

A Field Study On Mycotoxicoses In Horses

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ABSTRACT

The present study was conducted to investigate the etiology of sudden clinical manifestations and some mortalities in a large farm used for breeding of Arabian horses. The most prevalent symptoms were severe colic, distention, predominant depression, watery diarrhea with fetid odour, severe congestion of mucous membranes and excessive sweating. Affected animals were arched back with unpleasant odour of respiration. Some animals exhibited laminitis and other showed hemiplegia. Some of affected animals died within one week of onset of symptoms. The history of the case was associated the onset of the symptoms with the entrance of new feed lots of barley. Samples were collected from barley and subjected for mycological examination where some mycotoxin producing fungi e.g. *Aspergillus* species (*A. Flavus*; *A. Parasiticus*; *A. Ochraceus*) and *Fusarium* species were isolated and identified. The levels of some mycotoxins e.g. aflatoxins (B1; B2; G1; G2; M1); ochratoxin A, zearalenone and T2 toxin were estimated in barley and tissues of dead animals. Immunological investigations declared a degree of immunosuppressions in affected animals associated with haematological change and a significant decrease in alpha α 1 and gamma globulin. Histopathological examinations revealed congestion, hemorrhage, degeneration and necrosis with mononuclear inflammatory cellular infiltration in liver, kidney and heart. Focal areas of malacia, perivascular hemorrhage, edema; neuronal degeneration and gliosis were the main brain lesions. Bacteriological culture of fecal samples and intestinal content recorded salmonella, enterococci, pseudomonas and bacillus species.

INTRODUCTION

Mycotoxins are formed on animal feed when condition of moisture and temperature permits the growth of naturally occurring toxigenic fungi. Most frequent concentrations of mycotoxins in feed are below those that cause acute disease. It reduce growth rate of young animals and interfere with mechanisms of resistance and impair immunologic responsiveness. So the signs may be associated with infection rather than with mycotoxin that predispose the animal to infection (1).

Mycotoxins depress T and B lymphocyte activity, suppress immunoglobulin and antibody production, reduce complement or interferon activity and impair macrophage function (2). Signs of mycotoxicoses are vary and depend on species, system involved, dose and type of mycotoxin. The signs can range from acute death, immunosuppression, skin lesions and signs of hepatotoxicity, nephrotoxicity and neurotoxicity (3).

Although the effects of mycotoxins on horses are not well documented in scientific literature, in field situations apparent mycotoxin problems appear to be significant. Mycotoxins have been implicated in a variety of health problems including colic, neurological disorders, paralysis, hypersensitivity and brain lesions. The cumulative effect of feeding low levels of mycotoxins may also contribute to gradual deterioration of organ functions. This in turn affects growth rate, feed efficiency,

fertility, and the ability to perform work and life span (4).

A fatal clinical case of equine aflatoxicosis was recorded in a horse presenting by depression, icterus, shifting lameness and subcutaneous hemorrhage. Analysis of maize had been revealed aflatoxin B1 at 300 μ g/g (5).

Two episodes of acute aflatoxin poisoning in horses have been recorded. Lesions associated with exposure to aflatoxin included encephalomalacia of cerebral hemispheres, fatty degeneration, necrosis, bile duct hyperplasia and fibrosis of the liver, fatty infiltration of the kidney, hemorrhagic enteritis and myocardial degeneration (6).

An outbreak of aflatoxicosis in horses, mainly yearlings was investigated. The clinical signs included mild fever, anorexia, depression; uncoordination and marked swelling of the supraorbital fossae. The morbidity was high in yearlings and the mortality rate was 25%. Large quantities of aflatoxins B1 and B2 were detected in feed. All the affected yearlings showed various degrees of improvement after toxic feed was replaced and mortality ceased two months later (7).

Seventeen (27%) out of 63 mares on Tuling horse farm in shaanxi province, China aborted due to mycotoxins contaminated feed (8). Also, three horses died and others became sick and the cause of death was the maize

where mycological examination declared that 95% of colonies were *Aspergillus flavus* and chemical analysis of this maize was positive for aflatoxin B1, B2 and M1 at concentrations of 114, 10, and 6 ppb respectively (9).

In Egypt, cases of mycotoxicoses in equine at El-Shams club, Cairo, was recorded where 15 of 450 horses died after showing nervous symptoms and the examination of feed detected toxigenic strains of *Aspergillus flavus* from wheat bran and barley samples and aflatoxin B1 was detected in barley. Post mortem examination revealed changes in blood vessels, stomach, intestine, liver and kidneys. There was also congestion of blood vessel, edema, neurophagia, satellitosis and demyelination in the brain (10).

The present study was aimed to clarify the etiological determinants of sudden clinical manifestation of toxicity and mortality in a large governmental horse farm, which was parallel to the introduction of new lot of barley.

MATERIAL AND METHODS

Animals and history of the problem

A large governmental farm contains around 500 horses of both sexes, and different ages from which 20 horses developed sudden clinical manifestation of toxicity associated with feeding of newly introduced feed lot of barley. Six cases of these clinically ill horses died four of which between 1-2 years old beside two pregnant mares. The other horses were apparently normal. We noticed visually that the grains of barley were damaged. All animals were exposed to full clinical examination (11). The diseased animals were segregated into separate places then blood, serum and fecal samples were collected from living animals and samples from intestinal contents were taken from dead ones for bacteriological investigations. Moreover samples from intestinal content, liver, kidney, skeletal muscle were taken from dead animals for estimations of some mycotoxin residues. Also samples were collected from damaged barley and subjected to mycological investigations and their contents of some mycotoxins were also determined. Finally tissue samples were collected from some internal organs for histopathological investigations.

Analytical methods

Mycological examination of contaminated barley

Isolation, counting and identification of fungi in infested barley were carried out (12, 13, 14).

Determination of some mycotoxin in barley

We used immunoaffinity column for extraction and clean up of aflatoxins, Afla prep and for ochratoxin ochra prep (RHONE POULENC Diagnostic, France) followed by quantitative estimations of different types of aflatoxins and ochratoxins on HPLC according to the recommended methods by (15, 16). Zearalenone and T2 toxin are extracted with acetonitrile potassium chloride 4% (9:1) and proceeded as previously described (17) and the concentration of the mycotoxin was carried out by thin layer chromatography (TLC) against reference standards.

Determination of some mycotoxins residues

Samples of liver, kidneys and skeletal muscle beside intestinal content were collected from died animals where the residues of aflatoxins B1, B2, G1, G2, M1 and ochratoxin A were determined (18).

Hematological investigations

RBcs and WBCs counts as well as WBCs differential cell count were estimated (19) and phagocytic activity were determined (20). The following equation (21) was used for measurement of immunosuppression percentage (IS).

Where

$$IS\% = \frac{IS \text{ of control} - IS \text{ of diseased}}{IS \text{ of control}} \times 100$$

Also serum samples were separated for serum proteins fractionation by electrophoresis (22).

Microbiological examination

Fecal samples from diseased animals and intestinal contents from dead ones were examined bacteriologically (23).

Histopathological investigations

Tissue specimens from liver, kidneys, heart, lungs, spleen, brain, stomach and small intestines were collected from horses immediately after death and fixed directly in 10% buffered neutral formalin. After complete fixation these specimens were washed, dehydrated, cleared & embedded in paraffin. Then serial paraffin sections of 4-6 microns thickness were prepared and stained with hematoxylin and eosin (24).

Symptomatic and supportive therapy were adopted (25, 26) with isolation of affected animals into a separate stables and feeding of contaminated barley was stopped. Statistical analysis carried out (27).

RESULTS

Our results concerned with incidence and counting of mycotoxin producing fungi in barley; concentrations of some mycotoxins in barley; and concentration of different mycotoxin in intestinal content and tissues are tabulated in tables 1; 2; 3 respectively. Bacteriological investigations of fecal samples and intestinal content are presented in table 4. Hematological investigations and phagocytic activities of monocytes are tabulated in table 5 and protein fractionations are tabulated in table 6.

Concerning the postmortem examination of dead horses nearly similar gross pictures were recorded in all examined cases. Generalized congestion of s/c and visceral blood vessels was noticed. The liver was soft in consistency, pale in color and studded with petichial hemorrhages on its capsule. The cut section of the liver was yellow and mottled with red patches. Lungs showed multiple uniform consolidated reddish areas alternated with pale raised emphysematous areas.

The covering pleura was dull and covered with distinct layer of fibrinous exudates. The heart appeared enlarged, flabby and congested. The pericardium was also congested, rough and filled with serofibrinous exudate. The brain was soft in consistency and showed congested blood vessels with multiple petichiae on the cerebral and cerebellar hemispheres. Moreover, both kidneys were congested and showed pale spots and streaks on their cut sections. The stomach and intestine were congested and filled with pasty content.

Microscopically, the liver showed congestion of central veins and sinusoids with presence of multiple areas of hemorrhages replaced the hepatic parenchyma (Fig. 1) Focal areas of hydropic, vacuolar and fatty degeneration of hepatocytes were observed (Fig. 2). Disorganization of hepatic cords with presence of pale eosinophilic homogenous substance in between these dissociated hepatic cells were detected. Hyperplasia of the bile ductal epithelium with periductal fibrosis and few mononuclear inflammatory cellular infiltration were seen in the portal area (Fig. 3). Moreover, necrosis of hepatocytes represented by pyknosis of their nuclei was found in some examined liver.

Kidneys revealed dilatation and engorgement of renal blood vessels and capillaries with blood and focal intertubular

hemorrhage. Cloudy swelling and vacuolar degeneration of the lining epithelium of renal convoluted tubules were seen. Shrinkage of some glomerular tufts with pyknosis of nuclei of epithelium lining some renal tubules were observed. Focal inflammatory cellular infiltration of the renal cortex mostly lymphocytes was also found (Fig. 4). Moreover, desquamation of the lining epithelium of many renal tubules in the medulla was noticed.

Heart showed congestion of the myocardial blood vessels and intermuscular capillaries with focal hyalinosis of cardiac muscles and mononuclear inflammatory cellular infiltration (Fig. 5). Multiple areas of extravasations of erythrocytes between the myocardium were detected. Moreover, thickening of the pericardium due to presence of serofibrinous exudate was also found in some examined cases.

Lung showed severe congestion of the blood vessels and presence of pale eosinophilic homogenous substance mixed with inflammatory cells inside the alveolar luminae. In some examined lungs, the pulmonary alveoli were filled with network of fibrin mixed with macrophages, plasma cells and lymphocytes. Excessive deposition of golden yellow to brown granules of hemosiderin pigments inside the phagocytic cells and free within the pulmonary alveoli with presence of multiple areas of compensatory alveolar emphysema were detected (Fig. 6). Multiple areas of inflammatory cells mainly macrophages and lymphocytes were observed. Thickening of interlobular septa and pleura due to presence of serofibrinous exudate was noticed. Moreover, focal areas of calcification evidenced by presence of bluish structureless substance among the pulmonary parenchyma were also recorded in some cases. The spleen showed depletion and necrosis of the lymphoid tissues (Fig. 7), moreover focal areas of hemorrhages and hemosiderosis were also detected in the spleen.

The microscopic examination of stomach revealed necrotizing gastritis represented by severe congestion of blood vessel, with presence of focal areas of necrosis mixed with inflammatory cells in the gastric submucosa (Fig. 8).

Moreover, desquamation of mucosal epithelium and mononuclear inflammatory cellular infiltration of the mucosa were noticed (Fig. 9).

Intestine showed congestion of the submucosal blood vessel and capillaries with

Table (1): Incidence and counting of mycotoxin producing fungi in barley Samples (colony count/g).

Fungus species	No. of examined samples	No of positive samples	Percentage	Colony count per gram	Log. Count
<i>A. Flavous</i>	10	10	100%	6.5×10^4	4.8
<i>A. parasiticus</i>	10	10	100%	7.8×10^3	3.9
<i>A. Ochracious</i>	10	10	100%	3.4×10^2	2.5
<i>Fusarium</i>	10	10	100%	2.0×10	1.3

Table (2): Incidence and concentrations of mycotoxins (ppb) in barley samples

Mycotoxin	No. of examined samples	No of positive samples	Percentage	Range (ppb)	Mean \pm SE
<i>Aflatoxin B1</i>	10	10	100%	220-300	269 ± 2.69
<i>Aflatoxin B2</i>	10	10	100%	100-160	123 ± 1.23
<i>Aflatoxin G1</i>	10	4	40%	0-19	26 ± 0.26
Total aflatoxins	10	10	100%	340-471	398 ± 3.98
<i>Ochratoxin A</i>	10	10	100%	100-250	141 ± 1.42
<i>Zearalenon</i>	10	2	20	10-14	12 ± 0.13
<i>T-2 toxin</i>	10	2	20	12-15	13.53 ± 0.13

Table (3): Concentrations (mean \pm SE) of different aflatoxins (B1, B2, G1, G2, M1) and ochratoxin A in intestinal content and tissues (liver, kidneys and muscle) of dead animals.

Mycotoxin	Intestinal content	Liver	Kidney	Muscle
Aflatoxins				
B1	140.55 ± 1.41	14.62 ± 0.15	16.22 ± 0.16	20.41 ± 0.20
B2	54.39 ± 0.54	6.44 ± 0.06	6.23 ± 0.06	8.12 ± 0.08
G1	-----	4.32 ± 0.04	-----	6.32 ± 0.06
G2	-----	-----	-----	-----
M1	15.61 ± 0.16	10.43 ± 0.10	16.41 ± 0.16	-----
Ochratoxin A	57.62 ± 0.58	6.52 ± 0.07	8.21 ± 0.08	7.90 ± 0.08

Table (4) : Percentage of different types of isolated bacteria from focal samples of diseased animals as well as intestinal content of dead ones .

Type of isolated bacteria	Fecal samples	Intestinal content
<i>Salmonella. sp</i>	68.1 %	74.5 %
<i>Enterococci. sp</i>	18.1 %	25.4 %
<i>Pseudomonas. sp.</i>	15.7 %	22.8 %
<i>Bacillus. sp</i>	6.9 %	20.7 5
<i>Shigella. sp</i>	0.0 %	0.0 %

Table (5) : Mean values of some hematological changes in diseased as well as apparently healthy horses (control).

Parameters	Control Horses	Diseased horses
RBCs $10^{12}/l$	9.46 \pm 0.45	6.21 \pm 0.72**
Total WBCs $10^9/l$	10.95 \pm 0.63	7.96 \pm 0.54**
Lymphocytes %	41.8 \pm 0.75	32.1 \pm 0.62**
Neutrophils %	53.2 \pm 1.3	48.5 \pm 1.2 *
Monocytes %	2.13 \pm 0.38	1.6 \pm 0.43
Eosinophils %	2.49 \pm 0.53	1.8 \pm 0.6
Basophils %	0.86 \pm 0.21	0.73 \pm 0.13
Phagocytic %	42	26
Immunosuppression %	00	38.1

Significant at $P < 0.05^*$ Highly significant at $P < 0.01^{**}$

Table (6) : Mean values of serum proteins electrophoretic pattern of diseased as well as apparently healthy horses (control).

Statement	Control horses	Diseased horses
α_1 globulin g/dl	1.13 \pm 0.01	0.75 \pm 0.13*
α_2 globulin g/dl	0.86 \pm 0.31	0.61 \pm 0.2
β globulin g/dl	0.79 \pm 0.42	0.56 \pm 0.12
δ globulin g/dl	1.61 \pm 0.24	0.64 \pm 0.12 **

Significant at $P < 0.05^*$ Highly significant at $P < 0.01^{**}$

Fig. (1): Liver of horse suffered from mycotoxicoses showing focal area of hemorrhage replaced hepatic parenchyma. H & E stain X200



Fig.(1):

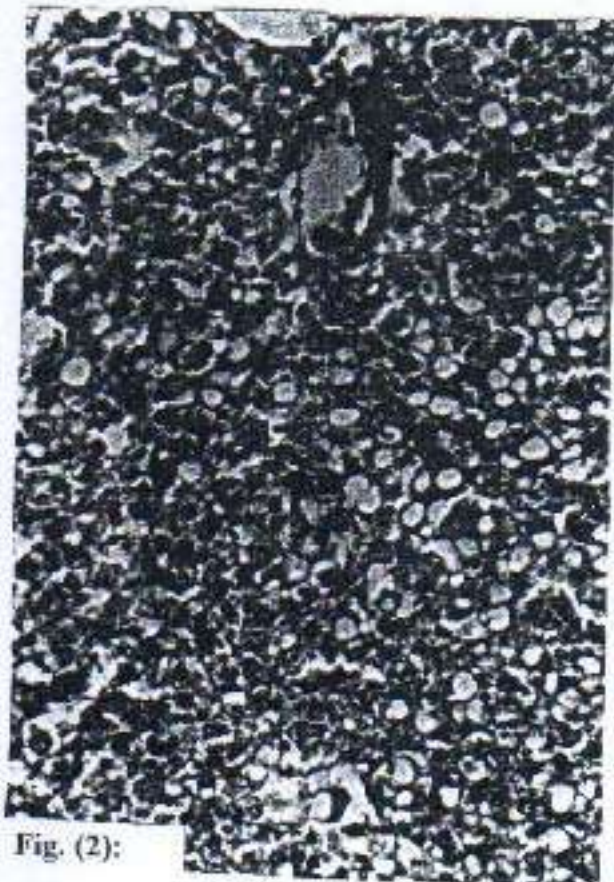


Fig. (2):

Fig. (2): Liver of horse suffered from mycotoxicoses showing fatty degeneration of the hepatocytes. H & E stain X200.

Fig. (3): Liver showing hyperplasia of bile ductal epithelium with periductal fibrosis and mononuclear inflammatory cellular infiltration X300

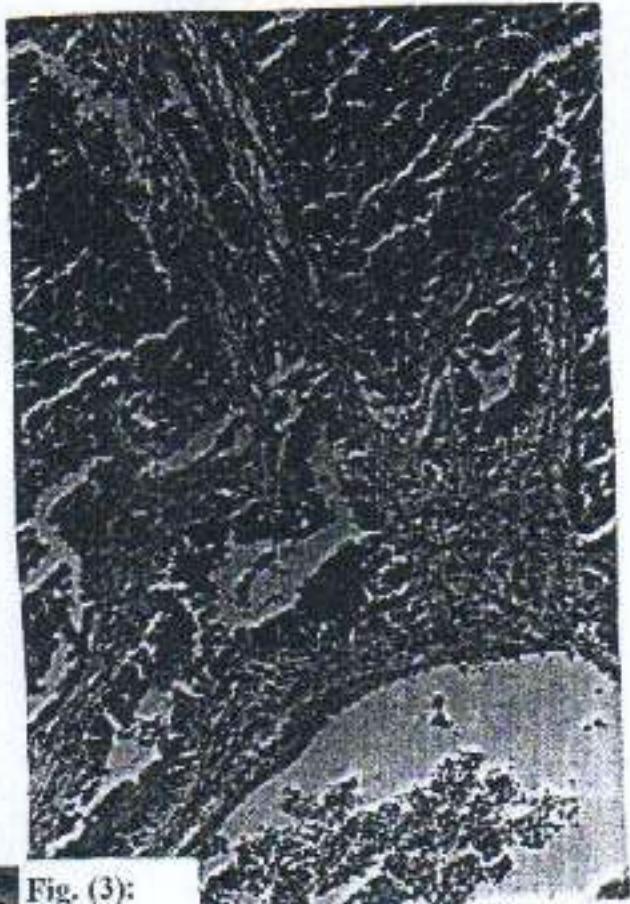


Fig. (3):



Fig. (4):

Fig.(4):Kidney showing focal inflammatory cellular infiltration mostly lymphocytes X300

Fig.(5):Heart showing myocardial hyalinosis and mononuclear cellular aggregation X200



Fig. (5):

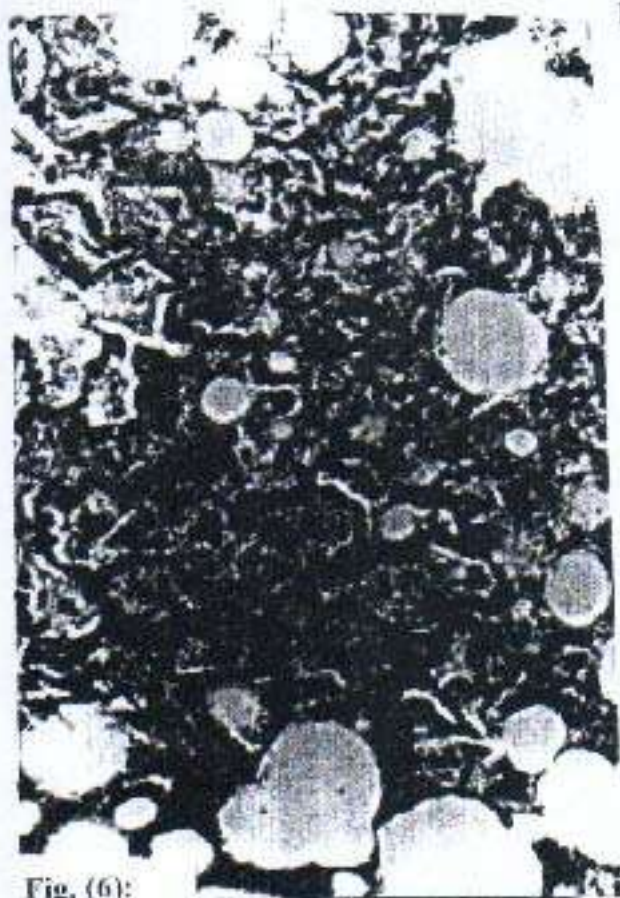


Fig. (6):

Fig. (6): Lung showing focal hemosiderosis X200

Fig. (7): Spleen showing lymphoid depletion of the white pulp X100

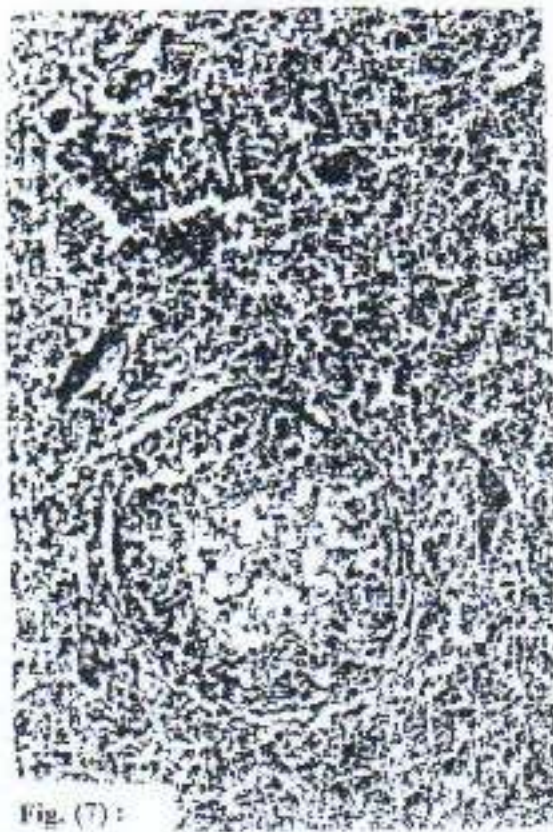


Fig. (7):



Fig.(8):

Fig.(8): Stomach showing necrosis of submucosa and inflammatory cellular infiltration X200

Fig. (9): Stomach showing desquamation of the mucosal epithelium with mononuclear inflammatory cellular infiltration X200



Fig. (9):

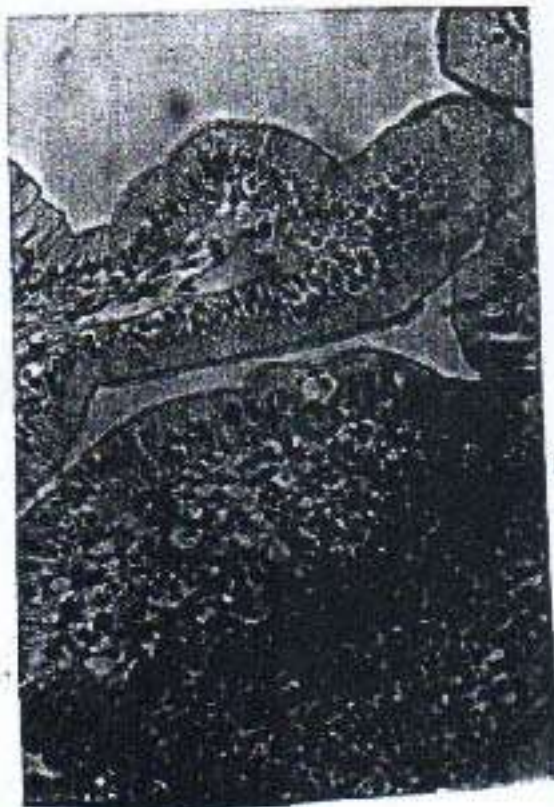


Fig.(10):Intestine showing lymphocytic cellular infiltration X 300.

Fig.(11):Brain showing perivascular hemorrhage X 200.

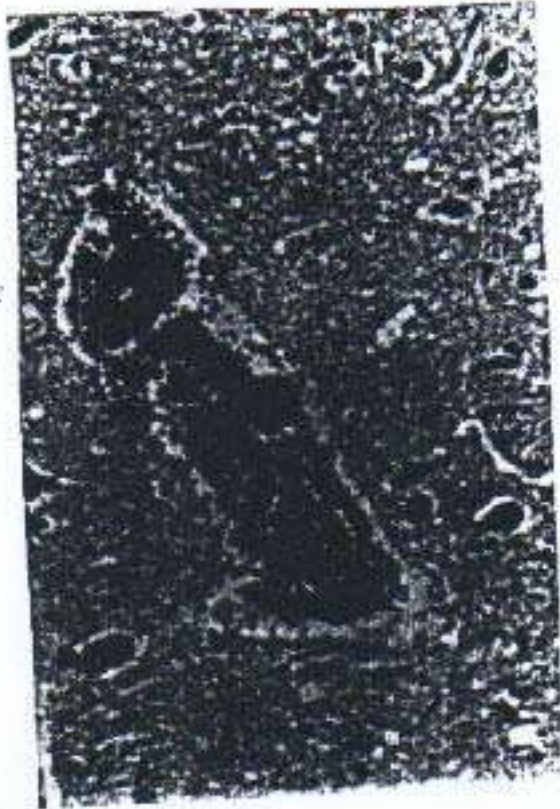


Fig.(12) : Brain showing focal inflammatory cellular aggregation mostly lymphocytes and macrophages X 200.

presence of focal areas of hemorrhage inside the submucosa. Moreover, mononuclear inflammatory cellular infiltrations of intestinal mucosa particularly lymphocytes was also recorded (Fig. 10). The examined brain showed severe congestion of blood vessels and capillaries with perivascular hemorrhages (Fig. 11). Endotheliosis and thrombosis of some meningeal blood vessels were seen. Perivascular and perineural edema with presence of multiple areas of encephalomalacia in the cerebral hemisphere were observed. Focal inflammatory cellular aggregation mostly lymphocytes and macrophages was found (Fig. 12). Moreover, multiple areas of neural degeneration, neurophagia and gliosis were also recorded.

DISCUSSION

Mycotoxins encompass a wide spectrum of different components and may effect many target organs and systems, notably the liver, kidney, the nervous system, the endocrine system and the immune system (28).

Since moldy forage are generally less palatable than normal forage, horses fed moldy forages typically refuse feed before ingesting enough feed to cause severe damage to intestinal tract. Mild colic is typically noted in such cases. Unfortunately, most molds associated with grains fed to horses don't readily affect palatability. Consequently, horses are most often exposed to the mycotoxins found in grains. Grain mycotoxins (as in the present study) are readily absorbed and should be considered to be potentially lethal for horses. (4).

Our study agreed with the previous suggestion (4) where horses in the equine farm under investigation fed large quantities of barley that was enough to succumb acute manifestations where affected horses exhibited severe colic, distention, predominant depression, slight swollen of supraorbital fossae, watery diarrhea with fetid odour, severe congestion of mucous membranes, excessive sweating, some animals were arched back with unpleasant odour of respiration, and other showed laminitis or hemiplegia. These clinical manifestations were nearly similar to those described by different authors (4; 5; 7; 10.) However, some animals fed contaminated barely didnt show signs of toxicity. In this respect subclinical disease was reported in which animals were exposed to moldy feed and only suffered a reduction in the productivity without overt clinical signs (29).

Table (1) shows the incidence and counting of some mycotoxin producing fungi from samples of barley collected from horses feed. All samples contained *Aspergillus flavus*; *Aspergillus parasiticus* that is known to produce aflatoxins and *Aspergillus Ochraceous* the producer of ochratoxins. Barley samples also contained *Fusarium* species which denoted the lowest colony count averaged (2X10 colony /gm) in comparison to other species of *Aspergillus*. However the percentage of contamination with the examined fungi reached to 100%. Similar multiconatmination were cited previously (10, 25, 30, 31, 32).

Table (2) revealed the presence of high concentrations of aflatoxins and ochratoxin A beside small quantities of other mycotoxin (Zearalenon and T-2 toxin). The highest concentrations was aflatoxin B1; ochratoxin A; aflatoxin B2 that recorded average concentrations of 269.0 ± 2.69; 141.0 ± 1.42 and 123.0 ± 1.23 ppb respectively. The total concentrations of aflatoxins was 398.0 ± 3.98 ppb and this was sufficient to induce acute aflatoxicosis in horses as previously recorded (5, 7, 9, 10.). The permissible limit of aflatoxin in animal feed should not exceed 25 ppb (32). However it was suggested that the maximum aflatoxin levels for mature, non-breeding horses should not exceed 50 ppb (4). We have got about eight fold higher concentrations in our study. Recovery of mycotoxins producing fungi (table1) and the mycotoxins (table 2) in contaminated barley was helpful in diagnosis the problem.

Further confirmation for the role of mycotoxins as etiological agents for the current problem is shown in table 3, the presence of high concentrations of aflatoxins (B1; B2; G1; G2; M1) and ochratoxin A were noticed in the intestinal content and some tissues (liver, kidney, muscles) of dead animals. High residues of aflatoxins B1 and B2 and ochratoxin A were recorded in liver kidney and muscle. Also aflatoxin M1 was recorded in these tissues, which probably is a metabolite of aflatoxin B1. Several works have been documented the occurrence of mycotoxin residues in soft tissues (16, 34, 35, 36, 37).

Regarding the evaluation of some immunological parameters of animals immune system a highly significant decrease in total leukocytic count with significant reduction in lymphocyte and neutrophil percentages were

recorded (table 4). Also there was suppression in phagocytes efficacy with 38.1 %. Serum protein fractionations showed significant decrease in alfa (α 1) and gamma globulin (table 5). These observations were coupled with previous findings (2, 38). The total RBcs count revealed highly significant decrease (table 4), similar findings was recorded in dairy cattle fed on aflatoxin contaminated ration (32).

Mycotoxins alter immune function when present in foods at levels below observable overt toxicity and impaired resistance to pathogenic organisms and may predispose animals to infectious diseases (35, 39, 40). Moreover alteration in gamma globulin was suggested to reflect a response of reticulo-endothelial system (19), also mycotoxins can cause immuno-suppression and appear to involve cellular immune phenomena and non specific humoral factors associated with immunity (41).

Bacteriological examination of fecal samples of diseased animals and intestinal content from dead ones (table 4) revealed presence of salmonellae, enterococci pseudomonas and bacillus species. The isolation of these agents might contributed to some sort of immunosuppression due to toxicosis but clinical advents of pathobiology of these infectious determinants might be confused or masked by clinical mycotoxicoses. Mycotoxins have a modulation effect on cell mediated immunity and alter gastrointestinal organisms population (42) and diagnosis is difficult due to non specificity of the lesions and masking of mycotoxic effects by secondary influences through immunosuppression or late appearance of the lesions (43). Moreover immunosuppression increases the animals susceptibility to various infectious agents and also interfere with the outcome of vaccination programs against infectious diseases (44).

Postmortem examination revealed generalized congestion with petechiae on the liver surface. Thickened pericardium and pleura with presence of pneumonic foci alternated with emphysematous areas on the lungs were also observed. Moreover, brain was congested and softer in consistency and showed multiple hemorrhagic foci. Similar findings were early cited (10, 25, 45).

The histopathological examination of different body organs revealed generalized congestion and hemorrhages which may be

considered to be a picture of acute mycotoxicoses. Lesions of equine mycotoxicoses depend on toxin levels and ranged from poor performance to general hemorrhagic syndrome (46).

Regarding to the microscopic hepatic lesions, degeneration and necrosis of hepatocytes were prevalent. Similar findings were previously observed (9, 47, 48, 49) in the liver of horses suffered from aflatoxicosis. Also examination showed an inflammatory cellular infiltration with biliary hyperplasia and fibrosis which are the characteristic lesions of mycotoxicoses. Equine acute aflatoxin poisoning should similar lesions (48, 50, 51). The kidneys showed nephritis evidenced by vacuolar degeneration and necrosis of renal tubules with inflammatory cellular infiltration. The diffuse renal fibrosis was the end result of ochratoxicosis in monogastric animals (51;52). The microscopic examination of the stomach revealed necrotic gastritis represented by destruction of the mucosa, inflammatory cellular infiltration with presence of focal areas of necrosis in submucosa. Nearly similar lesions were also reported due to ochratoxicosis (53).

Lesions in the brain; spleen; and heart were reported previously (47, 50, 53).

Concerning control of that problem, affected animals were segregated into a separate places, feeding on contaminated barely was stopped and replaced by good quality feed and symptomatic and supportive remedy was adopted.

Clinical improvement of affected animals was achieved in some diseased animals but others didn't respond well and die or still unthrifty for long period but the frequency of new clinical cases was ceased. In general, the control of aflatoxicosis in animals is still questionable and most of practical methods have been met limited success, also there was no specific antidote or detoxified agents available in field for controlling of these cases.

We conclude that, attention should be given for the occurrence of mycotoxins in animal feed and screening of mycotoxins in the newly introduced feed lot should be done by any of the available methods. The present study indicated that mycotoxins has a marked stress effect on the body immune system or causes some sort of immune alteration (immunosuppression) due to decrease number

and function of leukocytes permitting and enhancing secondary invaders. Hence, clinical or subclinical aflatoxicosis might interfere with immune response of vaccinated animals and these animals could achieved unprotective immune response post vaccination. Also, We recorded several pathological alterations due to mycotoxicoses especially in liver, kidney, spleen, stomach, intestine and lung with concomitant disturbances in functions of these organs and ultimately affect the body immunity. Finally, attention should be paid for careful surveillance of all stored feed evidence of mycotoxicoses and the suspicious or infested feed should be removed or discarded.

REFERENCES

- 1- *Pier, A. C. ; Richard, J. L. and Cysewski, S. J. (1980) : Implication mycotoxins in animal disease. J. Am. Vet. Med. Assoc., 176 (8) : 729-724.*
- 2- *Corrier, D.E.(1991) : Mycotoxicoses :mechanisms of immunosuppression. Vet Immunol Immunopathol., 30(1):73-87.*
- 3- *Hollinger, K. and Ekperigin, H.E. (1999) :Mycotoxicoses in food producing animals.Vet Clin North Amm Food Anim Pract., 15(1):133-165.*
- 4- *Mary, B.; Winston, M. ; Hagler, J.; Jeff, A. et al., (1999):Effect of mycotoxins on horse health and productivity. Internet at web site <http://gaston.ces.state.nc.us>*
- 5- *Asquith, R. and Edds, G. (1981): Investigations in equine aflatoxicosis Proceedings of the American Association of Equine Practitioners No.26 193*
- 6- *Angsubhakorn S.; Poomvises, P.; Romruen, K. et al., (1981) : Aflatoxicosis in horses.J Am Vet Med Assoc., 178 (3):274-278.*
- 7- *Poomvises, P.; Gesmankit, P. ; Inpanbutr, N. et al., (1982):Equine aflatoxicosis Proceedings of the scientific sessions (95-96).*
- 8- *Xie, Y.; Wang, Z. ; Qin, S. et al., (1991): Abortion in horses caused by aflatoxins Acta-Veterinaria et- Zootechnica - Sinica Vol. 22 (2) 145-149*
- 9- *Vesonder, R; Haliburton, J.; Stubblefield, R.; Gilmore, W. (1991): Aspergillus flavus and aflatoxins B1 , B2 and M1 in corn associated with equine death. Archives of Environmental Contamination and Toxicology Vol. 20 (1) 151-153.*
- 10- *Ragheb, R.; Maysa, H.; Shaker, K. ; and Rawia, K. (1995):Mycotoxicoses as a field problem in equine. Egyptian Journal of Comparative Pathology and Clinical Pathology Vol. 8 (2) , 39-57.*
- 11- *Kelly, W.R. (1974) : Veterinary clinical diagnosis.2nd Ed. Baillier Tindall, London.*
- 12- *Moussa, L.A., (1988): Effect of Gamma radiation on the biosynthesis of mycotoxins by certain microorganisms locally isolated from some medicinal plants.Ph.D. Thesis, Faculty of Science, Ain Shams University.*
- 13- *Samson, R.A. (1979) : A compilation of asperigilli described since 1965. Studies in mycology, No. 18 (P. 39).*
- 14- *Domsch, K.H.; Gams, W. and Andreson, T.H. (1980) Comperdium of soil funa (P 859). Academic press, New York*
- 15- *Mary, V. (1983): Method for determination of aflatoxin , ochratoxin A and zearalenone in mixed feed with detection by thin layer chromatography or high performance liquid chromatography. Proc. Int. Symp. Mycotoxins, 293-296.*
- 16- *Marley, E.; Nicol, W. and Candlish, A. (1995): Determination of ochratoxin A by immunoaffinity column clean-up and HPLC in wheat and pig liver. Mycotoxin Research, 11: 2 111*
- 17- *Roberts, B. and Patterson, D. (1975): Detection of twelve mycotoxins in mixed animal feed stuffs using a novel membranes clean up procedure .J. Association of Official Analytical Chemists. 58:1178.*
- 18- *Association of Official Analytical Chemists (1980) :Official methods of analysis of the association of official analytical chemists. 13th Edition.*
- 19- *Coles, E.H. (1986) : Veterinary clinical pathology. 4th Ed.W.B. Saunders. Comp. London.*
- 20- *Leibold, W. (1981): Cellulaere immunologie. Eine Praktische Einfuchrung. Hannover Veterinary School. West Germany.*
- 21- *Shalaby, M.; El- Sanousi, G.; El-Bagoury, A. (1992):Effect of Bovine virus diarrhea on the immune response of Egyptian buffalo calves Vet.Med.J., Vol. 40, No 3.*
- 22- *Ritzmann, S.E. and Daniels, J.C. (1979) : Diagnostic protecnology: In separation and characterization of proteins: Qualitative and quantitative assays in laboratory medicine.1st Ed. Herper and Row Inc., Hagerstown.*

- 23-Cruickshank, R.; Duguid, J.; Marminon, B. and Swain, R. (1975) : Medical microbiology. 12th Ed. Livingstone Limited, Edinburgh, London.
- 24-Drury, R. and Wallington, E. (1980): Carleton's Histological Technique 5th Ed. Oxford Univ. Press London, New York, Toronto
- 25-Radostits, O.M.; Blood, D.C and Gay, C.C (1994) : Veterinary medicine A textbook of the diseases of cattle, sheep, pigs, goats and horses. 8 th Ed. Baillier Tindall, England.
- 26-Kobluk, C.N.; Ames, T.R. and Geor, R.J. (1995) :The horse, diseases and clinical management. W.B.Saunders Comp, Philadelphia. London.
- 27-Snedecor, G. and Cochran, W. (1982) : 6th Edition, The Iowa State University Press, Ames, U.S.A.
- 28-Kuiper, G. (1995): Mycotoxins : risk assessment and legislation Toxicol lett Vol. Dec. 82 /853-859
- 29-Vanyi, A., et al. (1973) :Magy. Allator.Lap., 28 :303. Cited by Radostits, O.M.; Blood, D.C and Gay, C.C (1994) :Veterinary medicine A textbook of the diseases of cattle, sheep, pigs, goats and horses. 8 th Ed. Baillier Tindall, England
- 30-Jemmali, M. (1983) : Mycotoxins and food contamination. Arch Inst Pasteur Tunis., 60(3-4):381-392.
- 31- Abdel Hamid, A.M. (1990) : Occurrence of some mycotoxins (aflatoxin, ochratoxin A, Citrinin, Zearalenone and Vomitoxin) in various Egyptian feed. Arch Tierernahr., 40(7):647-664.
- 32-Toos, A.R. (1998) : Aflatoxicosis as a field problem in some cow farms in Kafer EL Sheikh province. Egypt. J. Agric. Res., 76 (1) : 303-311.
- 33-FDA. (1989) : Corn shipped in interstate commerce for use in animal feed: action levels for aflatoxin in animal feed. Fed. Reg. 54 :100 (No. 22622).
- 34-Micco, C.; Miraglia, A. ; Onori, R.; Ioppolo, A. et al., (1987) : Long term administration of low doses of mycotoxins in poultry. 1. Residues of ochratoxin A in broilers and laying hens. Poult. Sci. Jan. 66 (1) : 47
- 35-Selmi, A.; Abdel-Ghaffar, A.; and Abou Salem, M. (1996): Reproductive Performance, clinico-pathological findings and cytogenetic analysis in rabbits accidentally exposed to dietary aflatoxicosis. d Vet. Med. Cong. Zagazig. 8-10 october.
- 36-Elham, A. ; Mona, A. ; and Samia, M. (1997): Toxicity of aflatoxin and ochratoxin and their occurrence in feed and some animal products. Alex. J. Vet. Sci., Vol. 13 No. 3
- 37-Dimitri, R.; Gabal, M. and Saleh, N. (1998): Effect of aflatoxin ingestion in feed on body weight gain and tissue residues in rabbits Mycoses, 87
- 38-Pier, A.C. (1992) : Major biological consequences of aflatoxicosis in animal production. J Anim Sci., 70 (12): 3964-3967.
- 39-Sharma, R.P. (1993) : Immunotoxicity of mycotoxins. J. Dairy Sci., 76(3):892-897.
- 40-Pestka, J.J. and Bondy, G.S. (1990) : Alteration of immune function following dietary mycotoxin exposure. Can J Physiol Pharmacol., 68(7):1009-1016.
- 41-Smith, J.E.; Solomons, G.; Lewis, C. and Anderson, J.G. (1995) Role of mycotoxins in human and animal nutrition and health. Nat Toxins., 3(4):187-192.
- 42-Williams, P.P. (1989) : Effect of T-2 mycotoxin on gastrointestinal tissues : A review of in vivo and in vitro models. Arch Environ Contam Toxicol., 18(3):374-387.
- 43-Schiefer, H.B. (1990) : Mycotoxicoses of domestic animals and their diagnosis. Can J Physiol Pharmacol., 68(7):987-990.
- 44-Muneer, M.A.; Farah, I.O.; Nwman, J.A. and Goyal, S.M. (1988) : Review : Immunosuppression in animals. Br. Vet. J., 144 : 288-301
- 45-Hungerford, T.G. (1990) : Diseases of livestock. 9th Ed. McGraw-Hill Book Company Sydney, London.
- 46-Le Bars, J. and Le Bars, P. (1996) : Recent acute and subacute mycotoxicoses recognized in France. Vet Res., 27 (4-5):383-394.
- 47-Mc Gavin, D.; Knake, R. (1997): "Hepatic midzonal necrosis in a pig fed aflatoxin and a horse fed moldy hay. Veterinary Pathology Vol. 14 (2) 182-187.

- 48-Bortell, R.; Asquith, R.; Edds, G.; Simpson, C. (1983): Acute experimentally induced aflatoxicosis in the weanling pony. American Journal- of Veterinary Research 44 (11) 2110
- 49- Carlton, W. and M.C. Gavin, M. (1995) : Thamsons Special Veterinary Pathology 2nd Edition . Mosby, St. Louis, Boston, New York
- 50-Gabal, M.; Awad, Y.; Morcos, M.; Barakat, A. and Maïk, G. (1986): Fusariotoxicoses of farm animals and mycotoxic leucoencephalomalacia of the equine associated with the finding of trichothecenes in feed stuffs. Vet. Human Toxicol. June, 28 (3) ; 207- 212
- 51-Jones, T. and Hunt, R. (1983): Veterinary Pathology 5th ed. Lea&Febiger Philadelphia.

المخلص العربي

دراسة حقلية على التسمم الفطري في الخيول

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أجريت هذه الدراسة لإستكشاف أسباب وجود حالات أعراض تسمم فجائية مع حدوث وفيات في إحدى المزارع الكبرى المستخدمة في تربية الخيول العربية ودراسة تاريخ الحالات تبين أن هناك علاقة بين أعراض التسمم الفجائية واستخدام عليقة من الشعير في تغذية الخيول . تم أخذ عينات من الشعير وعزل بعض الفطريات المنتجة للسموم مثل بعض أنواع الأسراجيلس والفيوزريم . وتم قياس مستوى بعض السموم الفطرية مثل الأفلاتوكسين (أ ، ب ، ج ، ١ ، ٢ ، م ، ١) والأوكراتوكسين أ و الزيرالينون وثي ٢ توكسين في الشعير . وتم قياس مستوى بعض السموم الفطرية في أمسجة الحيوانات النافقة . أوضحت الدراسات المناعية انخفاض في مناعة الحيوانات المصابة حيث أنخفض مستوى الكرات الدموية الحمراء والبيضاء ، خصوصاً الخلايا الليمفاوية والنيتروفيل كما أنخفضت أيضاً معدلات الفا وانجاما الجلوبيولين مع انخفاض في النشاط الاكوتلي (الفاجوستيومي) لخليا الدم وبالفحص الباثولوجي وجد احتقان وانزفة وضمور مع تجمعات لخلايا إنتهاية في الكبد والكلى والمخ وأظهر الفحص البكتريولوجي لبراز الحيوانات المصابة ومحتويات أمعاء الحيوانات النافقة زيادة في ميكروبات السلمونيلا والميكروبات القلوانية والسيديموناس والباسيلاي وذلك بمقارنتها بالحيوانات السليمة .