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MYCOTIC INFECTIONS IN BIRDS AND RABBITS AND THEIR CONTROL

BY

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INTRODUCTION

Refai and Rieth (1966) described an outbreak of lung aspergillosis in turkey poults in a farm at Kafr-El-Sheikh with high mortalities. Aspergillus fumigatus was isolated from the caseated nodules in the lungs and air-sacs as well as from the walls of the egg incubators.

The improvement of the hygienic condition of the environment in the intensive poultry farms reduced the mortality drastically. In 1968; Bassiouni et al. proved experimentally the close relationship between haemorrhagic syndrome and the infection with A. flavus in poultry. El-Bahay et al. (1968) examined mycologically 56 samples of poultry feeds. They isolated 37 strains belonging to the genera Rhizopus, Aspergillus, Penicillium, Mucor Scopulariopsis and Paecilamyces.

Refai (1971) could isolated A. fumigatus, A. terreus, A. flavus, A. glaucus, A. niger, Paecilamyces, Fusarium and Stemphylium from dead egg embryoes and poulty feeds, and considered the feed to be the main source of infection. In 1974, Refai et al. succeeded in the isolation of *Mucor, A. niger, A. fumigatus* and *A. flavus* from the lungs, liver and heart of

hens, turkeys, ducks and rabbits and 30 feed stuff samples. In the same year, Saif noticed a problem of aspergillosis in Kafr-El-Sheikh, and reported a greater losses in turkeys. He could isolate *A. flavus*, *A. niger*, *Penicillium*, *Mucor* and *Alternaria* spp. from different organs of dead turkey poults, and he reported that *A. flavus* was the most common isolate.

Saleh (1976) recovered *A. fumigatus*, *A. niger*, *A. flavus*, *A. nidulans* from the respiratory and digestive tract of fowls.

Abou-Gabal et al. (1977) were able to isolate yeast and yeast like fungi included *Torulopsis*, *Rhodotorula Candida*, *Geatrichum* and *Cryptococus* spp. from the mouth, oesophogus, crop and small and large intestine of fowl using the swab method. Also, *A. flavus*, *A. miger*, *A. terreus*, *A. fumigatus* and *Penicillium* spp. were isolated.

Fungal hyphae were found in granulomatus nodules of the lungs, air-sacs, trachea, heart, liver and skeletal muscles. With regard to control of moulds, El-Bahay et al. (1968) used Isol. 1%, Tego 51 2%, Antigerm 50 5% and copper sulphate 8% as disinfectants against *A. flavus*, *A. fumigatus* and *A. niger*.

Refai (1971) applied Tego 51 as disinfectant in brooder rooms and obtained very satisfactory results with 1% solution especially when it was applied twice. The first application eliminated the moulds found on the walls, floors and feeders and the second eliminated the moulds in the air of the rooms.

Saif (1976) proved that thiobendazole and nyslatin had fungicidal effect on *A. flavus* culture, while cupper sulphate had no such effect. Thiobendazole and nystatin dipping solution prevented *A. flavus* infection of eggs during incubation.

Saif and Refai (1977) used thiobendazole for the control of moulds in poultry farm, having a history of high losses. They used the spray method as aerosol containing 19 mg/cubic meter once daily for 2 weeks.

The aim of the present work was to survey mycotic infections among birds and rabbits and to study the efficiency of antimycotic treatments.

MATERIAL AND METHODS

1. Isolation and identification of fungi:

a. Sampling:

Dead and ailing chicks, turkeys, ducks, pigeons, pheasants, parrots and rabbits of different breeds and sources, with a previous history of respiratory or nervous manifestations as well as ration and litter samples were collected from different poultry farms (Table 1).

b. Direct microscopic examination:

Tissue specimens from lungs, air-sacs, trachea and nodular lesions of ailing and dead birds and rabbits were examined directly microscopically using 15% potassium, hydroxide solution and lacto-phenol stain, for the presence of fungal elements.

c. Cultural examination:

All samples were aseptically inoculated into Sabouraud's glucese agar plates and incubated at 30°C for 10 days. Suspected fungal growth was subcultured onto sabouraud's glucos agar slants in order to obtain pure cultures and kept for further identification.

Suspected Aspergillus colonies were cultured onto Czapek's solution agar (3%) and incubated at 30°C

for 7 days, then examined microscopically.

2. In-vitro study of antifungal drugs against Aspergillus species:

Gentian violet, iodine solution, thiobenzole, formalin, vrown, FAM, antic, methylene blue, copper sulphate, diazinone, potassium permanganate, aflagin were tested in various dilutions against *A. fumigatus*, *A. flavus* and *A. niger*. The drugs were added to the molten Sabouraud dextrose agar (at 50°C) in plates, then the plates were inoculated with the fungi. (See Table 2 for the concentration of the drugs).

3. In-vivo study on the effect of antifungal drugs in experimentally infected chickens.

Three hundreds, one-day old Hubbard chickens were grouped into 16 groups in 2 rooms, 8 groups in each room. The first 7 groups in both rooms were infected intranasally with $1x10^6$ spores of either *A. fumigatus* or *A. flavus*. The remaining group in each room was kept as a non-infected control. The first 6 groups were treated either immediately after infection or 4 days thereafter with thiobenzol (60 mg/L in drinking water for 5 days), gentain violet (250 mg/L) or iodine solution (0.6 gm/L). The groups no. 7 and 8 were left without treatment. The infected chickens were observed twice daily torecord their general health condition and to notice their clinical signs. Post-mortem examination was performed on chickens which died after infection to record any nacked macroscopecal lesions of the internal organs and Mycological reisolation was attenpted. 10 days post - infection, the surviving chicks were slaug htered and examined.

4. Efficiency of antifungal drugs on feeds and litter:

Aflagin (150 mg). gentian violet (25 mg) and thiobenzole (6 mg) were added to flasks containing 100 g of previously autoclaved feeds and which were infected

with *A. funigatus*, *A. flavus*, or *A. niger*. All flacsks were cultured daily for 7 days for the growth of fungi.

The same was done on litter infected with the 3 species of fungi using Iodine (0.5%), formalin (0.3%), Crown solution (1.5%), FAM (1.5%) and Antic (1.5%).

RESULTS

1. Incidence of Aspergillus species in birds, feeds and litter:

As shown in Table 1, the highest rate of infection was reported in turkey poults (64%) and pigeons (37%). *A. fumigatus* was the most common in chickens, ducklings, pheasants and rabbits while *A. flavus* was common in turkeys. *A. niger* showed lower incidence in all birds.

In feeds, A niger was the most common, followed by *A. fumigatus* and *A. flavus*, where as in litter, *A. fumigatus* was the most common followed by *A. niger* then *A. flavus*.

The most obvious clinical signs observed among naturally infected birds were inappetence, emaciation, respiratory manifestation, inflamtion of eyes, nervous signs including torticollis, paralysis and incoordination, and the post-mortem pictures revealed caseous nodules as well as abscesses in the lungs and air-sacs. The trachea and bronchi were filled with mucous discharge, inflammatory lesions in the brain and meninges and lesions in the liver together with multiple nodular lesions throughout the body cavity detected. The microscopic examination revealed local proliferative pneumonia, microscopic granulomas were distributed in the hyperaemic and consolidated lung parenchyma, in addition, pleuritis and airsaculitis

Table 1: Frequency of isolation of fungi from the internal organs of poultry ration and litter.

Species	Total examined	total negative	Positive			
			Total %	A. fumigatus	A.flavus	A.niger
Baby chick	600	435 72.5%	165 27.5%	72 44%/12%	54 33%/ 9%	39 23%/ 7%
Adult chicken	115	92 80%	23 20%	12 52%/10%	7 30%/ 6%	4 17%/ 3%
Turkey poults	125	45 36%	80 64%	22 28%/18%	46 58%/37%	12 15%/ 8%
Adult turkey	22	16 33%	6 27%	2 33%/ 9%	4 67%/18%	- 0.0%
Ducklings	25	19 76%	6 24%	4 67%/16%	1 17%/ 4%	1 17%/ 4%
Pigeons	38	24 63%	14 37%	9 64%/24%	2 14%/ 5%	3 21%/ 8%
Pheasants	8	6 75%	2 25%	2 100%/25%	- 0.0%	- 0.0%
Parrots	2	1 50%	1 50%	1 100%/50%	- 0.0%	- 0.0%
Rabbits	46	33 72%	13 28%	6 46%/13%	3 33%/ 7%	4 31%/ 9%
Feeds	30	18 60%	12 40%	2 17%/ 7%	2 17%/ 7%	8 66%/27%
Litter	20	84%	12 60%	7 58%/35%	1 8%/ 5%	4 33%/20%
Total	1031	695 67%	325 31%	139 42%/13%	120 37%/12%	75 23%/ 7%

% / % = Calculated to total positive / calculated to total samples.

Table 2: Effective concentration % (causing complete inhibition) of various antifungal drugs on Aspergillus spp.

Antifungal	A. fumigatus	A. flavus	A. niger
1. Gentian violet	0.05	0.05	0.5
2. Iodine solution	0.125	0.125	0.125
3. Methylene blue	0.5	ineffective (0.5)	ineffective (0.5)
4. Thiobenzole	0.004	0.002	0.006
5. Formaline sol.	0.312	0.312	0.312
6. Crown solution	1.25	1.25	1.25
7. FAM solution	1.25	1.25	1.25
8. Antic solution	0.625	1.25	2.5
9. Copper sulphate	ineffective (0.1)	ineffective (0.1)	ineffective (0.1)
10. Potassium permenganate	ineffective (0.5)	ineffective (0.5)	ineffective (0.5)
11. Diazinone	ineffective (0.5)	ineffective (0.5)	ineffective (0.5)

were evident, colonies of fungal hypae could be demonstrated in sections. Lymphocytosis were evident (perivascular and peribronchial lymphocytosis) extravasation of erythrocytes.

2. In-vitro study of antifungal drugs:

From Table 2, it is clear that 7 out of the 11 tested drugs could cause inhibition at various concentrations of the three *Aspergillus species*. On the whole, *A. niger* was more resistant than the other two species. Copper sulphate in 0.1%, potassium permenganate and diazinone solution in 0.5% were not effective. In 0.5%, methylene blue could inhibit only *A. fumigatus*. It is also clear that thiobenzole was effective at very high dilution.

3. In-vivo study on the antifungal drugs:

Table (3) demonstrates that thiobenzole was the best drug where all treated chicks survived whether the treatment was immediate or 4 days after infection. In the control group 30-35% of chicks died between the 5th and 9th day post infection.

4. Efficiency of antifungal drugs on the fungi in feeds:

From Table (4), it is evident that the addition of alfagin to the feeds inhibited the growth of the 3 fungicomplete inhibition of *A. fumigatus* and *A. flavus* was achieved on the 6th day, whereas *A. niger* could grow up to the 8th day. Gentian violet and thiobenzole had little effect when they were mixed with the feeds.

5. Efficiency of the antifungal drugs on fungi in the litter:

The best results were obtained following application of iodine (0.5%) and formalin (0.3%). In both cases

Table 3: Efficieney of antifungal drugs on experimentally infected chicks.

	A. fumigatus		A. flavus	
	Total	mortality	total	mortality
1. Immediate treatment				
Thibenzole	0.0	0.0	0.0	0.0
Gentian violet	1.0	5.0	1.0	5.0
Iodine	2.0	10.0	1.0	5.0
2. Treat. on the 4th day				
Thibenzole	1.0	5.0	2.0	10.0
Gentian violet	3.0	15.0	3.0	10.0
Iodine	4.0	20.0	4.0	20.0
3. Control:				
Infected, untreated	7.0	35.0	6.0	30.0
Uninfected, untreated	0.0	0.0	0.0	0.0

Table 4: The efficiency of antifungal drugs on the fungi in feeds.

Drugs	Aspergillus species	Date 3rd	4th	5th	6th	7th	8th	9th day
1) Aflagin	*A.fumigatus*	+++	++	+	-	-	-	-
	A. flavus	+++	+++	+	-	-	-	-
	A. niger	+++	+++	++	++	+	+	-
2) Gentian violet	*A.fumigatus*	+++	+++	++	++	++	+	-
	A. flavus	+++	+++	++	++	+	-	-
	A. niger	+++	+++	+++	+++	++	++	++
3) Thibenzole	*A.fumigatus*	+++	+++	+++	++	++	+	-
	A. flavus	+++	+++	++	+	+	-	-
	A. niger	+++	+++	+++	+++	+++	+++	+++
control without drugs	*A.fumigatus*	+++	+++	+++	+++	+++	+++	+++
	A. flavus	+++	+++	+++	+++	+++	+++	+++
	A. niger	+++	+++	+++	+++	+++	+++	+++
control neither infected nor drugs	-	Neg	Neg	Neg	Neg	Neg	Neg	Neg

* 3rd day of infection (the day before adding the drugs).

+++ = Heavy growth

++ = Moderate growth

+ = Slight growth

- = No growth

no fungal growth was observed on the 4th day onwards. FAM (5%) and antic (1.5%) were similarly effective against *A. fumigatus* and to a lesser extent against *A. flavus*, whereas the growth of *A. niger* continued though in a reduced form.

DISCUSSION

The present study demonstrates clearly the role of aspergillus species in causing considerable losses in poultry. This role has been already emphasized by Refai and Rieth (1966). The isolation of the causative *Aspergillus species* from feeds and litter in this work and from the air of egg incubators by Refai and Rieth (1966) substantiate the importance of considering the environment and feeds as sources of infection. *A. fumigatus* is well known as aetiological agent of respiratory infections in poultry (Refai and Rieth, 1966, refai, 1971, Saif, 1976, Abou-Gabal et al. 1977, El-Batrawi, 1980 and Ibrahim, 1983). On the other hand, *A. flavus* which is primarily known to produce aflatoxins that causes aflatoxicoses was isolated from caseated nodules in the lungs, i.e. causing infection particularly in turkeys. this result strengthen the data mentioned by Saif (1976). It is worthy to mention that El-Badry (1979) reported a high incidence of *A. fumigatus* in turkeys. *A. niger* which is frequent in the environment in Egypt (Youssef and Refai, 1986) could be also isolated from lesions in infected birds. The isolation was also confirmed by crushing of the nodules and detection of the fungal hyphae microscopically and by histopathological examination of sections prepared from the affected organs.

The testing of various drugs available indicates the efficiency of thiobenzole in the treatment of experimentally infected. This has been reported for the first time in Egypt by Saif and Refai (1977) who also reported the drastic reduction of fungal load in the environment of poultry farms.

Table 5: The efficiency of antifungal drugs on the fungi in the litter.

Drugs	Aspergillus species	Date 3rd	4th	5th	6th	7th	8th	9th day
1) Iodine 0.5%	*A.fumigatus*	+++	-	-	-	-	-	-
	A. flavua	+++	-	-	-	-	-	-
	A. niger	+++	-	-	-	-	-	-
2) Formalin 0.3%	*A.fumigatus*	+++	-	-	-	-	-	-
	A. flavus	+++	-	-	-	-	-	-
	A. niger	+++	-	-	-	-	-	-
3) Crown 1.5%	*A.fumigatus*	+++	++	+	-	-	-	-
	A. flavus	+++	++	+	-	-	-	-
	A. niger	+++	+++	+	+	-	-	-
4) FAM 1.5%	*A.fumigatus*	+++	-	-	-	-	-	-
	A. flavus	+++	+	-	-	-	-	-
	A. niger	+++	+	+	+	+	+	+
5) Antic 1.5%	*A.fumigatus*	+++	-	-	-	-	-	-
	A. flavus	+++	+	-	-	-	-	-
	A. niger	+++	+	+	+	+	+	+
Control without drugs	*A.fumigatus*	+++	+++	+++	+++	+++	+++	+++
	A. flavus	+++	+++	+++	+++	+++	+++	+++
	A. niger	+++	+++	+++	+++	+++	+++	+++
Control neither infected nordrugs		Neg	Neg	Neg	Neg	Neg	Neg	Neg

* 3rd day of infection (the day before adding the drugs).

On the other hand, iodine and formalin were the best in disinfecting the litter. Formaline is however cheaper and is commonly used as universal disinfectant.

As mould inhibitor in the feeds aflagin appears to be the best. This was expected as this drug contains propionic acid which is known to inhibit mould growth (Refai, 1988). Moreover, it contains vermiculite which is the carrier for the propionic acid which releases the propionic acid that inhibits the mould growth' and at the same time adsorbs the toxins thus reducing their deliterious effect.

SUMMARY

A total of 956 samples dead and diseased chicks, turkeys, ducks pigeons, pheasants, parrots and rabbits as well as 30 samples of feeds and 20 samples of litter were examined mycologically. Aspergillus fumigatus was the most common in chickens, ducklings, pheasants and rabbits while *A. flavus* was common in turkeys. Of the 11 tested antifungal drugs, thiobenzole was the most effective in inhibiting the growth of the examined *Aspergillus species*. It also showed high efficiency in the treatment of experimentally infected chickens. In feeds, alfagin had the best effect and in the litter both iodine and formalin killed the fungi within 3 days after application.

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