معمل بحوث صحة الحيوان ـ قنـا ٠
معهد بحوث صحة الحيوان ـ الدقي٠
رئيس المعمل: د٠ أبو المجد محمود محمد٠

فطريات تجمعات الدواجن بمحافظة قنـا

عبدالفتاح البدري ، ابراهيم سكر*

١٠٣٨ عينة أخذت من أعضاء الجهاز الهضمي والتنفسي لعدد ٣٥٢ فراخة ميتـة جمعت من المزارع المختلفة بقنا وفحصت فطريـا ٠

وعلى الجانب الآخر عزلت ٦٠ عترة اسبرجلس ، ٣٣ عترة عفن اخرى بالاضافة الـى ٤ عترات خمائر من العلائق ومياه الشرب وقشر البيض والمفرخات وعنابر التربية مـن تلك المزارع المختلفة بقنـا ٠

اجريت الدراسة المرضية ببعض العترات الفطرية المعزولة على كتاكيت فيومـي عمر ٣ يوم وقد سجلت الاعراض المرضية ونسبة النافق والاعراض التشريحية لتـلك الكتاكيت المعديـة ٠

وقد درست تأثير بعض العقاقير على بعض الفطريات المعزولة معمليا ، وثبـت أن عصارة نبات الثوم ودواء الثيوبنزول أكثر تأثيرا من دواء الميكوستاتين ومركـب كبريتات النحـاس٠

---

*: قسم أمراض الدواجن ـ كلية الطب البيطري ـ جامعة أسيوط٠

Animal Health Research Lab., Kena.
Head of Lab. Dr. M.M. Aboul-Maged.

MYCOTIC FLORA OF CHICKEN POPULATION IN KENA GOVERNORATE

(Wit 4 Tables)

By
A.A. EL-BADRY and I.M. SOKKAR*

(Received at 1/6/1987)

SUMMARY

A total of 1038 samples were taken from the digestive and respiratory organs of 352 dead chickens collected from different farms in Kena. They were examined for mycotic flora, 228 Aspergillus species (A.fumigatus, A.flavus, A.niger) as well as 306 other moulds belonging to Mucor, Rhizopus, Paecilomyces, Penicillium and Sporotrichum were recovered. In addition to a 23 yeast species (Rhodotrula and Sacchromyces) were isolated from the examined organs. On the other hand 60 Aspergillus species A. fumigatus, A.flavus, A.niger and A. flavipes) and 33 other mould species (Mucor, Rhizopus, Sporotrichum and Paecilomyces), together with 4 yeast species belonging to Geotrichum and Rhodotrula were isolated from feed, drinking water, egg-shell, hatcharies and brooder rooms. Pathogenicity of some isolates were studied in 3 days-old Fayoumi chicks, clinical signs, mortality and P.M. lesions were recorded. The effect of drugs on some isolates was studied in vitro. Garlic and thiobenzole were more effective than mycostatin and copper sulphate.

INTRODUCTION

In the last few years, the poultry population in Kena Governorate has increased considerably. Mycotic diseases of poultry caused high economic losses particularly when associated with other infections. However, pathogenic fungi has been isolated by many workers from chickens and the surrounding environments. In Egypt (REFAI et al., 1966, 1968, 1971 and 1976 isolated several species of fungi from poultry farms). SAIF, 1976 reported great losses of turkeys due to A.flavus, SAIF et al., 1977 found that A. fumigatus was common in chicken farms. ABOU-GABAL et al., 1977 studied the incidence of pathogenic fungi in poultry. EL-BADRI, 1979 isolated a number of Aspergillus species from turkeys. EL-BATRAWI, 1980 isolated C.albicans from the crop of chickens. EL-BADRI, 1983 isolated C.albicans and other mould species from chicken farms in Kena. IBRAHIM et al., 1983 were able to isolate Aspergillus species, Penicillium spp. and C.albicans from lungs, crops and intestinal samples from a broiler flock in Assiut.

The incidence of mycotic infection in the wide population of chicken farms in Kena called for further studies attempting to the :

1- Isolation and identification of the mycotic flora from chickens and their surrounding environment.
2- Study the pathogenicity of some isolated fungi to susceptible chicks.
3- Study the effect of some drugs on the growth of some isoalted fungi in vitro.

* : Dept. of Poultry Diseases, Fac. of Vet. Med., Assiut University.

MATERIAL and METHODS

Samples were taken from the esophagus, proventriculus, trachea and lungs, as well as dead in shell chicken embryos (135 adult and baby Fayoumi chicks, 60 Fayoumi dead embryo, 90 adult and baby L.S.L. chicks, 30 adult high-six, 25 adult Matrooh and 12 baby Dokki 4 chicks). In addition, 20 ration samples, 20 drinking water samples, 120 egg-shell samples, 20 samples of the atmosphere of brooder rooms and 20 samples of hatcheries atmosphere were all examined for fungi.

Media : Sabouraud's agar "tubes".
- Czapeks agar "plates".
- Corn-meal agar (BACKERSPIGEL, 1954).
- Sugar assimilation media (BISPIN, 1961).
- Raw - egg white (BUCKLEY and VAN UDEN, 1963).
- Normal saline containing 250 mg streptomycin and 250 mg chloramphenicol/Liter.

Drugs for sensitivity test :

- Copper sulphate	(El- Nasr).
- Thiobenzole	(M.S.D.)
- Mycostatine	(Memphes)
- Garlic juice	(Plant).

Birds for experimental infection :

50 Fayoumi chicks, 3 days-old were subjected-d to experimental infection.

Mycological isolation and identification was conduted by direct swabs from digestive and respiratory organs. Swabs were streaked on slope Sab. agar containing 250 mg. streptomycin and 250 mg. chloramphenicol/liter. Egg-shell and ration samples were suspended in normal saline to which streptomycin and chloramphernicol were added for 2 hours on 37°C., a loop-full was inculated on slope Sab. agar. Plates of Czapeks agar were exposed in the atmosphere of brooder rooms and hatcharies for 2 hours. All the cultured media were incubated at 37°C for 7 days before recording the result. Fungal Growth was identified morphologically and physiologicaly.

Pathogenicity study :

Subcultures from A.fumigatus, A.flavus, A.niger and Paecilomyces spp. were made on Czapeks agar plates and incubated at 37°C for 7 days. Spore suspensions were made by adding 10 ml. distelled water containing 0.1 ml glycerol as a wetting to the cultures. Fifty apparently healthy 3-day-old Fayoumi chicks were obtained from Saedi Abdel Rheem poultry farm. The chicks were divided into Five groups each of 10 birds. They were treated as follows:
- Brids of group "I" were injected with 0.25 ml. A.fumigatus spores/bird via heart.
- Brids of group "II" were inoculated by the same dose and route using A.flavus spores.
- Birds of group "III" were given the same dose and route using A.niger spores.
- Birds of group "IV" were injected with Paecilomyces spores by the same dose and route.
- Birds of group "V" were injected by the same dose and route using normal saline. All chicks were kept under observation for 3 weeks. Clinical signs, mortality, and P.M. lesions were recorded. Reisolation of the injected fungi were carried out from organs showing gross P.M. lesion.

The effect of some drugs on some isolated fungi :

Known concentration of drugs were dissolved in Sab. agar at 60°C. Garlic juice was mixed with the media in serial conc. (0.1, 0.2 and 0.4 ml. 10 ml. media) as well as the tested fungal spores were mixed in serial dilutions of garlic juice (0.1, 0.2 and 0.4 ml./10 ml. dist water for 2 hours before inoculation on Sab. agar media, the inoculated plates were incubated for 7 days at 37°C. the control plates of fungi without drugs were also incubated.

RESULTS

Results of the isolation and identification as well as the distribution of the different organisms are summarized and presented in tables I & II.

Pathogenicity test :

The daily mortalities as well as the total deaths appear in Table III.

It is worth stating that depression, diarrhoea and gasping started to occur after 2 days in birds inoculated with A. fumigatus. Those receiving A. flavus had closed eyes, ruffled faethers, paralyses of legs, twesting of head and neck followed by mortalities after 3 days and there after (see Table III). Group 4 infected with paecilomyces appeared sleepy and depressed after 2 days post infection followed by mortalities. Neither symptoms nor mortalities occured in group III or V. Post mortem changes in dead or killed birds showed airsaculitis, pneumonia, necrotic foci in lungs and heart as well as distension of the gall bladder. Yellowish necrotic foci occured in the liver and brain of birds inoculated with A. flavus.

In vitro trials to determine the sensitivity of some isolated fungi to some antifungal drugs are shown in table IV.

DISCUSSION

Isolation of fungi from the upper digestive and respiratory tracts of dead birds without P.M. lesions indicates that some of fungal flora may be picked up from the surroundings and harboured by the fowls without causing any apparent ill-effects. In this investigation, Rhizopus (24%), Mucor (19%), Paecilomyces (0.07%), Penicillium (0.03%), Sporotrichum (0.009%) as well as A.niger (18%), A.fumigatus (13%) and A.flavus (10%) in addition to Rhodotorula (0.02%) Sacchromyces (0.018%) were isolated from the respiratory and digestive tracts of the dead birds without P.M. lesions. It was found that the different fungi isolated from dead birds were also isolated from feed, drinking water, hatcharies, egg-shell and brooder-rooms. thus it appeared that the surrounding play a role in being a source of infection and the problem of poultry mycosis is mainly hygienic. This idea is supported by REFAI and RIETH, 1966. Our isolates are similar to those isolated by JORDAN, 1954; CHUTE et al., 1956; RAJAN, 1965; SHARMA et al., 1971 JAND et al., 1973. In Egypt REFAI et al., 1976; SAIF et al., 1979; and IBRAHIM et al., 1983. On the other hand Sporotrichum, Rhodotorula and Sacchromyces spp. were not isoalted by any of the above mentioned authors.

Mould species isolated from poultry feed, drinking water, hatcharies, egg-shell and brooder rooms were A.niger (24%), A.flavus (14%), A.flavipes (0.02), Mucor (22%), Rhizopus (0.08%), Sporotrichum (0.02%) and Paecilomyces (0.02%), in addition to Geotrichum spp. (0.01%) and Rhodotrula spp. (0.03). The present results may agree with those discribed by CARLL et al., 1955; CHUTE et al., 1964; NIKOLEAV, 1965; REFAI et al., 1968; REFAI, 1971; JAND et al., 1973 and SAIF, 1979. Sporotrichum and A.flavipes appeared to be isolated from the chicken envirnoment in Egypt for the first time. In the pathogenicity studies, only A.niger

was found to be without clinical signs or mortalities during the observation period. P.M. lesions in all injected birds were similar, while in chicks infected with A.flavus, yellowish necrotic hard nodules appeared on the liver and yellowish necrotic focci occured on the brain. The results of this experiment are similar to the results recorded by CHUTE and O'MEARA, 1958; MITRIOU et al., 1962; RAJAN et al., 1965; MATUKA, 1968; SINGH and HALHOTRA,1974; NAFADY 1978; El-BATRAWI, 1980 and IBRAHIM et al., 1983. The pathogenicity of Paecilomyces on 3 days old chicks in Kena was described for the first time by the authors. Cultured growth of the tested fungi could be inhibited in vitro by thiobenzole 5 mg./ml. and Garlic juice 0.2 and 0.4 ml./10 ml. media. Copper sulphate 30 mg/ml. media was of moderate effect on the tested fungi. Mycostatine 100 i.u./ml. media had no fungicide or fungistatic effect. Similar results to some extent were reported by TARLATZIS et al.,1957;DEVOS et al.,1967;STANKUSHEV and DUPARIREVA, 1971; SAIF, 1967; and SAIF & REFAI, 1977. Our results disagree with those recorded by STEWART et al., 1977 and IBRAHIM et al., 1983. The effect of the Garlic juice on the tested fungi in this study is considered to the best of our Knowledge, the first record in Egypt. From the obtained results of this work, it could be concluded that the fungal flora of chickens vary considerably both in species and in the amount in which they were present in the digestive and respiratory tracts although not causing disease. They may be considerd as a stress factors affecting the hatchability, growth and development of birds. Hygienic conditios of the flock played an important role in complication of this fungal infections. Also, it can be concluded that Garlic is an efficient drug which can be used successfully in controlling mycotic infection.

REFERENCES

Abou-Gabal, M.; Enab, S.M.; El-Agroudi, M.A. (1977): Studies on the incidence of pathogenic fungi in poultry. J. Egypt. Vet. Med. Ass. 36 (1), 90-102.

Bakerspigel, A. (1954): A perferred method for the routine identification of Candida. Jour. Inf. Dis., 94, 141-143.

Bisping, W. (1961): Zbl. Vet. Med. 10: 22.

Buckely, H.R. and Van Unden, N. (1963): Identification of C.albicans within two hours by the use of an Egg-white slide preparation. Sab. 2: 205.

Carll, W.T. Forgace, J.; Herring, A.S. and Mahlandt, B.G. (1955): Toxicology of A.fumigatus subistrates to animals. Vet. Med. 50, 210.

Chuted, H.L. and Barden, E. (1964): The fungus flora of chicks hatcharies. Avian Dis, 8, 13.

Chute, H.L. and O'Meara, O.C. (1958): Experimental fungus infections in chickens. Avian Dis.2,154.

Chute, H.L.; D.C.O'Meara, H.D. Tresner and E. La. Combe, (1956): The fungus flora of chickens with infections of the repiratory tract. Am. J. Vet. Res. 17: 763.

Devos, A. Hooreus, J. Viaene, N.; Spanoghe, L. and Van Impe, J. (1967): Aspergillosis of the repeiratory system and brain of turkey poults. Vlaams. Diergeneesk Tijdschr., 36, 1, 16.

El-Badri, A.A. (1979): A study of mycotic diseases in turkeys. Thesis, Assiut Univ., Fac. of Vet. Med.

El-Badri, A.A. (1983): Chicken Candidiasis in Upper Egypt. Thesis, Assiut Univ., Fac. of Vet.Med.

El-Batrawi, A.M. (1980): Studies on infections in poultry with particular reference to A.fumigatus infections in intensive poultry farms. Thesis, Cairo Univ., Fac. Vet. Med.

Ibrahim, A.A.; Atia, M.A. Shahata, M.A. and Mousa, S. (1983): Some studies on fungi isolated from broiler flock in Assiut. Ass. Vet. Med. Jour. 10 (20): 173-177.

Jand, S.K. and Dhillon, S.S. (1973): Fungi associated with respiratory diseases of poultry. Indian Vet. J. 50: 211.

Jordan, F.T.W. (1954): Incidence of fungi in th lungs of poultry. Br. Vet. J. 100, 25.

Matuka, O.; Aganovic, N. and Forsek. S. (1968): Veterinaria, Saray, 17, 167.

Mitroiu, P. Jivoin, P. and Sirbu, Z. (1962): Inst. Cero. Vet. Bioprep. Pasteur, 1, 473.

Nafady, A.A. (1978): Pathological studies on Aspergillosis in poultry. Thesis, M.V.Sc, Fac. Vet. Med. Assiut Univ.

Nikoleav, K. (1965): The species distribution of Aspergillus on poultry farm. Vet. Med. Nauki, Sof. 2, 209.

Rajan, A. Seshadri, S.J. Chandrosekharan, Nair, K.P. and Rai, B.N. (1965): Avian Aspergillosis-Studies on natural cases and experimental transmission. Indian Vet. J. 42, 390.

Refai, M. (1971): On the incidence of moulds in the poultry industry and determination of pathogenicity and disinfection trials. Proceeding of Mycological Congress Frankfort.

Refai, M.; El-Bahy, G.M. (1968): Incidence of Moulds in poultry feed in Egypt. Mykosen 11,954.

Refai, M.; El-Bahy, G.M. and Mostata, F.M. (1976): Investigations on the role of moulds in poultry industry. Jour. Egypt. Vet. Med. Assoc. 35 (3): 66-76.

Refai, M. and Rieth, H. (1966): Epidemic occurrence of pulmonary aspergillosis in Turkey hen chick in Egypt. MyKosen, 9 (4): 163-165.

Saif, A.A. (1976): Studies on respiratory infections with particular reference to Aspergillosis in turkeys in Egypt. Thesis presented to Fac. Vet. Med. Cairo Univ.

Saif, A.; Abou-Khier (1979): Studies on the Epidemiology of Aspergillosis in poultry and poultry-men. J. Egypt. Vet. Med. Assoc. 39 (3): 5-19.

Saif, A. and Refai, A. (1977): The use of thiobendazol to countrol moulds in poultry forms. Castellania, 5 (9): 185-187.

Sharma, V.D.; Sethi., M.S. and Negi, S.K. (1971): Fungal flora of the respiratory tract of fowls. Poultry Sci., 50 (4) 1041-1044.

Singh, D.; Halhotra, R.C. (1974): Experimental studies on pathology and pathogenesis of Aspergillosis in chicks. Indian, J. of poultry Sci. 9 (1): 64.

Staukushev, Kh. and Duparinova, M. (1971): Disinfection of straw (for use as poultry litter) contaminated with Aspergillus. Vet. Sbir., Sof., 68, 4 pp. 11-13 (B).

Stewart, R.G.; Wyatt, R.D. and Ashmore, M.D. (1977): The effect of various antifumgal agents on aflatoxin production and growith characteristics A.parasiticus and A.flavus in liquid medium. Poult. Sci. 56 (5): 1630-1635.

Tarlatzis, C.B.; Panetses, A.G. and Dragenas. P.N. (1957): The effect of some antibiotics and chemical drugs on the growth invitro of A.Fumigatus. Amer. J. Vet. Res. 18, 214-215.

Table (1): Incidence & Distribution of fungi in organs of different breeds of chicken.

Sources of samples	breed, state & ages	Organs	No. of birds	+ve isolates	%	Species of isolated fungi									
						A. fumigatus	A. flavus	A. niger	Rhizopus	Mucor	Paecilo-myces	Sporotr-ichum	Penicill-ium	Rhodoto rula	Sacchro-myces
Saedi-Abdel Rheem poultry farm.	fayoumi dead adult.	Oesophagus	45	33	73.3	5	4	–	10	10	–	2	–	2	–
		provent		22	48.9	–	7	6	6	3	–	–	–	–	–
		trachea		35	77.8	2	9	10	2	5	7	–	–	–	–
		lung.		20	44.4	1	7	4	–	5	3	–	–	–	–
	fayoumi dead b.chick.	Trachea lung.	90	50	55.5	13	–	–	8	25	4	–	–	–	–
				47	52.2	20	–	3	2	17	5	–	–	–	–
	fayoumi dead embryo.	Oral cavity lung.	60	20	33.3	5	–	2	–	13	–	–	–	–	–
				15	25.0	3	–	7	–	–	5	–	–	–	–
	L.S.L dead adult	Oesophagus	15	14	93.3	–	2	3	7	–	–	–	1	1	–
		trachea		7	46.7	–	3	2	2	–	–	–	–	–	–
		lung.		10	66.6	8	2	–	–	–	–	–	–	–	–
	Hig-six dead adult	Oesophagus	30	28	93.3	–	1	6	1	4	3	–	2	9	2
		trachea		22	73.3	–	4	12	1	5	–	–	–	–	–
		lung.		17	56.7	–	2	8	–	–	2	–	5	–	–
	Matrooh dead adult	Oesophagus	25	22	88.0	4	–	2	10	–	–	2	1	–	–
		trachea		18	72.0	3	8	5	2	–	–	–	–	–	–
		lung.		20	80.0	7	7	3	3	–	–	–	–	–	–
Egg-producti-poultry-farm.	L.S.L dead bab. chick.	Oesophagus	75	40	53.3	–	–	–	25	6	3	–	4	–	2
		provent		55	73.3	–	–	–	35	7	5	1	3	1	3
		trachea		20	26.7	–	–	15	2	3	–	–	–	–	–
		lung.		15	20.0	–	1	6	1	4	3	–	–	–	–
Special-chicken farm.	Dokk-4 dead bab. chick.	Oesophagus	12	11	91.7	–	–	–	8	–	–	–	–	–	3
		provent		8	66.7	–	–	1	5	2	–	–	–	–	–
		trachea		5	41.7	–	–	3	1	–	–	–	1	–	–
		lung.		6	50.0	–	–	2	3	–	–	–	1	–	–
			352	560		71	57	100	134	109	40	5	10	13	10

Table (II): Isolated fungi from different poultry environments.

Kind of sample	No. of cultures	+ve isolates	%	The species and number of isolates									
				A. fumig-atus	A. flavus	A. niger	A. flavi-pes	Mucor	Rhiz-opus	Sporo-trichum	Paecil-omyces	Rhod-ottula	geotr-ichum
Feed	20	14	70.0	2	-	5	-	5	2	-	-	-	-
Drinking water	20	7	35.0	-	3	-	-	3	-	-	-	-	1
Egg-shell	120	50	41.7	12	10	15	-	10	-	-	-	3	-
Hatcharies	20	17	85.0	5	1	2	-	-	6	2	1	-	-
Brooder room	20	9	45.0	2	-	1	2	3	-	-	1	-	-
	200	97	48.5	21	14	23	2	21	8	2	2	3	1

Table (III): Experimental infection of baby chicks with the different isolates.

Group No.	Injected fungi	No.of infected bird	Daily deaths																				Total deaths	Mortality rate
			1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th	17th	18th	19th	20th	21st	
I	A.fumigatus	10	3*	-	2	-	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	80%
II	A.flavus	10	-	-	3	3	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	8	80%
III	A.niger	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IV	Paecilomyces	10	-	-	1	-	4	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	70%
V	Saline	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* deaths due to sudden shock.

Table (IV): Effect of different drugs on some of the isolated fungi.

Sources of fungi	Species of fungi	Chemical drugs			Garlic juice		
		Copp.sulphat 40mg/ml	Thiobenzole 5mg/ml	Mycostatin 100 i.u./ml	0.1ml/ 10ml.	o.2ml./ 10ml.	0.4ml./ 10ml.
Hatchar	A.fumigatus	+	++	-	++	++	++
Egg-shell	A.flavus	+	++	-	++	++	++
High-six-fowl	A.niger	+	++	-	-	++	++
L.S.L.fowl	Paecilomyces	+	++	+	++	++	++
Brooder room	Sporotrichum	+	++	-	++	++	++

(++) Complete inhibition of growth.

(+) Tew growth of fungi.

(-) No effect of the drug on fungus growth.