

معمل بحوث صحة الحيوان - بقنا •
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PIEZO MYCOSES WITH PARTICULAR REFERENCE TO CRYPTOCOCCUS
NEOFORMANS INFECTIONS IN KENA PROVINCE
(With 3 Tables)

فطريات الحمام والاشارة الى الاصابة بكريبتوكوكس نيوفورمنس في محافظة قنا

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أمكن عزل كريبتوكوكس نيوفورمنس ، كنديدا ألبيكانس ، أسبرجلس فلافس ، أسبرجلس
فلافيس ، أسبرجلس نديلانس ، أسبرجلس تيجر ، أسبرجلس فيمويجتس ، أسبرجلس
أورناتس ، بنسليم وفطر الفيساريم من الحمام المريض والميت ومن أجنة الحمام الميتة
داخل البيض ، كذلك أمكن عزل كريبتوكوكس نيوفورمنس ، سكروميسس ، أسبرجلس فيمويجتس
أسبرجلس نيجر ، أسبرجلس نديلانس ، أسبرجلس فلافيس ، ميكور والبنسليم من زرق
الحمام داخل وخارج الاعشاش ومن مياه شرب الحمام •

وتم دراسة تأثير بعض مضادات الفطريات على كريبتوكوكس نيوفورمنس في المعمل
ووجد أن الشوم أكثر تأثيراً من الميكوستاتين •

MATERIAL and METHODS

- 1- Birds, including liver, lungs and intestines, were kept in the form of twigs in the food, mainly for living and diseased birds, as well as dead birds, pigeons, or other birds, in well-ventilated and airy conditions and psychological examinations.
- 2- All pigeons, including those which were not diseased, were kept in a shaded place as well as in a dark place, water and food were taken under aseptic conditions.

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PIGEON MYCOSIS WITH PARTICULAR REFERENCE TO *CRYPTOCOCCUS* *NEOFORMANS* INFECTIONS IN KENA PROVINCE

(With 3 Tables)

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SUMMARY

Cryptococcus neoformans, *C.albicans*, *A.flavus*, *A.flavipes*, *A.nidulans*, *A.niger*, *A.fumigatus*, *A.ornatus*, *Penicillium* and *Fusarium* spp. could be isolated from diseased, dead pigeons and dead in shell-pigeon-embryos. On the other hand, *Cryptococcus neoformans*, *Sacchromyces*, *A.fumigatus*, *A.niger*, *A.nidulans*, *A.flavipes*, *Mucor* and *Penicillium* spp. were isolated from pigeon excreta (inside and outside nests) and from pigeon drinking water. The effect of some antifungal drugs on the isolated *Crypt. neoformans* were studied in vitro. Garlic was more effective than Mycostatin.

INTRODUCTION

Mycotic infections in birds is to be expected particularly in the open and dusty yards as the spores are ubiquitous in nature, and birds frequently come in contact with them through inhalation and/or ingestion.

Mycosis have been reported from all species of birds in many parts of the world (MASTR-
EFRANCISCO and RAIME, 1940; AINSWORTH and AUSTWICK, 1955; EMMONS, 1955; KAO and
SCHWARZ, 1957, HUBBEN, 1958, SILVA, 1960; McDONOUGH *et al.*, 1966, LITTMAN and BOROK,
1968; SINGH and SINGH, 1970, KUTTIN *et al.*, 1975). In Egypt REFAI and RIETH, 1966, BAHGAT
et al., 1971, REFAI, 1971, ABOU-GABAL *et al.*, 1977, EL-BADRI, 1979, 1983 and EDRIS, 1986).

Although, up till now there are no complete references about mycotic infection especially
Cryptococcosis among pigeons in Kena Province.

Therefore, the present investigation was planned to put on record the presence of
the mycotic infections in pigeons for the first time in Kena province.

MATERIAL and METHODS

1- Brains, Intestines, Livers, Lungs and air-sacs of 5 native pigeons with clinical signs
in the form of twisting of the head, inability to flying and decreased body weight, as well
as 3 dead native pigeons, in addition to 20 dead in shell embryos were exposed for P/M
and mycological examinations.

2- 120 pigeon droppings collected from inside and out-side feral pigeon nests at Abou-
Shoosha village as well as 100 drinking water samples were taken under aseptic conditions
for mycological examinations.

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- Media :**
- Sabouraud's dextrose agar (tubes).
 - Czapeks agar plates.
 - Corn-meal agar (BACKERSPIGEL, 1954).
 - Raw-egg white (BUCKLEY and VAN UDEN, 1963).
 - Littman ox-gall agar Difco (PAUL and ROBERT, 1972).
 - Sugar assimilation media (BISPING, 1961).
 - Niger-seed agar *Guizotia abyssinica* seeds (STAIB and SENSKA, 1973).
 - Normal saline containing streptopenicol, 250 mg/liter.

- Stains :**
- Gram's staine.
 - Indian-ink (diluted staine).

- Drugs for sensitivity test :

- Copper-sulfate 200 ug/disc (El-Nasr).
- Thiobenzole 200 ug/disc (M.S.D.).
- Mycostatin 250 i.u./disc (Upjohn).
- Neomycin 30 ug/disc (Upjohn).
- Linco-spectin 30 ug/disc (Upjohn).
- Garlic Juice (*Allium-Sativum*).

- Identification of the isolated fungi :

Fungi were identified on the Sabouraud's agar tubes and Czapeks agar plates according to their morphological appearance.

Yeasts were identified morphologically and physiologically by using Indian-ink staine, nigerseed agar (*Guizotia-abyssinica* seeds, Littman ox-gall agar as well as Corn-meal agar and Raw-egg-white.

Sensitivity of the isolated *Crypt. neoformans* to some antifungal drugs in vitro:

Filter paper discs were soaked in the above mentioned drugs of known concentration, and left to dry for 24 hr. the dried discs were put on the surface of *Cryptococcus neoformans* freshly prepared cultures on Sabouraud's dextrose agar and incubated at 37°C for 72 hours, at the same time control culture of *Cryp. neoformans* without drugs was also incubated.

RESULTS

The species of the isoalted fungi and their percentage from the examined samples of pigeons and embryos are recorded in Table (1).

The isoalted fungi from pigeon excreta and drinking water are shown in Table (2).

Six antifungal agents of known concentration were used to determine their effect on the isoalted *Crypt. neoformans* from pigeons in vitro. The results are illustrated in Table (3).

DISCUSSION

In Egyptian villages, pigeons are reared in all houses as a rapid economic source of meat. In Egypt there were many studies on mycotic infections in poultry, (BAHGAT et al., 1971; SAIFE, 1976; REFAL et al., 1976; ABOU-GABAL et al., 1977; EL-BADRI, 1979, 1983 and EDRIS, 1986). In Kena province no complete study on mycotic diseases was done in pigeons, this fact necessitated carrying out the present work.

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From diseased, dead pigeons and dead in shell embryo, the fungal species isolated from brain, intestine, liver, lungs, air-sacs and embryonic fluid were Cryptococcus neoformans, C. albicans, A. flavus, A. flavipes, A. nidulans, A. niger, A. fumigatus, A. ornatus, Penicillium and Fusarium spp. Crypt. neoformans was isolated from (19.7%) of the samples examined, C. albicans from (9.1%) A. flavus (21.3%), A. flavipes (1.5%) A. nidulans (1.5%) A. niger (7.6%) A. fumigatus (10.6%) A. ornatus (16.7%) Penicillium (3.0%) and Fusarium spp. (9.6%). These results were in agreement to a certain degree with other investigators, MASTREFRANCISCO and RAIME (1940); HAJSIG (1962); LITTMAN and BOROK (1968) who isolated Crypt. neoformans from the beak and feet of feral pigeons, POLYSIK et al. (1968); BAHGAT et al. (1971); SHARMA et al. (1971); REFFAI et al. (1976); ABÔU-GABAL et al. (1977) and EDRIS (1986). Concerning the isolation of fungi from pigeon excreta and from the drinking water the fungal species were Crypt. neoformans (11.3%) Sacchomyces (16.5%), A. fumigatus (6.5%) Aniger (24.8%) A. nidulans (3.5%) A. flavipes (6.5%) Mucor (12.6%) and Penicillium spp. (18.2%). The present findings are in agreement with EMMONS (1955); KAO and SCHWARZ (1957); SILVA (1960) who isolated Crypt. neoformans from soil collected inside and close to chicken yards, NIKOLAEV (1965); McDONOUGH et al. (1966); REFAI (1971); JAND and DHILLON (1973); BENNETT et al. (1977); MISHRA et al. (1981) and EL-BADRI (1983).

Cultured growth of the tested Crypt. neoformans could be inhibited in vitro by garlic juice 1/20 ml/disc and Mycostatin 250 i.u./disc, while copper sulfate 200 ug/disc, Thiobenzole 200 ug/disc, Neomycin 30 ug/disc and Linco-spectine 30 ug/disc had no effect. Similar results but to some extent were recorded by EL-BADRI (1983) who studied the effect of some anti-fungal drugs on C. albicans in vitro.

In the same time there are no available references in our hand about the effect of the antifungal drugs on the Crypt. neoformans in vitro. The effect of Garlic on the tested yeast in this study is considered to the best of our knowledge, the first record in upper Egypt. From the obtained results of this work it could be concluded that the fungal species which were isolated from diseased and dead pigeons were similar to those isolated from pigeon droppings and drinking water, as well as Crypt. neoformans could be isolated from the brain of the pigeons which had nervous signs, thus there is a great probability of such cause of C.N.S. infection. Also, it can be concluded that garlic juice is an efficient drug in vitro and should be tested in infected birds to determine its value experimentally.

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Table 1: Distribution of isolates among different organs and percentage.

Specimens and number	Isolates	Sources, Number and Percentage of the isolate										Total No.	% of each isolate	
		Brain	%	Intestine	%	Liver	%	Lung	%	Air-sac	%			Embryonic fluid
I11-pigeons (5)	<u>C. neoformans</u>	1	33.3	2	66.6	-	-	-	-	-	-	-	3	4.5
	<u>C. albicans</u>	-	-	2	40.0	2	40.0	1	20.0	1	-	-	5	7.6
	<u>A. flavus</u>	-	-	2	40.0	1	20.0	1	20.0	1	20.0	-	5	7.6
	<u>A. flavipes</u>	-	-	1	100.0	-	-	-	-	-	-	-	1	1.5
	<u>A. nidulans</u>	-	-	2	40.0	-	-	2	40.0	1	100.0	20.0	5	7.6
	<u>Penicillium</u>	-	-	2	100.0	-	-	-	-	-	-	2	3.0	
Dead-pigeons (3)	<u>C. neoformans</u>	2	40.0	2	40.0	1	20.0	-	-	-	-	5	7.6	
	<u>C. albicans</u>	-	-	-	25.0	1	100.0	-	-	-	-	1	1.5	
	<u>A. flavus</u>	1	20.0	2	40.0	-	-	2	50.0	1	25.0	4	6.1	
	<u>A. ornatus</u>	-	-	1	50.0	1	50.0	-	-	2	40.0	5	7.6	
	<u>Fusarium</u>	-	-	-	-	1	50.0	-	-	-	-	2	3.0	
Dead in shell embryos (20)	<u>C. neoformans</u>	-	-	-	-	-	-	-	-	-	-	5	7.6	
	<u>A. fumigatus</u>	-	-	-	-	-	-	-	-	-	-	7	10.6	
	<u>A. flavus</u>	-	-	-	-	-	-	-	-	-	-	7	10.6	
	<u>A. ornatus</u>	-	-	-	-	-	-	-	-	-	-	5	7.6	
	<u>Fusarium</u>	-	-	-	-	-	-	-	-	-	-	6	9.1	
		4		17		6		6		6		27		
												66		

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Table 2: Showing the isolated fungi from pigeon excreta and drinking water as well as the percentage of each isolate.

The isolated species	Pigeon excreta				Drinking water (100)		Total No. of isolates	% of each isolate
	inside-nest No. of isolate	%	out-side nest No. of isolate	%	No. of isolate	%		
<i>Crypt. neoformans</i>	15	57.7	6	23.1	5	19.2	26	11.3
<i>Sacchromyces</i>	3	7.9	-	--	35	92.1	38	16.5
<i>A. fumigatus</i>	4	26.7	1	6.7	10	66.7	15	6.5
<i>A. niger</i>	20	35.1	12	21.1	25	43.8	57	24.8
<i>A. nidulans</i>	8	100.0	--	--	--	--	8	3.5
<i>A. flavipes</i>	12	80.0	2	13.0	1	6.7	15	6.5
<i>Mucor spp.</i>	8	27.6	1	3.4	20	68.9	29	12.6
<i>Penicillium spp.</i>	15	35.7	5	11.9	22	52.4	42	18.2
	85		27		118		230	

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Table 3 : In vitro effects of some antifungal agents on Crypt. neoformans.

Sources of Crypt. neoformans	Breeds	Cooper sulfate	Thio-benzole	Mycostatin	Neomycin	Linco-spectine	Garlic juice
Ill-pigeons (Kena-city)	native	-	-	+	-	-	++
Pigeon excreta (insid nest) (abou-shoosha)	Feral	-	-	+	-	-	++

++ : Complete inhibition zone. (2-2.5 cm. diameter).

+ : Slight inhibition zone. (1-1.5 cm. diameter).

- : No inhibition zone.