure OA in chicks revealed severe nephrotoxicity, but liver lesions were limited to lymphocyte infiltration [10]. These differences in target organ toxicity might be partially explained by a work that examined just 4 US isolates of *A. ochraceus* with known toxicity when fed to chicks [9]. It was found that the 2 most toxic isolates both produced OA and caused acute mortality. However, mortality was also recorded from 2 isolates not producing OA, but which induced subacute, severe growth suppression; fatty changes and necrotic foci in the liver were related to the presence of OA and perhaps an unidentified hepatotoxin. One of the non-ochratoxigenic isolates produced asci, hydropediculorum and edema in 4 of 8 birds, compared with none of these findings in the 20 birds fed the ochratoxigenic isolates. These data support our hypothesis that this field case was caused by OA and (at least) one other mycotoxin, that was a potent hepatotoxin, but...
which remains unidentified.

The changes in blood parameters measured in the feeding trial using suspect feed [40] were different from those recorded in ochratoxicoses in fowl elsewhere [19, 35, 43]; of particular concern was the severe reduction in body weight in both field cases and in the feeding trial is typical of changes in serum parameters. The severe reduction in body weight in both field cases and in the feeding trial is typical of ochratoxicoses in poultry [41]. The low incidence of OA contamination [46], with corn specifically present in nonagricultural areas (Arctic) of Norway. Mycopathologia 105: 143-151.

References


