

MYCOSIS IN TURKEYS

By

ATEF A. HASSAN

Department of Mycology, Animal Health Research Institute, Dokki, Cairo

SUMMARY

Sudden death in a turkey farm in Alexandria governorate was investigated. The P. M. examination of twenty representative cases, showed enlargement and hemorrhages of liver, spleen, lung, kidney and muscles. *Aspergillus flavus*, *A. niger*, *A. fumigatus* and *Penicillium citrinum*, were isolated in dominating frequency but *Fusarium sporotrichioides*, *E. tricinatum* and *F. oxysporum* rarely isolated. The same fungi were isolated from houses with turkey deaths (feed, litter, air, water and droppings). From one hundred and three of these isolates eighty were mycotoxigenic (77.4%). *A. flavus* (74.4%), *A. ochraceus* (80.0%), *F. sporotrichioides*, *F. tricinatum* and *F. oxysporum* (100%) and *P. citrinum* (90%). The majority of these isolates were encountered in feeds and litter which give a large probability that feeds are source of these toxicosis. The hygienic significance of fungal pollution for turkeys has been discussed.

INTRODUCTION

The environmental pollution by fungi and

mycotoxins are recognized as public health problem of considerable importance. This is based mainly on its carcinogenicity to mammals. Toxicity to poultry by feed invaded by fungi has been recorded by several investigators (Meronuck et al., 1970; Hassan, 1994 and Hassan and Omran, 1996). Out of several flocks of fowl including turkeys affected with broader pneumonia, trachitis and air sacculitis, *A. fumigatus* and *A. flavus* were the most commonly isolated fungi from lesions but *Penicillium* and *Mucor* species were also isolated in rare frequency (Barton et al., 1992 and Singh et al., 1993). In other cases *F. oxysporum* isolated from feeds produced tibial dyschondroplasia and immuno suppression in poultry (Chu et al., 1995).

The mycotoxins which are produced by most of fungi in feeds, litter and water resulted in severe growth depression and increased water consumption; and such toxins were detected in liver and kidney tissues (Manning et al., 1985 and Debay et al., 1995).

The goat of this paper was to screen the affected turkey houses for fungal pollution with toxigenic isolats associated with sudden death in turkeys.

MATERIALS AND METHODS

Material:

1. **Samples:** Sudden death occurred in a turkey farm in Alexandria governorate, from which 234 samples were collected as follows: feeds (98), litter (34), droppings (54), water (34) and air (14 samples), and 20 representative cases of suddenly died turkeys for investigating their internal organs for fungal infection.

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

Methods:

1. Isolation and identification of fungi: for isolation of fungi methods of A. P. H. A. (1966) and Conner et al., (1992) were used, the isolates were briefly identified as reported by Wyllie and Morehouse (1977) and Pitt (1981).
- 2- Screening of isolated fungi for mycotoxin production. As recommended by Trenck et al. (1971) and Misilivec et al. (1975). The individual isolates of mould was grown on sabouraud's dextrose agar slant at 28°C for up to 7 days. Then spore suspension was prepared and inoculated into flasks containing 50 grams of natural substrates (rice seeds in case of *Penicillium* species and yellow corn in case of other isolates). The moisture content was raised to 100% and 50%, respectively and all flasks incubated at 28°C for 10-15 days before mycotoxins were extracted and analyzed (AOAC, 1975).

RESULTS AND DISCUSSION

A variety of conditions in poultry characterized primarily by sudden onset of increased mortality without premonitory signs (sudden death syndrome) have been described (Frank et al., 1991). In this study, cases of sudden in turkeys were investigated, when these cases were necropsid, the gross lesions were dominating in enlargement and hemorrhages of the main organs (liver, kidney, spleen, lungs, skeletal muscles and pancreas) (Table, 1). Similar post-mortem lesions were seen by Hammiton et al., (1972) and Reams et al., (1997). 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. fumigatus* were isolated from lung and liver frequently together with *P. citrinum* (Table, 3). Similar findings were also reported by Iskander et al., (1993); Singh et al., (1993) and Marquardt (1996) who obtained these fungi from lung and liver in cases of brooder pneumonia. Also, *E. sporotrichioides*, *F. tricinctum* and *F. oxysporum* could be isolated from intestinal mucosa (Table, 3). The fusarial culture material were reported to cause hemorrhages and redness in breast muscle by 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 water (Table, 2). (*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) and Reams et al., (1997) and produced mycosis as

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	20	100	20	100	20	100	14	70	7	35	9	45	20	100
Penicillium species	20	100	7	35	-	-	7	35	7	35	-	-	7	35
Fusarium species	-	-	-	-	-	-	-	-	-	-	7	35	-	-
Scopulariopsis species	-	-	-	-	-	-	-	-	7	35	-	-	-	-
Mucor species	-	-	-	-	-	-	-	-	7	35	-	-	-	-
Rhizopus species	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cladosporium species	14	70	-	-	-	-	-	-	-	-	7	35	-	-
Candida species	-	-	7	35	7	35	-	-	-	-	7	35	-	-

No. = number of positive samples

% = percent.

Table (2): Mycoflora of turkey house (feed, air litter, water and stool).

Genera of isolated fungi	Prevalence of fungi in turkey house													
	Feed		Litter		Air		Water		Droppings					
	No.	%	No.	%	No.	%	No.	%	No.	%				
Aspergillus species	74	75.5	14	41.2	14	100	16	47	22	40.7				
Penicillium species	44	44.9	4	11.7	10	71.4	8	23.5	18	33.3				
Fusarium species	18	18.4	-	-	-	-	-	-	-	-				
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				
Gioitrichum species	-	-	2	5.9	-	-	-	-	-	-				

Table (3): Members of Aspergillus (A) Penicillium (P) and Fusarium (F) species in internal organ 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	20	100	14	70	14	70	7	35	7	35	7	35	7	35				
A. niger	20	100	14	70	14	70	8	40	-	-	7	35	7	35				
A. fumigatus	14	70	-	-	-	-	-	-	-	-	-	-	14	70				
A. terreus	-	-	-	-	-	-	-	-	-	-	-	-	7	35				
A. candidus	-	-	7	35	7	35	-	-	-	-	7	35	7	35				
P. citrinum	14	70	3	15	-	-	2	10	-	-	-	-	3	15				
P. rubrum	-	-	-	-	-	-	-	-	3	15	-	-	2	10				
P. verucosum	4	20	-	-	-	-	-	-	-	-	-	-	-	-				
P. rugulosum	-	-	3	15	-	-	-	-	-	-	-	-	-	-				
Unidentified P.	4	20	2	10	-	-	4	20	4	20	-	-	3	15				
F. sporotrichioides	-	-	-	-	-	-	-	-	-	-	3	15	-	-				
F. oxysporum	-	-	-	-	-	-	-	-	-	-	4	20	-	-				
F. tricinum	-	-	-	-	-	-	-	-	-	-	1	5	-	-				

Table (4): Individual isolates (A), Penicillium (P) and Fusarium (F) in turkey houses.

Genera of isolated fungi	Prevalence of fungi in turkey house									
	Feed		Litter		Air		Water		Droppings	
	No.	%	No.	%	No.	%	No.	%	No.	%
A. Flavus	58	59.2	4	11.8	10	71.14	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	-	-	-	-	-	-	2	3.7
P. purpurogenum	-	-	1	2.9	-	-	-	-	13	24.0
Unidentified P.	16	16.3	-	-	10	71.4	7	20.6	-	-
F. sporotrichioides	4	4	-	-	-	-	-	-	-	-
F. tricinctum	14	14.3	-	-	-	-	-	-	-	-
F. oxysporum	2	2	-	-	-	-	-	-	-	-

Table (5): Prevalence of mycotoxigenic fungi in house and organs of suddenly died turkeys.

Isolates tested for mycotoxins production	Incidence of toxigenic isolates													Mycotoxin produced		
	Feed	Litter	Air	Droppings	Liver	Lung	spleen	Kidney	total							
	Sc	+	Sc	+	Sc	+	Sc	+	Sc	+	Sc	+	Screen	+	%	
A. flavus	20	20	4	4	3	1	2	2	4	2	2	2	47	35	74.4	Aflatoxins
A. ochraceus	12	8	4	4	4	4	-	-	-	-	-	-	20	16	80	ochratoxins
P. citrinum	12	12	2	2	-	-	2	1	1	2	2	2	21	19	90	citrinin
P. islandicum	1	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-
P. verrucosum	-	-	-	-	-	-	-	-	-	1	-	-	1	1	-	-
P. rugulosum	1	-	-	-	-	-	-	1	-	-	-	-	2	2	-	-
Unidentified P.	-	-	-	-	-	-	-	-	-	1	-	-	1	1	-	-
F. tricinum	4	4	-	-	-	-	-	-	-	-	-	-	4	4	100	T2 toxins
F. oxysporum	2	2	-	-	-	-	-	-	-	-	-	-	2	2	100	T2 toxins
F. sporotrichioides	2	2	2	2	-	-	-	-	-	-	-	-	4	4	100	T2 toxins
Total	60	48	12	12	7	5	4	3	6	4	5	4	7	4	80	77.6
Percentage	80	100	71.4	75.4	66.6	80	57.1	0	-	-	-	-	-	-	-	-

T2 toxin= trichatecene

- Other mycotoxins not reported due to lack of reference standards.

Sc= Numbers of samples screened for mycotoxin production.

+ = Number of positive samples.

well as sudden death in turkeys.

Out of 103 isolated culture materials, 80 were found to be mycotoxigenic (Table, 5). The ratio between the number of isolates screened for toxin production versus the number found to be positive is of interest, (74.4%) of *Aspergillus flavus* produced aflatoxins, 80% of *A. ochraceus* produced ochratoxin A, 100% *E. sporotrichioides*, *F. tricinctum* and *F. oxysporum* produced T-2 toxins and 90% of *P. citrinum* produced citrinin (Table, 5). Other isolates and other mycotoxins were not investigated due to lack of reference standards. The majority of mycotoxic fungi were detected in feed and litter which could be considered as the main source of these toxicosis (Table, 5). 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 mycotoxin producing fungi in feed and litter of harmful significant effect (Chang et al., 1992; Kathuria et al., 1993; Abarco et al., 1994; Kubena et al., 1995 and Adam, 1997). The liver is considered to be the primary target organ of active toxigenic fungi (Table, 5). These findings were also reported by Edrington et al., (1997).

In conclusion, the importance of feed as a source of toxogenic fungi should make it imperative that feed and feed handling equipment kept dry to prevent fungal growth and toxin formation. Routine cleaning of watering devices into which feed may be carried on birds beaks in a precaution against mycotoxicosis which should not be

overlooked.

REFERENCES

- Abarco, M. L.; Margulat, M. R.; Castella, G. and Cabances, F. J. (1994): "Mycoflora and aflatoxin producing strains on animal mixed feeds". *J. Food Protection*, 57 (3): 256.
- Adam, S. (1997): "Comparative studies on microbiological analysis of feeds for fungi." Ph.D. Thesis, Bact. Imm. And Mycology Dep., Fac. of Vet. Med., Cairo University.
- American Public Health Association (APHA) (1966): "Recommended methods of microbiological examination of food". New York.
- Association of Official Analytical Chemistry (1975): Page 462-469 in *Official Methods of Analysis*, 12th ed., Harwitz W. ed., AOAC, Washington, D.C.
- Barton, J. T.; Doft, B. M.; Read, D. H.; Kinde, H. and Bichford, A. A. (1992): "Tracheal aspergillosis in 5-6 week old chickens caused by *A. Flavus*". *Avian Diseases*, 36 (4): 1081-1085.
- Chang, Y. I.; Bidasee, K. and Rampersad, G. (1992): "Mycoflora and mycotoxin producing potential of fungi from poultry feeds and feed ingredients in Trinidad." *J. and Science of Food and agriculture*, 60 (3)L: 283-286.
- Chu, Q.; Wu, W. D.; Cook, M. E. and Smally, E. B. (1995): "Induction of tibial dyschondroplasia and suppression of cell mediated immunity in chickens by *F. oxysporum* grown on sterile corn." *Avian Dis. (USA)* Jan-Mar, 39 (1): 100-107.
- Conner, D. E.; Samson, R. A.; Hocking, A. D.; Pitt, J. I. and King, A. D. (1992): "Evaluation of methods for selective enumeration of fusarium species in foodstuffs, Modern methods in food mycology." *Development in Food Sci.*, 31: 299.

- Debay, M. C.; Trampel, D. N.; Richard, J. L.; Bundy, D. S.; Hoffman, L. J.; Meyer, V. M. and Cox., D. F. (1995): Effect of environmental variable in turkey confinement houses on air born *Aspergillus* and mycoflora composition, "Poult. Sci., 74 (3): 463-471.
- Dyar, P. M.; Fletcher, O. K. and Page, R. K. (1984): "Aspergillosis in turkeys associated with use of contaminated litter," Avian Dis., 28 (1) 250-255.
- Edrington, T. S.; Kubena, L. F.; Harvey, R. B. and Rottinghaus (1997): "Influence of a supRACTIVATED charcoal on the toxic effects of aflatoxin on T-2 toxin in growing broiler," Poult. Sci., 76: 1205-1211.
- Frank, R.; Newman, K. J. and Ruth, G. R. (1991): "Lesions of perirenal haemorrhage syndrome in growing turkeys," Avian Dis., 35: 523-534.
- Hassan, A. A. (1994): Detection and control of ochratoxin in food and foodstuffs," Ph. D. Thesis, Bact., Immu. And Mycology Dept., Fac. and Vet. Med., Cairo University.
- Hassan, A. A. and Omran R. M. A. (1996): "Seasonal variation in mycoflora and pathoogical changes due to ochratoxins." J. Egypt. Vet. Med. Ass., 56 (1) : 73-96.
- Hamilton, P. B.; Tung. H. T.; Harris, J. R.; Gainer, J. H. and Donalds (1972): "The effect of dietary fat in aflatoxocosis in turkey," Poult. Sci., 51:165-170.
- Iskander, T.; Ghalib, D.; Hostine, S. and Penyakit, H. (1993): "Pathological aspecis of lung aspergillosis in broiler chickens," Indonesia, 24 (43): 48-51.
- Kathuria, P. C.; Jand, S. K.; Singh, N. and Sandhu, K. S. (1993): "Toxicological screening of fungi and detection of aflatoxins from litter used for ruminant feed," Ind. J. and Animal Sci., 63 (8): 668-670.
- Kubena, L. F.; Edrington, S.; Kamps, C.; Harvey, R. B.; Eliscalde, M. H. and Hatingt, G. E. (1995): "Influence of FBI present in *F. monillform* culture material and T-2 toxin in turkey poults." Poult. Sci., 74: 306-313.
- Manning, R. Brown, T. P.; Wyatt, R. D. and Fletcher, O. J. (1985): "The individual and combined effects of citrinin and ochratoxin A. in broiler chicks." Avian Dis., 29 (4): 986-997.
- Marquardt, R. R. (1996): "Effect of molds and their toxin on livestock perfomance.: An. Feed Sci. and Tech., 58 (1-2): 77-89.
- Meronuck, R. A.; Gorren, K. H.; Nelson, G. H. and Fern Batees, B. S. (1970): "Effects on turkey poults and chicks of ration containing corn invaded by penicillium and fusarium species." Am. J. Vet. Res., 31:3.
- Misilivec, P. B.; Dicter, C. T. and Bruce, V. R. (1975): "Mycoloxin producing potential of mold flora of dried beans." App. Micro., Apr. p. 522-526.
- Pitt, J. I. (1981): "Food spoilage and biodete-rioration." New York, Academic Press, 2:111.
- Reams, R. Y.; Thacker, L. H.; Harrington, D. D.; Milton, N.; Novilla, G.; Rattinghaus, G.; Bennett, A. and Jeffery, H. (1997): "Sudden death syndrome induced in poults and chicks fed diets containing *F. fujikuroi* with known concentration of moniliformin." Avian Dis., 41: 20-35.
- Singh, N.; Grewal, G. S.; B. and Dhaliwal, B. (1993): "Outbreak of mycotoxic trachitis in domestic fowl." Mycosis, 36 (1-2): 65-67.
- Trenck, H. Mary, E.; Butz, E. and Chu, F. S. (1971): "Production of ochratoxins". in different cereals produced by *A. ochraceus*. App. Micob., 21 (6): 1032-1035.
- Wu, W.; Jermoe, D. and Nagarj, R. (1994): "Increased redness in turkey breast muscle induced by fusarial culture material." Poult. Sci. Sci., 73 (2): 331.
- Wyllie, T. D. and Morehouse, L. G. (1977): "Mycotoxic fungi, Mycotoxins and mycotoxicosis." Vol. I, Mycotoxic fungi and chemistry of mycotoxins." Marcel Dekker, New York.