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CRYPTOCOCCUS NEOFORMANS OF SAPROPHYTIC ORIGIN.

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Cryptococcus neoformans was isolated on 276 occasions as follows: from pigeon faeces, 159 (45%) of 349 samples; pigeon crops, 39 (50%) of 80 crops; from drinking water, 37 (74%) of 50 samples; from the atmosphere, 3 strains in a contaminated loft.

It was also recovered from 38 (19%) of 200 samples of canary faeces. It is possible that *C. neoformans* survives in the crop of pigeons and occasionally passes to the faeces where it multiplies.

The factors influencing its presence are: the irregular cleaning of lofts, overcrowding and air-pollution from poor ventilation.

Emmons (1954) was first to isolate *Cryptococcus neoformans* from pigeon droppings and his investigations were the start of many others. Its high frequency in pigeon droppings is closely linked to the presence of substances favourable to its growth (Staib, 1962). However the same cannot be said of its presence in the digestive tract. Very few authors, among them Weiland, Böhm & Abdallah (1968) have actually succeeded in isolating *C. neoformans* from that habitat.

This work concerns the isolation of *C. neoformans* from pigeon and canary droppings from the digestive tract of pigeons with droppings positive for *C. neoformans* and from the environment of such pigeons.

MATERIALS AND METHODS

Culture Medium

The results were obtained on *Guizotia abyssinica* medium containing 0·1% diphenyl as recommended by Staib & Seeliger (1968), but slightly modified. Our medium did not contain penicillin or streptomycin and also, chloramphenicol and diphenyl were added before autoclaving. This facilitated the preparation and the distribution of the medium in the test tubes.

The medium approximately 10 ml per dish, was poured into Petri dishes (diam 9 cm).

After seeding, they were incubated at 25°C and examined after 8 and 15 days.

Determination of the Yeasts

These were purified and identified according the criteria of Lodder (1970) and the techniques of Vanbreuseghem (1966). A specific criterion for *C. neoformans* is its pathogenicity for white mice.

Bird droppings

One ml of droppings was emulsified in 9 ml of sterile physiological saline and a swab immersed 3 times in this mixture was rubbed on the surface of the culture medium. The faeces used throughout these experiments were from 4 sources. The

first (PPL) was 18 private pigeon lofts. The second (LP) was lost pigeons maintained together in different premises (n°19, 20, 21) but each of them being in an individual cage. If not reclaimed, the pigeon was killed 15 days later. The third group (C.D.) consisted of canary droppings.

The canaries were kept together, but in separate cages, with hundred of birds of diverse origins for song competition. The canaries were usually maintained in darkness for 7 days and our samples were collected on the 8th day. The fourth group (DBD or different bird droppings) was made of:

a) 46 samples of starling droppings collected from elm leaves near roost areas.
b) 36 samples of droppings of exotic birds (chinese quail, parrots, frugivorous pigeons, colibris) and jays maintained in spacious aviaries. They were partially free.
c) 36 samples of canary droppings also partially free or belonging to private breeders where cleaning was regularly performed.

Pigeons Crops

Specimens of the content of the crops of living pigeons obtained with swabs, were inoculated on the surface of the culture medium. These pigeons were from pigeon loft 21, in August 1972, where the droppings were 80 % positive for *C. neoformans*.

Digestive Tract of Pigeons

The intestine immediately posterior to the gizzard was sectioned at 3 levels: duodenum, intestine, rectum and the contents were squeezed separately into 3 Petri dishes. Five ml of physiological saline was added and the suspension directly spread on the culture medium.

Isolation from Air

Two techniques were used:

a. With the "Casella slit sampler" (C. F. Casella and Co. Ltd., Britannia Walk London), known volumes of air were aspirated on Petri dishes filled with *Guizotia abyssinica* medium.
b. Negative droppings dispersed in 12 Petri dishes were exposed to the air in a known positive pigeon house (LP21). The dishes remained open in 12 empty cages for periods of 8 and 15 days. Twelve control dishes were placed in a clean room for the same period.

Water Sampling

A swab, dipped in 2 ml of drinking water from cages containing pigeons whose faeces were positive, was used for inoculating medium.

Results

Isolation of C. neoformans from Bird's Faeces

From Table 1, it is seen that droppings of 149 pigeons of group 1 (PPL) had 8 positive samples (5·3%). Droppings from the second group (LP) showed 151 positives in 200 samples (75%). (Table 2).

The results reported in Table 3 show large variations in positive samples not only from place to place (19, 20, 21) but also from month to month e.g. locale 21 in

TABLE 1.—STRAINS OF *C. neoformans* ISOLATED FROM SAMPLES OF PIGEON DROPPINGS BELONGING TO GROUP 1 — PRIVATE PIGEON LOFTS

Pigeon loft	*No. of samples*	*No. of isolates of C. neoformans*
1	10	0
2	10	0
3	3	0
4	12	0
5	9	0
6	8	1
7	2	0
8	2	0
9	2	2
10	7	4
11	60	0
12	1	0
13	7	0
14	1	1
15	1	0
16	5	0
17	4	0
18	5	0
	149	8 (5·3%)

TABLE 2.—STRAINS OF *C. neoformans* ISOLATED FROM SAMPLES OF PIGEON DROPPINGS BELONGING TO GROUP 2 – LOST PIGEONS.

Pigeon loft	*No. samples*	*No. isolates of C. neoformans*	*% age*
19	25	16	64
20	25	8	32
21 (May 1972)	50	46	92
21 (April 1973)	50	44	88
21 (July 1973)	50	37	74
	200	151	75·5

May 1972, April 1973 and July 1973. This last place is remarkable for having produced more than 1000 colonies *C. neoformans* per Petri dish. It is interesting that the number of *C. neoformans* colonies was in inverse ratio to the other yeast colonies. *Candida albicans* was present in 36% of the 150 specimens collected in May 1972, April 1973 and July 1973 of pigeon loft No. 21. The examination of canary faeces revealed that 3 (3%) of 100 cages were positive at Borsbeek while 35 (34·5%) were positive at Wijnegem. We think that the difference could be due to the large number of contaminants on the Borsbeek plates. From the fourth group (DBD) all 118 specimens were negative.

Pigeon Crops

In natural conditions, *C. neoformans* has never been found in the crop of pigeons

TABLE 3.—COLONY COUNT OF *C. neoformans* ISOLATED FROM PIGEON DROPPINGS OF GROUP 2 (LP).

Pigeon loft	*No. of colonies of C. neoformans counts per dish*				
	> 1000	> 100	> 10	> 1	0
19	3	5	6	2	9
20	2	2	2	2	7
21 (May 1972)	29	11	3	3	4
21 (April 1973)	11	21	8	4	6
21 (July 1973)	9	10	14	4	13

and Emmons (1955) inoculated unsuccessfully the content of crops of 20 pigeons to white mice.

The pigeons from which crop samplings were taken, were all from locale No. 21. We isolated *C. neoformans* from 39 of 80 crops (nearly 50%). However in 26 cases of 39, the number of colonies per Petri dish was lower than 10.

In 7 other ones, we had respectively : 70, 23, 20, 16, 14, 12, 10 colonies. In 6 cases we could not accurately count the positive colonies and so dilutions and subcultures were necessary to isolate the organism. Interestingly also *C. albicans* was present in 18 of the 80 crops.

Digestive Tract of Pigeons

In none of the 3 sections of 220 digestive tubes of pigeons collected haphazardly at poultry stores have we isolated *C. neoformans* but *C. albicans* was isolated in 13 of 130 digestive tubes.

Air Samples

With the Casella slit sampler on 5 occasions we collected 150 litres of air from locale 21 (Table 2) where the percentage of positive droppings varied from 74-92. On each of the 5 Petri dishes collected from the sampler, we isolated respectively : 17, 3, 5, 1 and 9 colonies of *C. neoformans*. If each colony developed from 1 *C. neoformans* blastospore we estimate that the medium content of *C. neoformans*/m^3 was 46.

It is interesting to note (Table 4) that negative droppings became positive when exposed in locale 21 whereas controls remained negative.

Water Samples

In July 1973, in locale 21, 37 of 50 droppings samples were positive for *C. neoformans*. Simultaneously we collected the drinking water of the same 50 cages and

TABLE 4.—CONTAMINATION OF PIGEON DROPPINGS BY AIR CONTAINING *C. neoformans*.

Place	*Exposure time in days*	*No. negative droppings at the start*	*No. positive droppings after exposure*
Pigeon loft No. 21 (air positive)	8	6	1
	15	6	1
Control locale (air negative)	8	6	0
	15	6	0

TABLE 5.—*C. neoformans* IN PIGEON DRINKING WATER.

Droppings (+) and water (+)	27
Droppings (—) and water (—) ..	3
Droppings (+) and water (—)	10
Droppings (—) and water (+)	10
	50

tested it for *C. neoformans*. The results are shown in Table 5. It is impossible to relate the positivity of water to that of droppings, χ^2 not being significant. However the drinking water of 2 pigeon lofts of group 1 (No. 6 & 9) whose droppings were positive, was never positive.

DISCUSSION

Using the medium of Staib & Seeliger slightly modified, we obtained 276 isolates of *C. neoformans*. The distribution of these isolates is extremely variable according to their origin : 159 from pigeon droppings, 39 from pigeon crops, 37 from drinking water used by pigeons, 3 from the air of pigeon house No. 21 where the prevalence of *C. neoformans* in the pigeons droppings was at its highest and 38 from canary's droppings.

Droppings from a large number of wild birds taken in their natural milieu gave completely negative results.

The factors favourable to the presence of the yeast in bird droppings are:

a) the daily accumulation of faeces in cages.
b) a large population of birds in the same pigeon or canary house.
c) insufficient aeration which favours an increase in the frequency of *C. neoformans* in the air.

We have proved (Table 4) that negative droppings may become positive for *C. neoformans* if kept in an atmosphere where *C. neoformans* is present.

The sequence of events could be as follows:

a) presence in the pigeon house of a bird (theoretically 1 would be sufficient) of which droppings were positive.
b) the *C. neoformans* passes in the surrounding air and infects other bird droppings, which acts as a favourable environment.
c) the lack of aeration and the movements of the birds favour the dispersion.

Their accumulation by lack of cleaning favours their development. Where do they come from? None of the 660 specimens of 220 intestinal contents, examined at 3 levels, was positive. With different techniques, other workers e.g. Emmons (1955) has the same results, but the crop of the pigeons—we have not investigated the crop of other bird species—gave positive results in a high percentage of cases. We may assume provisionally that the crops of the pigeons are the place where *C. neoformans* survive at least for some time if not for very long periods. From there it may pass from time to time in the droppings where the conditions are favourable to its further development and that without leaving any trace in the digestive tube. When the conditions are favourable : over-crowded pigeon loft, lack of regular cleaning and, consequently, accumulation of droppings, and may be an elevation of the surrounding temperature which favour the growth of *C. neoformans,* this yeast, starting from 1 or 2

cages, may contaminate droppings which have remained so far negative, contaminate the air and finally contaminate the crops of some pigeons where the organism will be able to survive for some time.

Besides the working hypothesis just presented it may be concluded from these experiments that:

1) the first cultures of the isolates of *C. neoformans* from the drinking water are of a much lighter tint that the first cultures of *C. neoformans* of other origins. However, they resume the usual rather darkish brown, yellow tint from the second culture onwards.

2) although apparently *C. neoformans* grows well on the Staib-Seeliger medium, it is easy to show that the growth is less profuse than on Sabouraud medium. This is due to the presence of diphenyl since by reducing its concentration, the growth improves.

3) besides *C. neoformans,* we have isolated from the crops and from the pigeon droppings *C. albicans* often in great quantities. The great difference between the 2 yeasts however is that *C. albicans* is often present in the digestive tract.

4) although many extraneous materials were examined, as possible sources of *C. neoformans,* it was present only in pigeon crop and faeces, canary faeces, in the air of the immediate environment of the birds and in the drinking water of pigeons.

Resume

Cryptococcus neoformans a été isolé 276 fois, dont 159 (45%) fois à partir de fientes de pigeons pour 349 échantillons examinés; 39 (50%) fois à partir de jabots de pigeons pour 80 échantillons examinés; à partir d'eau de boisson des pigeons, 37 (74%) échantillons sur 50 étant positifs; à partir de l'air ambiant d'un pigeonnier (3 souches). Il fut également isolé à partir de 38 (19%) échantillons sur 200 de fientes de canaris.

Nous considérons comme possible la survie de *C. neoformans* dans le jabot de pigeon, suivie de son passage occasionnel dans les fientes ou il se multiplie.

Les facteurs favorisant la positivité de ces fientes étant: leur accumulation dans les cages irrégulièrement nettoyées, une surpopulation des locaux, une mauvaise aération qui provoque une augmentation de la fréquence de la levure dans l'air.

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