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PREVALENCE AND ANTIFUNGIOGRAPHY OF ASPERGILLOSIS IN BROILERS

(With 4 Tables & 4 Figs.)

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انتشار الاصابة بمرض الاسبرجلوزيس
في بداري التسمين وتأثير المضادات الفطرية على العترات المعزولة

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فحص عدد ٣٥٧ قطيع ـ يحتوى على ٣٧٢٨٠٧ طائر ـ لاستكشاف الاصابة بفطر الاسبرجيلس ، حيث ثبتت الاصابة في عدد ٣٧ قطيع ـ يحتوى على ٣٧٢٧٥ طائر ـ بنسبة اصابة ٩٫٨٪ واختلفت شدة الاصابة تبعا للعمر وفصول السنة ونظم التغذية والرعاية . ومن كل العينات الايجابية فقد تم عزل فطر الاسبرجليس فيوميجاتس A. Fumigatus من الرئة (١٠٠٪) والأجنة النافقة (٨٧٪) والأكياس الهوائية (٦٨٪) والغشاء البريتوني (٣٧٪) والعلائق (٩٠٪) والفرشة (٧١٪) ووجد أن ١٥٦ من بين ١٧٩ عترة من الفطر المعزول أظهرت حساسية عالية لكبريتات النحاس في حين أن ١٥٤ عترة كانت حساسة للجنشيان فيوليت (Gentian violete) وعدد ١٥٠ عترة حساسة لحامض البروبيونيك (Propionic acid).

SUMMARY

Among 357 diseased flocks (372807 birds) aspergillosis was recorded in 35 (37275 birds) indicating an overall prevalence of 9.8 per cent. Prevalence of aspergillosis varied in various age groups, seasons of the year, in birds fed on different feeds and kept under different managemental conditions. From all the postive samples only Aspergillus fumigatus was isolated from lungs (100%), dead in shells (87%), air sacs (68%), peritoneum (37%), feed (90%) and litter (71%). Sabouraud's dextrose agar was found to be the best media for isolation studies. Among 179 isolates 156 were highly sensitive to copper sulphate, 154 to gentian violet and 150 to propionic acid.

INTRODUCTION

Avian aspergillosis occurs in almost all parts of the world and is usually caused by Aspergillus fumigatus (GEMEINHARDT and IPPEN, 1982). Aspergillus flavus and

Aspergillus nidulans very rarely found to have aspergillosis. Aspergillus fumigatus is highly pathogenic and frequently causes pulmonary infections in poultry birds (SHOYINKA and ONYEKWEODIRI, 1987). The organism is widely distributed in nature and found on damp grains, food stuff and litter leading to contamination of incubators and brooders. HASTIONO (1979) examined 187 chicks during 6 years of routine mycological examination. He found 110 cases positive for aspergillosis. SIDDIQUE, et al. (1987) reported 1.2 per cent prevalence of aspergillosis in commercial poultry in Pakistan.

Birds of almost all species may be affected commonly by inhalation of fungal spores from mouldy litter or feed. Aspergillus spores reach the lungs by inhalation and germinate there leading to obstruction of bronchi and alveoli (SHOYINKA and ONYEKWEODIRI, 1987). The disease may be eggborne and infection of hatchery origin is seen in day old chicks, either by egg contamination or through contamination at hatching time (IVETIC, et al. 1984).

SAIF and REFAI (1977), found that thiabendazole inhibited the growth of Aspergillus fumigatus. ZIGER (1971) reported fungistatic activity on Aspergillus fumigatus of nystatin, postassium iodide and thiabendozole in vitro.

The investigations were initiated to record the prevalence of aspergillosis in young chickens, isolation efficacy of various selective media and response to different antifungals.

MATERIAL and METHODS

A total of 357 broiler farms in and around Rawalpindi, Pakistan were surveyed and the birds showing respiratory distress were selected to study the prevalence of aspergillosis. History of the flocks including breed, age, feeding pattern and managemental conditions were recorded. Various organs including lungs, peritoneum and air sacs were collected from affected birds. Feed, dead in shells and litter samples were also obtained to isolate Aspergillus fumigatus. Morbid tissues were examined under microscope by potassium hydroxide method (O'MEARA and WITTER, 1971). Macro and microscopically positive samples were confirmed for the presence of Aspergillus fumigatus by inoculating on Sabouraud's dextrose agar (Difco), Malt extract agar (B.B.L), Czapek's dox agar (oxoid). Plates were incubated for 2 to 5 days at 30°C. Identification, characteristic growth and morphology of fungus was studied by single spore isolation technique, tease mount technique with lactophenol cotton blue dye, scotch tape technique and microslide culture technique (KONEMANN and FANN, 1971). Drug sensitivity of each isolate of Aspergillus fumigatus was performed by disc method (TAMARA, et al. 1989).

RESULTS

Are presented in tables (1-4) and figures (1-4).

DISCUSSION

During the period of one year from February, 1988 to January, 1989, a total of 357 broiler flocks (372807 birds) upto 5 weeks of age were surveyed for the prevalence of aspergillosis. Among these, 35 flocks (37275 birds) suffered from aspergillosis (Table I). An overall prevalence of aspergillosis was recorded 9.8 per cent. Maximum (14.04%) prevalence was recorded in 3 week old chicks, the minimum (8.16%) being in the 1st week of age BHATTACHARYYA, et al. (1984) also recorded maximum preponderence of aspergillosis during 2 to 5 weeks of age. Low rate of prevalence in 1st week of age might be due to the incubation period of fungus, while presence of disease in first week can not be excluded due to eggborne infection.

Table (I)
Prevalence of aspergillosis in broiler chickens of different age groups

Age (weeks)	Number Examined		Number Affected		Prevalence (%)	
	Flocks	Birds	Flocks	Birds	Flocks	Birds
1.	64	88267	4	7211	6.25	8.16
2.	63	72412	6	6793	9.52	9.38
3.	65	44821	10	6295	15.38	14.04
4.	78	67254	8	7387	10.25	11.72
5.	87	100053	7	9089	8.05	9.08
Total:	357	372807	35	37275	9.8	9.99

Environmental factors play an important role in the epidemiology of aspergillosis. Maximum (12.14%) prevalence was recorded in Autumn followed by Summer (11.34%), Winter (8.86%) and the minimum (6.59%) in Spring. Maximum incidence of aspergillosis might be due to favourable humidity and temprature as well as lack of attention paid during brooding and faulty storage of grains. Management has a pivotal effect on the incidence of various infectious diseases. Prevalence of aspergillosis decreases

with the improvement of managemental conditions (Table II).

Table (II)
Prevalence of aspergillosis under various managemental conditions

Managemental conditions	Number Examined		Number Affected		Prevalence (%)	
	Flocks	Birds	Flocks	Birds	Flocks	Birds
Poor	168	175430	21 (60%)	22365	12.50	12.74
Satisfactory	127	132620	13 (37%)	13845	10.23	10.43
Good	30	31340	1 (2.8%)	1065	3.33	3.39
Excellent	32	33417	-	-	0.00	0.00
Total	357	372307	35	37275	9.80	9.99

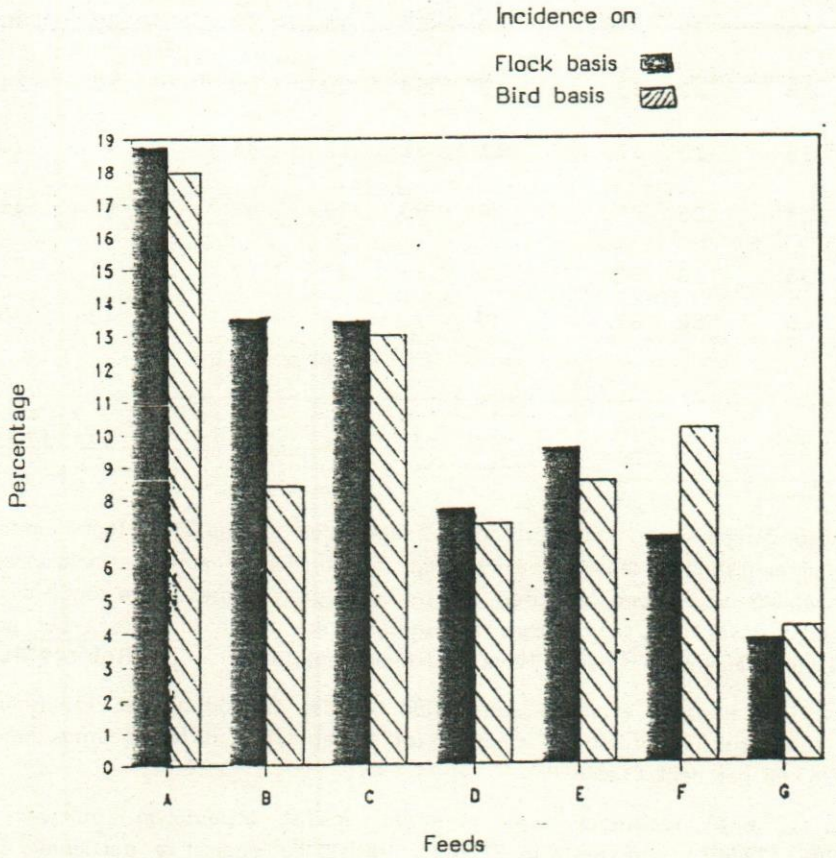
According to the condition of littre, storage of feed, cleanliness of drinkers and feeders these farms were catagorised in various managemental groups accordingly. Among 35 affected farms, the maximum (60%) prevalence was recorded on farms with poor managemental conditions followed by satisfactory management (37%). Non of the case was recorded in farms with an excellent management. JAVED and HAMEED (1989) has indicated that poorly constructed sheds, poor brooding system and insufficent ventilation, improper feed storage, poor litter management, contamination of feed and water may lead to higher prevalence of aspergillosis.

One of the potential sources of infection is the feed. Aspergillosis varied greatly among birds fed on various commercial feeds. It varied from 18.75 to 13.14 per cent in birds fed on common commercial feeds, while in well reputed commercial feeds it ranged from 9.52 to 3.70 per cent (Fig. I). Chances of feed contamination and occurrance of aspergillosis can be avoided by good quality and proper storage of feed ingredients, hot pelleting addition of vitamin E and antifungals. SAIF and REFAI (1977) reduced fungal spores load from brooders and feed by using antifungals.

Aspergillus fumigatus is known to localize in lungs air sacs, kidneys and peritoneum, so the involvement of various visceral organs is definite (CUBILLOS and PRUSSING, 1979). For isolation purposes various tissues including lungs, air sacs and peritoneum were collected along with a few samples of litters, feed and dead in shells embryos. Lungs proved to be convenient tissue, followed by air sacs and peritoneum (Table

III). Regarding the isolation on various media the maximum (51.14%) isolates were obtained from Sabouraud's dextrose agar followed by Czapek's dox agar (46%), Malt extract agar (14.71%) and Rose Bengal agar (37.71%). Aspergillus fumigatus took more time (3 - 5 days) to grow on Czapek's dox agar as compared to other media (WILLOMITZER, et al. 1985).

Fig. (1): Bar diagram mentioning aspergillosis in birds fed on different commercial feeds available in Pakistan.



Aspergillus fumigatus appeared as white fuzzy growth, which turned blue green fuzzy and cottony turning to grey green with age. The growth was dull green, velvety on Czapek dox, Malt

Table (III)

Efficacy of different media for isolation of Aspergillus fumigatus

Sources	No of samples	Sabouraud's agar		Czapek agar		Malt extract agar		R. Bengal agar	
		No	(%)	No	(%)	No	(%)	No	(%)
Lungs	35	35	100	30	86	28	80	28	80
Air sacs	35	22	63	19	54	15	43	12	34
Peritoneum	35	13	37	11	31	11	31	9	26
Litter	35	25	71	22	63	20	57	16	46
Feed	35	32	91	30	86	27	77	25	71
DIS (Embryos)	60	52	87	49	82	45	75	42	70
Total	235	179	51.14	161	46	146	41.71	132	37.71

extract and Rose Bengal agars. On microscopic examination hyphae were found uniform (Figs. 2, 3) hyaline, distinctly septate, having many nuclei in each portion. Conidiophores were smooth walled and green coloured at terminal part, spores were budded out in chains in mono layer (Fig. 4). Mature ascospores were round in surface but pully like in latteral view. Indentical findings have also been reported by COTTRAL (1978).

Various antifungal drugs available were tried by disc method against 179 isolates of Aspergillus fumigatus. According to spectrum of susceptibility, different drugs ranged from 87.15 to 63.68 per cent (Table IV).

Maximum (25.69%) resistance was observed against griseofulvin, followed by potassium iodide (22.90%) and nystatin (6.70%). Resistance against a particular drug develops with passage of time due to the prevalence of various strains and type of antifungals used frequently in feed and drinking water (BAINS, 1979).

It can be concluded that spergillosis is one of the major health hazard of our poultry industry. However, well planed elaborative studies in other poultry raising areas would be of significant importance to know the real magnitude of the disease.

Improvement of the managemental conditions. Hatchery hygiene should be adopted alongwith mould free feed can play an important role in the prevention of apergillosis. For the control of aspergillosis in feed copper sulphate, gentian violet and propionic acid may be cheaper antifungals. Various physical methods needs further investigation to control aspergillosis.

Table (IV):
Antifungal susceptibility to various isolates of Aspergillus fumigatus

Sr. No.	Antifungal Drugs	Sensitive No.	Sensitive (%)	Intermediate No.	Intermediate (%)	Resistant No.	Resistant (%)
1.	Griseofulvin	114	63.68	19	10.61	46	25.69
2.	Copper Sulphate	156	87.15	23	12.84	-	-
3.	Nystatin	129	72.06	38	21.22	12	6.70
4.	Propionic acid	150	83.79	25	13.96	4	2.23
5.	Tribrissen	152	84.91	24	13.40	3	1.67
6.	Potassium iodide	123	68.71	15	8.37	41	22.90
7.	Gentian violet	154	86.03	22	12.84	2	1.11
Total		978	78.05	166	13.24	108	8.61

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Fig. (2): Photograph showing uniform hyphae (X 350).

Fig. (3): Branch and septate hyphae having many nuclei in each portion (X 1400).

Fig. (4): Photograph showing chains of sterigmata (X 1400).