Diverse toxicological manifestations due to different spectra of fungal toxins in two mycotoxicoses caused by *Aspergillus* spp. In Israel

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

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, hepatoxicidad y no tuvieron lesiones renales. Estos descubrimientos se pueden explicar al encontrar otros aislamientos de *A. ochraceus* que produjeron ocratoxina y causaron motalidad, mientras que otras dos líneas no produjeron ocratoxina, y aún así causarin 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 hepatopathy and 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, ochratoxicosis.

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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. Mycotoxigenic 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 fusariotoxins (out of > 100 known such toxins) examined in wheat from 79 farms. Of 85 samples, 94 % contained 2-6 toxins, showing that multiple fusariotoxin 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, fumonisin 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, fumonisin, 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 fumonisin 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* sp. 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 mass of the lateral aspect of the hind legs, and the shoulder and neck area. When quiescent animals were lifted, they often developed fits, comprising marked tremor and clonic convulsions. Recumbent sheep died within 72 hours. New 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 palpation did not reveal any focus of pain, and a lifting of the feet onto raised objects, which in many instances was a recumbent sheep. Gait 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 hyperesthesia 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

chromatolysis and/or margination of chromatin were consistently found in the hippocampus and medulla, and a few glial foci in these areas were also present.

This highly lethal neurotoxic syndrome with marked latency resembled, but was far from mirroring, the *A. clavatus* toxicoses recorded in sheep in South Africa [21, 22], England [15] and Australia [37] (Table 1).

Exposure to ochratoxin

A mycotoxin 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 liver, resulted in condemnation of the geese and their products for food. Several flocks of goslings (3-6 weeks-old) showed poor feathering, ataxia or lameness, tremor, depression, diarrhea, paresis, a severe reduction in growth rate, and a greatly increased mortality. The affected broiler flocks showed a mild reduction in growth rate with a greatly increased mortality, severe dyspnea and ruffled feathers. All affected geese showed marked multifocal liver necrosis but kidney lesions were present only in some birds. Broilers had a diffuse liver necrosis, with no lesions in any kidneys examined. This syndrome was diagnosed as a mycotoxicosis based on the finding that imported corn fed only to affected 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 mycotoxicoses [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 1. Comparative frequency of clinical manifestations in *A. clavatus* neurotoxicoses in sheep in 4 countries where cases were recorded (+++ = marked, ++ = moderate, + = mild, - = no manifestations).

Manifestation	Israel	South Africa	England	Australia
Latency	+++	-	-	-
High mortality	+++	++	-	++
Recumbency	+++	++	+	+++
Lameness	+++	+	-	-
Tremor	++	+++	-	+++
Abnormal gait	++	++	+++	+++
Chewing	+	++	-	-
Hyperesthesia	-	++	-	+++
Loss of weight	-	++	+++	+++

Aspergillus clavatus is a commonly encountered fungus in the environment, producing a number of mycotoxins including patulin, kojic acid, cytochalasins and tremorgens [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 98% 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 toxicosis by another toxin group, the plant pyrrolizidine alkaloids [38], these toxins being severe hepatotoxins. However, the terminal clinical syndrome induced by these alkaloids is that of an hepatoencephalopathy, that was not found in any of the *A. clavatus* toxicoses. 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 heterogenous 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 toxicosis with pure OA in chicks [34], the main post-mortem finding was visceral gout 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-ochratoxinogenic isolates produced ascites, hydropericardium 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 the changes, only elevated uric acid levels were present in all 4 works. The hepatopathy seen at necropsy and microscopically in both species in the trial was not reflected in changes in serum parameters. The severe reduction in body weight in both field cases and in the feeding trial is typical of ochratoxicosis in poultry [9, 12], but is also non-specific and found in a plethora of conditions in poultry [41]. The low diagnostic value of these potential diagnostic parameters further confounds the diagnosis of OA exposure. In the present cases, OA was not considered to be the primary cause of the severe syndrome induced, but the toxin served as an easily identified indicator of mycotoxin contamination. Zearalenone and deoxynivalenol are 2 mycotoxins which likewise have been proposed as being easily analysed "biomarkers" for mycotoxin contamination of grains or feeds, without actually identifying the other specific toxins [28, 36].

The most potentially harmful *Aspergillus* mycotoxins to human health are aflatoxin B1 and OA. This is due to the fact that both are carcinogenic and immunosuppressive, particularly worrying in the light of their well-known capacity to exist in food of animal origin, aflatoxin being a particularly threat in milk (and cheese) and liver, and OA mainly in the kidney [26]. However, demonstrating the carcinogenic and immunosuppressive role of these toxins in man is very difficult epidemiologically. The main causes of practical concern with OA are the proven role of OA in causing mycotoxic porcine nephropathy [25] from OA residues in porcine and avian tissues eaten by man [13, 25], and the link between endemic Balkan nephropathy in man and exposure to OA [13]. Pigs and dogs are the most sensitive of domestic animals to OA [13], with ruminants being the most resistant [30]. Toxicoses in farm animals other than porcines are rarely reported [11], with most cases, including mass toxicoses involving millions of birds, having been recorded in 9 episodes in poultry in the USA, mainly in the 1970's, caused in 8 cases by OA contaminated corn [18]. In meat - type birds, one case of ochratoxicosis in the Ukraine involved ducks [4] and another case was in broilers in Canada [2]. Since the 1970's, no cases of ochratoxicosis have been recorded in meat-type poultry in the USA (P. Hamilton, pers. comm.), for no readily apparent reason. The predominance of mycotoxic porcine nephropathy and endemic Balkan nephropathy in Europe is said to be due to prevalent climatic regions of greater than 65 % humidity, with fog and rain, particularly at grain harvest time [13]; these conditions are seen in Denmark, Sweden, the British Isles, the German coastal area and the endemic Balkan nephropathy endemic areas of Yugoslavia, Romania and Bulgaria [13]. Surveys of animal feedstuffs in the USA revealed a very low (< 2%) incidence of OA contamination [46], with corn specifically having 0.35 % and 1.02 % contamination [6].

Apart from standard residue testing of animal tissues, conducted at random, at best in a very small sample size, there is virtually no efficacious means of detecting harmful mycotoxin residues before distribution of potentially-contaminated foodstuffs to the public. There is therefore no reliable protocol of ensuring that food is free of harmful residues. An ancillary way to improve the rate of residue detection would be for the farmer or attending veterinarian to be led by typical clinical manifestations to a differential diagnosis that includes exposure to a specific mycotoxin, and thence to analysis of feed and tissues to determine potential contamination of food of animal origin during production (milk, eggs) and before slaughter of the animals (meat, organs). It is therefore of much concern that "typical" clinical and pathological manifestations of mycotoxicoses, such as ochratoxicosis, are apparently not so easily characterized with finality, and do vary not a little. It is particularly worrying that some of these mycotoxins that confound clinicians, i.e. the hepatotoxin co-produced with OA, or the toxin(s) causing the highly lethal, delayed toxicity in sheep, are still unknown, cannot be analyzed, and could perhaps appear in food intended for man. As the toxins are as yet unknown, the degree of their toxicity to man is not known.

In conclusion, the hypothesis that different spectra of mycotoxins in feeds or foods can induce widely differing clinical syndromes seems to be valid, as exemplified by these 2 cases in farm animals in Israel. The potential deleterious effects of such manifestations could be that inexplicable clinical syndromes are not diagnosed as the field data do not match the data from the literature, or the occurrence of mycotoxin residues in foods derived from animals, where the initial exposure of the farm animals was not manifested by "typical" signs of a mycotoxicosis. In the light of these conclusions, it should also be borne in mind that the older contention that mycotoxins are found only uncommonly in foods and feeds should be replaced by a more modern philosophy that mycotoxins are ubiquitous environmental contaminants, frequently present at low (g/kg) concentrations.

References

1. Abbas, H.K., C.J. Mirocha, R. Gunther, 1989. Mycotoxins produced by toxic Fusarium isolates obtained from agricultural and nonagricultural areas (Arctic) of Norway. Mycopathologia 105: 143-151.
2. Abramson, D., J.T. Mills, B.R. Boycott, 1983. Mycotoxins and mycoflora in animal feedstuffs in western Canada. Canadian Journal of Comparative Medicine 47: 23 - 26.
3. Austwick, P.K.C., 1983. Fungal nephrotoxins. Veterinary Research Communications 7: 145 - 154.
4. Bodnarchuk, A.I., I.M. Kaspruk, 1984. Ochratoxicosis in ducks. Veterinarya Moscow 2: 67 - 68 (Poultry Abstracts 1984 10: no. 1092).
5. Boorman, G.A., NTP technical report on the toxicology and carcinogenic studies of ochratoxin A [CAS no. 303-47-9] in F344/n rats [gavage studies]. NIH publication no. 89-2813. Dept. Hlth. Hum. Serv., NIH, Research Triangle.
6. Buckle, A.E., 1983. The occurrence of mycotoxins in cereals and animal feedstuffs. Veterinary Research Communications 7: 171 - 186.
7. Burns, R.B., P. Dwivedi, 1986. The natural occurrence of ochratoxin A and its effects in poultry. A review. Part II. Pathology and immunology. World´s Poultry Science Journal 42: 48 - 55.
8. Clardy, J., J.P. Springer, G. K. Matsuo, R. Wightman, 1975. Tryptoquivaline and tryptoquivalone, two tremorgenic metabolites of Aspergillus clavatus. Journal of the American Chemical Society 97: 663-665.
9. Doupnik, B. Jr., J.C. Peckham, 1970. Mycotoxicity of *Aspergillus ochraceus* to chicks.
10. Dwivedi, P., R.B. Burns, 1984. Pathology of ochratoxicosis in young broiler chicks. Research in Veterinary Science 36: 92 - 103.
11. Dwivedi, P., R.B. Burns, 1985. Immunosuppressive effects of ochratoxin A in young turkeys. Avian Pathology 14: 213 - 225.
12. Dwivedi, P., R.B. Burns, 1986. The natural occurrence of ochratoxin A and its effects in poultry. A review. Part I. Epidemiology and toxicity. World´s Poultry Science Journal 42: 32 - 47.
13. Frank, H.K., 1991. Risk estimation for ochratoxin A in European countries. *In:* Castegnaro, M., R. Plestina, G. Dirheimer, IN, Chernozemsky, N. Bartsch, (eds.), Mycotoxins, endemic nephropathy and urinary tract tumours. International Agency for Research on Cancer (IARC) Scientific Publications Lyon pp. 321 - 325.
14. Fukui, Y., K. Hoshino, Y. Kameyama, T. Yasui, C. Toda, H. Nagano, 1987. Placental transfer of ochratoxin A and its cytotoxic effect on the mouse embryonic brain. Food and Chemical Toxicology 25: 17 - 21.
15. Gilmer, J.S., D.M., Inglis, J. Robb, M. Maclean, 1989. A fodder mycotoxicosis of ruminants caused by contamination of a distillery by-product with *Aspergillus clavatus.* Veterinary Record 124: 133-5.
16. Glinsuron, T., 1974. Isolation and purification of cytochalasin E and two tremorgens from Aspergillus clavatus. Plant Foods for Man 1: 113-9.
17. Hamilton, P., 1982. Mycotoxins and farm animals. Refuah Veterinarith 39: 17 - 45.
18. Hamilton, P.B., W.E. Huff, J.R. Harris, R.D. Wyatt, 1982. Natural occurrences of ochratoxicosis in poultry. Poultry Science 61: 1832 - 1841.
19. Huff, W.E., L.R. Kubena, R.B. Harvey, 1988a. Progression of ochratoxicosis in broiler chickens. Poultry Science 67: 1139-1146.
20. Huff, W.E., L.F. Kubena, GE. Rottinghaus, 1988b. Toxic synergism between aflatoxin and T-2 toxin in broiler chickens. Poultry Science 67: 1418-1423.
21. Kellerman, T.S., S.J. Newsholrne, J.A. Coetzer, G.C. Van Der Westhuizen, 1984. Tremorgenic mycotoxicosis of cattle caused by maize sprouts infested with *Aspergillus clavatus*. Onderstepoort Journal of Veterinary Research 51: 271-4.
22. Kellerman, T.S., J.G Pienaar, G.C. Van Der Westhuizen, L.A. Anderson, T.W. Naude, 1976. A highly fatal tremorgenic mycotoxicosis of cattle caused by *Aspergillus clavatus*. Onderstepoort Journal of Veterinary Research 43: 147-54.
23. Kubena, L.F., T.S. Edrington, C. Kamps-Holtzapple, R.B. Harvey, M.H. Elissalde, G.E. Rottinghaus, 1995. Influence of fumonisin B, present in *Fusarium moniliforme* culture material, and T-2 toxin on turkey poults. Poultry Science 74: 306313.
24. Kubena, L.F., W.E. Huff, R.B. Harvey, T.D. Phillips, G.E. Rottinghaus, 1989. Individual and combined toxicity of deoxynivalenol and T-2 toxin in broiler chicks. Poultry Science 68: 622-626.
25. Krogh, P., B. Hald, E.J. Pedersen, 1973. Occurrence of ochratoxin A and citrinin in cereals associated with mycotoxic porcine nephropathy. Acta Pathologica et Microbiologica Scandinavica B 81: 689 - 695.
26. Kuiper-Goodman, T., 1990. Risk assessment to humans of mycotoxins in animal-derived food products. Veterinary and Human Toxicology 32: 6-14.

27. Langseth, W., A. Bernhoft, T. Rundberger, B. Kosiak, M. Garies, 1999. Mycotoxin production and cytotoxicity of *Fusarium* strains isolated from Norwegian cereals. Mycopathologia 144: 103-113.
28. Leeson, S.G.J., J.D. Diaz, 1995. Trichothecenes. In: Leeson, S.GJ., Diaz, J.D. Summers, S. (eds.), Poultry Metabolic Disorders and Mycotoxins. University Books, Guelph. pp. 190-226.
29. Loretti, A.P., E.M. Colodel, D. Driemeier, A.M. Correa, J.J. Bangel Jr., L. Ferreiro, 2003. Neurological disorder in dairy cattle associated with consumption of beer residues contaminated with *Aspergillus clavatus*. Journal of Veterinary Diagnostic Investigation 15: 123-132.
30. Marquardt, R.R., A.A. Frohlich, 1992. A review of recent advances in understanding ochratoxicosis. Journal of Animal Science 70: 3968 - 3988.
31. McLean, M., 1996. The phytotoxicity of Fusarium metabolites: an update since 1989. Mycopathologia 133: 163-179.
32. Muller, H.M., K. Schwadorf, 1993. A survey of the natural occurrence of *Fusarium* toxins in wheat grown in a southwestern area of Germany. Mycopathologia 121: 115-121.
33. Parry, H.B., 1983. Scrapie Disease in Sheep. Academic Press, London.
34. Peckham, J.C., B. Doupnik Jr., J., Jones, 1971. Acute toxicity of ochratoxins A and B in chicks. Applied Microbiology 21: 492 - 494.
35. Raina, J.S., K.S. Roy, B. Singh, 1991. Biochemical and histochemical studies in experimental mycotoxicosis in chicks. Indian Journal of Animal Science 61: 1276-1281.
36. Romer, T., 1990. Advances in mycotoxin analysis: marking *Fusarium*. Feed Management 41: 30-32
37. Ross, A.D., G. Morice, I. Masters, 2003. Mouldy feed neuropathy in feedlot sheep. Annual Meeting, Australian Society for Veterinary Pathology, City, pp. 33-34
38. Shlosberg, A., M.N. Egyed, S. Perl, B. Yakobson, M. Abrahamson, H. Weinberg, 1981. Chronic poisoning in calves caused by ingestion of *Heliotropium europaeum.* I. Epidemiological investigations. Refuah Veterinarith 38: 80-88.
39. Shlosberg, A., I. Zadikov, S. Perl, B. Yakobson, D. Elad, E. Rapoport , V. Handji, 1991. *Aspergillus clavatus* as the probable cause of a lethal mass neurotoxicosis in sheep. Mycopathologia 114: 35 - 39.
40. Shlosberg, A., V. Handji, M. Malkinson, U. Orgad, E. Bogin, M. Meroz, R. Bock, 1991. Severe nephropathy and hepatopathy in geese and broilers associated with ochratoxin in their feed. Mycopathologica 138: 71-76.
41. Shlosberg, A., 2001. Toxicants in Poultry. *In*: Jordan, F., Pattison, M., Alexander, D., Faragher, T., (eds.), Poultry Diseases. 5th ed. W.B.Saunders, London, pp. 505-514.
42. Singh, G.S.P., H.V.S. Chauhan, G.J. Jha, K.K. Singh, 1990. Immunosuppression due to chronic ochratoxicosis in broiler chicks. Journal of Comparative Pathology 103: 399-410.
43. Sreemannarayana, O., R.R. Marquardt, A.A. Frohlich, D. Abramson, G.D. Phillips, 1989. Organ weights, liver constituents, and serum components in growing chicks fed ochratoxin A. Archives of Environmental Contamination and Toxicology 18: 404-410
44. Vanyi, A., G Salyi, G. Majoros, R. Glavits, G Sandor, G Bago, 1989. Interaction of T-2 fusariotoxin and monensin in broiler chickens infected with coccidia. Acta Veterinaria Hungarica 37: 327-333.
45. Varga, J., K. Rigó, J. Molnár, B. Tóth, S. Szencz, 2003. Mycotoxin production and evolutionary relationships among species of *Aspergillus* section clavati. Antonie van Leeuwenhoek 83: 191-200.
46. Wood, GE., 1992. Mycotoxins in foods and feeds in the United States. Journal of Animal Science 70: 3941 - 3949.
47. Zhou, H.R., J.R. Harkema, J.A. Hotchkiss, D. Yan, R.A. Roth, J.J. Pestka, 2000. Lipopolysaccharide and the trichothecene vomitoxin (deoxynivalenol) synergistically induce apoptosis in murine lymphoid organs. Toxicological Sciences 53: 253-263.