Studies On Acute Mycotoxicosis In Turkey

Hassan, A. A*.[1]; Nahed, M.El Mokhtar[1] and Samira, A.M. Snousi [2]

Mycology Deptartment, [1] Animal Health Research Institute, Agriculture Research Centre, Dokki and [2] Veterinary Laboratory, El-Dakhla , El-Wadi -El-Gadid Governorate.
*atefhassan2000@yahoo.com

Abstract : The present study was undertaken to investigate the causes of Sudden death in a turkey farms at El-Wadi El-Gadid and Giza Governorates. The Post mortems examination of forty representative cases, showed enlargement and hemorrhages of liver, spleen, lung, kidney and muscles. *Aspergillus flavus, A.niger, A.funigatus and Penicillium citrinum*, were isolated in dominating frequency but *Fusarium sporotrichioides, F. tricinctum and F. oxysporum* were rarely isolated from these organs. The same fungi were recovered from houses of dead turkey (Feed, litter, air, underground water and dropping). From 122 of these strains isolated from houses of turkey, 110 were mycotoxigenic (90.1%), the ratio between the numbers of isolates screened for toxin production versus the number found to be positive is of interest. 85.3% of *Aspergillus flavus* produced aflatoxins, 80% of *A. ochraceus* produced ochratoxin A and 80% of *F. Sporotrichioides* and 100% of *F. tricinctum* and *F. oxysporum* produced T-2 toxins. The majority of mycotoxic fungi were detected in feed (90%) and 100% in litter which could be considered as the main sources of this toxicosis. On the other hand, the mycotoxins were detected in utilized feeds in significant levels. The mean levels of aflatoxins 35.0±1.5ppb were detected in 76.6% of examined wheat, 80.0±1.0 ppb of ochratoxins detected in 40% while 19.5±0.02 ppb of T-2 was recovered from 15% of examined wheat samples. Also, the higher significant mean levels were observed in utilized yellow corn in feeding of diseased turkey (42.5±2.6 ppb of aflatoxins and 51.0±0.01 ppb of ochratoxins). The same mycotoxins were also detected in sera of diseased live birds, 40% of turkey sera had a mean levels of 4.74±0.01 ppb aflatoxins, 20% had 1.200±0.03 ppb of ochratoxins and 50% of examined diseased turkey sera had a mean levels of 52.0±0.2 ppb T-2 toxins. The foregoing results gave a large probability that the feed, water, litters are the sources of these toxicosis. The biochemical parameters and electrophoresis patterns were significantly altered in diseased birds. The hygienic significance of fungal and mycotoxins pollution for turkeys health had been discussed.

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1. Introduction:

Great attention has been paid to the increased importance of fungi and their mycotoxins contamination in food and feed, which are serious for animal productivity. Toxicity to poultry by feed invaded with fungi had been previously recorded by several investigators (Hassan *et al*., 1997 and Hassan, 1998). Out of several flocks of fowl including turkeys affected with broader pneumonia, trachitis and air saculitis, *A. fumigates and A. flavus* were the most commonly isolated fungi from lesions but *Penicillium and Mucor* species were also isolated in rare frequency (Singh *et al*., 1993 and Hassan, 1998). In other cases, *F. oxysporum* isolated from feeds produced tibial dyschondroplasia and immune suppression in poultry (Chu *et al*., 1995 and Hassan *et al*., 2007; 2009 and 2010 a & b). On the other hand, mycotoxins are formed by certain fungal species, whenever environmental factors are conductive during the growth of these frequently occurring mycomycetes on foodstuffs and animal feeds; the process takes place as a secondary metabolism. The *Fusarium* mycotoxin inhibits cell division, RNA/ DNA synthesis and apoptosis (Rotter *et al*., 1996).Growth retardation and immune suppression are the major toxic effects induced by *Fusarium and aflatoxins* ingestion in farm animals (Edrington *et al*., 1997 and Widestrand *et al*., 2003). The mycotoxins resulted in severe growth depression and increased water consumption; and such toxins were detected in liver and kidney tissues and affect upon the growth rate and health of human being and animals including carcinogenic, tremorgenic, haemorrhagic, dermatitic, pulmonary edema, immunosuppressive and hormonal effects *(Hassan*, 1998 and 2003*;* Mogda *et al.,* 2003 and *Hassan et al.,* 2002*;* 2004; 2005; 2007; 2009 and 2010 a &b). Therefore, the goals of this paper were to investigate the turkey affection and their houses with fungal pollution and their toxins associated with cases of sudden death in turkeys. In addition detection the role of environment in such condition.

2. Materials and Methods:

Material:

Samples: Sudden death occurred in a turkey farms at El-Wadi El Gedid and Giza Governorates, from which 234 samples were collected as followed: feeds (98) (50 wheat and 48 yellow corn), litter (34), dropping (54), underground water (34) and air (14 samples).

Sudden dead turkey: Forty representative cases of suddenly died turkeys were collected for investigating their internal organs for fungal and their toxins contamination.

Other Turkey in farms: One hundred blood samples were collected from diseased and healthy turkeys in these farms (50 of each) for measurement of mycotoxins and biochemical parameters.

Standard of mycotoxins: Aflatoxins B1, B2, G1 and G2, Ochratoxin A and T-2 toxins, were purchased from Sigma Chem. Comp., U.S.A.

Methods:

Isolation and identification of moulds: The samples of feed, litters, underground water and internal organs of suddenly dead turkeys were subjected for isolation and identification of fungi as recommended by (Conner *et al.,* 1992).

Production of mycotoxins: The mycotoxins were produced using the isolated fungi from the present samples as recommended by Smith (1997) and D'Mello *et al*. (1997): A flasks, each containing 100 gm of finely ground corn and 40-50 ml of distilled water was mixed and autoclaved at 121°C for one hour. The flask was shaken to prevent cooking of yellow corn. It was inoculated with corresponding fungus for required mycotoxins and incubated for 4 weeks at 25-28°C. In case of *Fusarium* toxins production, the flasks were transferred to 8-10°C for additional 2 weeks. After end of incubation period, the corn was removed from flasks, dried, finely ground and 50 g of each was subjected for estimation using immune-affinity column of toxin as recommended by Hansen (1993).

Detection of mycotoxins: Detection of mycotoxins in serum of turkey and feed stuffs were applied by fluroumetric method as described by Hansen (1993).

Biochemical investigations: A blood sample was collected from each suspected case of turkey and taken without anticoagulant in centrifuge tube, allowed to clot, and then centrifuged at 3000 rpm for 10 minutes for separation of serum which used to assay the biochemical parameters. Serum analysis of turkey included estimation of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities according to Reitman and Frankel, (1957), serum urea according to Wybenga *et al.* (1971), total serum protein and albumin as described by Pesce and Kaplan (1978) and electrophoresis pattern according to O'Farrell (1975).

Statistical analysis: The obtained date were computerized and analyzed for significance. Calculation of standard error and variance according to (SPSS 14, 2006).

3. Results and Discussion

Avariety of conditions in poultry characterized primarily by sudden onset of increased mortality without obvious clinical signs (sudden death syndrome) had been described (Hassan, 1998). In this study, cases of sudden deaths in turkeys were investigated, when these cases were necropsy, the gross lesions were dominating in enlargement and hemorrhages of the main organs (liver, kidney, spleen, lungs, skeletal muscles and pancreas). Similar post-mortem lesions were seen by Reams *et al*. (1997) and Hassan (1998). Members of genus *Aspergillus* were isolated from almost all internal organs (dominating in lung, liver, kidney, gizzard and spleen) (Table, 1). *Aspergillus flavus, A.niger, A. funigatus* were isolated from lung and liver frequently together with *P. citrinum* (Table, 2). Similar findings were also reported by Iskander *et al*. (1993); Marquardt (1996) and Hassan (1998) who obtained these fungi from lung and liver in turkey cases of broader pneumonia. Also, species of *F. sporotrichioides, F. tricinctum and F. oxysporum* could be isolated from intestinal mucosa (Table, 2). The *Fusarium* species culture material was reported to cause hemorrhages and redness in breast muscle (Wu and Nagarj, 1994). From houses of these suddenly died turkeys, genera of *Aspergillus, Mucor, penicillium and Rhizopus* species were isolated from feed, litter, droppings, air and used underground water (Table,3). *Penicillium citrinum, F. sporotrichioides, F. tricinctum and F. oxysporum* were also isolated from some feed samples (Table, 4). Parallel findings were obtained by Dyar *et al*. (1984); Debay *et al*. (1995); Reams *et al*. (1997) and Hassan (1998) who detected a case of mycosis and mycotoxicosis associated with Sudden death in turkeys. Out of 118 isolated culture materials recovered from turkey houses, 106 were found to be mycotoxigenic (93.1%)(Table, 5). The ratio between the numbers of isolates screened for toxin production versus the number found to be positive is of interest; 85.3% of *Aspergillus flavus* produced aflatoxins, 80% of *A. ochraceus* produced ochratoxin A and 80% of *F. Sporotrichioides* and 100% of F. *tricinctum* and *F. oxysporum* produced T-2 toxins (Table, 5). The majority of mycotoxic fungi were detected in feed (90%) and (100%) litter which could be considered as the main sources of this toxicosis (Table, 5). On the other hand, the mycotoxins were detected in utilized

feeds in significant levels. The mean levels of aflatoxins (35.0±1.5ppb) were detected in 76.6% of examined wheat, 80.0±1.0 ppb of ochratoxins detected in 40% while 19.5±0.02ppb of T-2 was recovered from 15% of examined wheat samples. Also, the higher significant levels were observed in utilized yellow corn in feeding of diseased turkey, 42.5±2.6ppb of aflatoxins and 51.0±0.01ppb of ochratoxins (Table, 6). This is supported by the last awareness from use of litter as a nitrogen source for animal and poultry in diet, which makes the potential mycotoxins producing fungi in feed and litter of harmful significant effect (Kubena *et al*.,1995; Hassan, 1998 and Hassan *et al*., 2009 and 2010, a & b; Girish *et al*., 2008; Girish *et al*., 2010 and Prasath *et al*., 2010) were concluded that the feeding of grains naturally contaminated with *Fusarium* mycotoxins results in adverse effects on gut immunity and mucosal cell proliferation. Whereas, the T-2 toxin-induced apoptosis necrosis in the thymus and spleen of turkey poults. Also, they reported that the feeding of contaminated diet had no significant effects on the concentrations of neurotransmitters and metabolites in hypothalamus and cortex. They added that the consumption of grains naturally contaminated with Fusarium mycotoxins adversely altered the pons serotonergic system of turkeys and caused adverse effects on intestinal morphology during early growth phases of turkeys. On the other hand, the same mycotoxins were also detected in sera of diseased live birds, 40% of turkey sera had a mean levels of 4.74±0.01 ppb aflatoxins , 20% had 1.200±0.03 ppb of ochratoxins and 50% of examined diseased turkey sera had a mean levels of 52.0±0.2 ppb T-2 toxins. These results supported by the findings of (Hassan, 1998; 2009 and 2010 a &b) who detected these mycotoxins in sera of toxicated sheep, cattle and turkeys. The mycotoxins of greatest agricultural and public health significance include aflatoxins, ochratoxins, trichothecenes, fumonisins, zearalenone, and ergot alkaloids. The world health organization (WHO Technical Report series, 2002) had classified aflatoxin as a group 1 human and animal carcinogen; ochratoxins and fumonosins as group 2 possible human and animal carcinogens; and trichothecenes and zearalenone as non carcinogens (group 3). Trichothecenes are highly toxic to humans and animal and zearalenones are recognized endocrine disruptors (WHO Technical Report series, 2002).

The current results in table (8) showed significant decreased of functions of vital internal organs enzymes, where the serum albumin and total protein concentrations were decreased in suspected toxicated birds. Raju and Devegowda (2000) and Don and Kaysen (2004) reported lower serum albumin, and total protein concentrations in swine and they suggested that AF impaired protein synthesis. Concerning the effect of the infection on the serum liver enzyme activity, the present data revealed that there were a significant increase in AST and ALT, with a significant decrease in serum urea compared to healthy turkey this was reflected organ damage (Cheng *et al.*, 2001) and hepatic degeneration and subsequent leakage of enzymes into circulation. Chen *et al*. (2008); Wang *et al.*(2008) and Aravind *et al.* (2003) reported that mycotoxins particularly AF increase AST and ALT activities, this increase clearly indicated the liver damage. Moreover, in a subsequent study with laying hens, they found that feeding contaminated grains led to reduced hepatic fractional protein synthesis rates (Chowdhury and Smith, 2005).

Bilgiç *et al.* (1998) reported that chicks had petecial hemorrhages in liver and kidneys by feeding diets containing AFB1. This was in agreement with results obtained by *Celyk et al.* (1999); Eraslan *et al.* (2006) and Chowdhury and Smith (2007). They showed that the performance and plasma chemistry of turkeys were sensitive to the feeding of a diet containing a combination of mycotoxins arising from naturally contaminated grains. The results cleared the hepatotoxic effect of fungi and their toxins in the diseased turkey. In addition, fungi and mycotoxins has immunosuppressive effect inhibit nearly cellular and humeral immunologic reaction (Pestka *et al*., 2004)which including disruption of normal cell function by inhibiting RNA, DNA, and protein synthesis; inhibition of cell division; stimulation of ribotoxic stress response; and activation of mitogen-activated protein kinesis. It has been found that T-2 toxin is a potent member of the trichothecene group of mycotoxins produced by *Fusarium* fungi (Bamburg *et al*., 1970). T-2 toxin is a mycotoxin with immunomodulatory activity as it can stimulate (immunostimulation) or inhibit (immunosuppression) the activity of the immune system (Shinozuka *et al*., 1997and Pestka *et al*., 2004).

Results in table (9) showed the effects on the total protein and its electrophoretic pattern in diseased turkey serum levels in comparison to healthy birds. The reported impairment of protein synthesis due to mycotoxicosis was in agreement with that reported by Raju and Devegawda (2000); Aravind *et al.* (2003); Don and Kaysen (2004) and Eraslan *et al.* (2006).

Table (1): Mycoflora of internal organs of suddenly died turkeys

Genera of isolated fungi	Prevalence of fungi in organs													
	Lung		Liver		Kidney		Spleen		Heart		Intestine		Gizzard	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Aspergillus species	40	100	40	100	40	100	28	70	14	35	18	45	40	100
Penicillum species	40	100	14	35	-	-	14	35	14	35	-	-	14	35
Fusarium species	-	-	-	-	-	-	-	-	-	-	14	35	-	-
Scopulariopsis species	-	-	-	-	-	-	-	-	14	35	-	-	-	-
Mucor species	-	-	-	-	-	-	-	-	14	35	-	-	-	-
Cladosporium species	28	70	-	-	-	-	-	-	-	-	14	35	-	-
Candida species	-	-	14	35	14	35	-	-	-	-	14	35	-	-

No. = number of positive samples % = percent

Table (2): Members of Aspergillus (A) Penicillium (P) and Fusarium (F) species in internal organs of suddenly died turkeys:

Genera of isolated fungi	Prevalence of isolates in organs													
	Lung		Liver		Kidney		Spleen		Heart		Intestine		Gizzard	
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
A.flavus	40	100	28	70	28	70	14	35	14	35	14	35	14	35
A.niger	40	100	28	70	28	70	8	20	-	-	14	35	14	35
A.funigatus	28	70	-	-	-	-	-	-	-	-	-	-	28	70
A.terreus	-	-	-	-	-	-	-	-	-	-	-	-	14	35
A.candidus	-	-	14	35	14	35	-	-	-	-	14	35	14	35
P.citirinum	28	70	6	15	-	-	4	10	-	-	-	-	6	15
P.rubrum	-	-	-	-	-	-	-	-	6	15	-	-	4	10
p.verucosum	8	20	-	-	-	-	-	-	-	-	-	-	-	-
p.rugulosum	-	-	6	15	-	-	-	-	-	-	-	-	-	-
Unidentified p.	8	20	4	10	-	-	8	20	6	15	-	-	6	15
F.sporotrichioides	-	-	-	-	-	-	-	-	-	-	6	15	-	-
F.oxysporum	-	-	-	-	-	-	-	-	-	-	8	20	-	-
F.tricinctum	-		-	-	-	-	-	-	-	-	2	5	-	-

Table (3): Mycoflora of turkey house (feed, air litter, underground water and droppings)

Genera of isolated fungi	Prevalence of fungi in turkey house									
	Feed (98)		Liter (34)		Air (14)		Water (34)		Dropping (54)	
	No.	%	No.	%	No.	%	No.	%	No.	%
Aspergillus species	74	75.5	14	41.2	14	100	16	47	22	40.7
Penicillum species	44	44.9	4	11.7	10	71.4	8	23.5	18	33.3
Fusarium species	18	18.4	10	29.4	-	-	-	-	-	-
Mucor species	62	63.3	14	41.2	10	71.4	2	5.9	54	100
Rhizopus species	34	34.7	18	52.9	4	28.6	-	-	2	3.7
Cladosporium species	4	4	6	17.6	-	-	2	5.9	2	3.7
Scopulariopsis species	4	4	6	17.6	-	-	-	-	-	-
Candida species	2	2	6	17.6	-	-	10	29.4	4	7.4
Giotrichum species	-	-	2	5.9	-	-	-	-	-	-

Table (4): Individual isolates Aspergillus, Penicillum and Fusarium in turkey house.

Genera of isolated fungi	Prevalence of fungi in turkey house									
	Feed(98)		Liter(34)		Air(14)		Water(34)		Dropping(54)	
	NO	%	NO	%	NO	%	NO	%	NO	%
A.flavus	58	59.2	4	11.8	10	71.4	18	52.9	12	22.2
A.niger	18	18.4	10	29.4	10	71.4	12	35.3	8	14.8
A.fumigatus	2	2	-	-	4	28.6	2	5.9	4	7.4
A.candidus	18	18.4	-	-	4	28.6	2	5.9	8	14.8
A.ochraceus	8	8.2	2	5.9	4	28.6	2	5.9	2	3.7
A.ustus	2	2	-	-	-	-	-	-	-	-
A.glaucus	-	-	-	-	-	-	6	17.6	2	3.7
A.terreus	-	-	-	-	2	14.3	-	-	-	-
A.clavatus	-	-	-	-	-	-	-	-	2	3.7
P.citrinum	23	23.5	3	8.8	-	-	1	2.9	2	3.7
P.islandicum	2	2	-	-	-	-	-	-	-	-
P.rubrum	4	4	-	-	-	-	-	-	-	-
P.rugulosum	2	2	-	-	-	-	-	-	-	-
P.purpurogenum	-	-	1	2.9	-	-	-	-	2	3.7
Unidentified P	16	16.3	-	-	10	71.4	7	20.6	13	24.0
F.sporotrichioides	4	4	3	8.8	-	-	-	-	-	-
F.tricinctum	14	14.3	3	8.8	-	-	-	-	-	-
F.oxysporum	2	2	4	11.8	-	-	-	-	-	-

Table (5, a): prevalence of mycotoxigenic fungi in house of suddenly died turkeys.

Isolates tested for mycotoxins production										Total		Mycotoxin produced & mean level(ppb)± SE
Fungi	Feed		Litter		Air		Dropping		Total			
	Sc	+	SC	+	SC	+	SC	+	Screen	+	%	
A.flavus	40	40	8	8	6	2	4	4	58	54	85.3	Aflatoxins (12±0.1)
A.ochraceus	24	16	8	8	8	8	-	-	40	32	80	Ochratoxins (22±2.00)
F. tricinchtum	8	8	3	3	-	-	-	-	8	8	80	T2 toxins (18±0.02)
F.oxysportum	4	4	3	3	-	-	-	-	4	4	100	T2 toxins (16±.0.02)
F.sporotrichioides	4	4	4	4	-	-	-	-	8	8	100	T2 toxins (25±0.2)
Total	80	72	26	26	14	10	4	4	124	110	93.1	
Percentage	90		100		71.4		100		-	-	-	

T2 toxin = trichothecene Sc = Numbers of samples screened for mycotoxin + = Number of positive samples.

Table (5, b): prevalence of mycotoxigenic fungi in organs of suddenly died turkeys

Isolates tested for mycotoxins production										Total		Mycotoxin produced & mean level (ppb))± SE
Fungi	Liver		Lung		Spleen		Kidney		Total			
	Sc	+	SC	+	SC	+	SC	+	Screen	+	%	
A.flavus	8	8	4	4	8	4	4	-	24	16	66.6	Aflatoxins(9±0.3)
F. tricinchtum	-	-	-	-	2	-	-	-	2	-	0.0	T2 oxins(13±0.2)
Total	8	8	4	4	10	4	4	-	26	16	61.5	
	100		100		40		0		-	-	-	

T2 toxin = trichothecene Sc = Numbers of samples screened for mycotoxin + = Number of positive samples

Table (6): Prevalence of mycotoxins in turkey feeds (ppb)

Mycotoxins	Aflatoxins			Ochratoxin A			Trichocethene (T-2)		
Feed	+ve	%	Mean levels	+ve	%	Mean levels	+ve	%	Mean levels
Wheat (30)	23	76.6	35.0±1.5	12	40	80.0±1.0	5	16.6	19.5±0.02
Yellow corn (30)	25	83.3	42.5±2.6	11	36.6	51.0±0.01	00	00	00

Table (7): Determination of mycotxoins in serum of representative cases in diseased farm.

Turkey	Aflatoxins			Ochratoxin A			T-2		
	+ve cases	%	Mean levels ppb	+ve cases	%	Mean levels ppb	+ve cases	%	Mean levels ppb
Apparently healthy (50)	5	10	0.57±0.2	4	8	0.100±0.01	8	16	5.7±0.03
Diseased cases(50)	20	40	4.74±0.1	10	20	1.200±0.03	25	50	52.0±0.2

Ppb: Part per billion

Table (8): Some serum biochemical parameters in healthy and diseased turkey (M± SE).

Turkey	T.P.(g/l)	Albumin.(g/l)	AST(u/l)	ALT(u/l)	Urea(mg%)
Healthy	3.31±0.09	1.26±0.07	33.67±3.04	12.03±1.19	21.56±1.27
Diseased	2.63±0.17	0.91±0.09	61.96±2.56	25.87±2.24	11.24±0.91
F.Calculated	43.2#	150.64#	35.5#	60.5#	65.8#

Table (9): Serum protein electrophoresis pattern of in healthy and diseased turkey (M± SE).

Turkey	Alpha 1	Alpha 2a	Alpha 2b	Beta 1	Beta 2	Gama 1	Gama 2a	Gama 2b
Healthy	0.523 ± 0.016	0.41 ± 0.016	0.646 ± 0.018	1.217 ± 0.007	0.533 ± 0.006	1.2 ± 0.021	0.67 ± 0.019	0.353 ± 0.004
Diseased	0.544 ± 0.009	0.398 ± 0.008	0.54 ± 0.004	1.06 ± 0.01	0.50 ± 0.007	1.096 ± 0.012[a]	0.834 ± 0.014[a]	0.39 ± 0.027
F.Calculated	1.9	32.32#	31.07#	55.02#	39.49#	17.45#	41.21#	14.20#

Significant at $P < 0.05$ using ANOVA test

The presence of fungi and their toxins in feed, water and food reflected unhygienic measure during cultivation, irrigation harvesting transportation, handling, and storage and processing of feed and food. Therefore, frequent testing programs of food during different steps of production and routine cleaning of watering devices into which feed may be carried on birds is a precaution against mycotoxicosis which should not be overlooked before given to animals or human for consumption. The first aim of such this study was increasing the quality of human food and animal's wealth and the routine measurements of the toxin levels in foods and feeds should be carried out to prevent their harmful effects on health.

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