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CRYPTOCOCCUS NEOFORMANS AND THE EPIDEMIOLOGY OF CRYPTOCOCCOSIS

by

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Summary — Direct plating of pigeon droppings on selective medium has allowed the isolation of *C. neoformans* from droppings collected in pigeon lofts and in premises where lost pigeons are kept in Belgium.

C. neoformans was also isolated from canary droppings. There is no doubt that the way of life of the bird influences the positivity of the droppings. It should also be observed that drinking water of pigeons in those overcrowded premises, and air can be positive and that negative droppings exposed to the air of a positive place can become positive.

C. neoformans was also found in crops of pigeons living in pigeon houses where the droppings are positive. Introduced in a pigeon house where the droppings are positive, a pigeon with negative crop can develop a positive one.

C. neoformans has always been considered an exclusively exosaprophyte.

Our study proves the existence of an endosaprophytic phase in the pigeon crop.

It is possible that the same endosaprophytic phase exists in other birds.

KEYWORDS : *Cryptococcus neoformans*; Epidemiology; Pigeons.

Introduction

A. *Epidemiology of yeast infections*

The yeasts that cause parasitic diseases called mycoses (Langeron and Vanbreuseghem, 1952) have not only a parasitic life, but also a saprophytic one.

Vanbreuseghem names these yeast infections « levuroses » from the French word « levure ». This saprophytic life can develop either free in nature or in the digestive tract of man and animals.

The yeasts that have their saprophytic life free in nature are called exosaprophytes by Vanbreuseghem (1964), in opposition to endosaprophytes that live as saprophytes in the intestine.

Candida albicans is a yeast that as a saprophyte can only be found in the intestine of man and certain aminals (Rose and Harrison, 1970; Vanbreuseghem *et al.*, 1970).

Its isolation in the exterior surroundings has to be imputed to human or animal contamination.

Candidosis occurs after taking antibacterial antibiotics or when a pathological (diabetes) or physiological state (pregnancy) settles itself (Vanbreuseghem, 1963, 1966).

Exosaprophytes, on the contrary, penetrate man and animal only accidentally through inhalation, ingestion or traumatism, while their biotope is free in nature.

Cryptococcus neoformans was, until now, considered to be exclusively exosaprophyte (Drouhet and Segrétain, 1950; Langeron and Vanbreuseghem, 1952; Emmons, 1969).

Emmons (1954) was the first to isolate *C. neoformans* from pigeon droppings.

When inhaled by man or animal and the surrounding is modified as in the case of candidosis, this yeast can invade the tissues and become a parasite.

B. *Cryptococcus neoformans*

1) *History and description*

The discovery of *C. neoformans* took place at the end of the previous century.

In Sardegna, Sanfelice (1895) isolated out of fruit juice a yeast that was pathogenic for the cavia. Almost at the same time in Germany, Busse (1895) and Buschke (1895) isolated a yeast out of cutaneous ulcers, located at the tibia in a patient with systemic mycosis.

These two cases were caused by the yeast mentioned in Lodder and Kreger van Rij index (1952) under the binomial of *Cryptococcus neoformans.*

C. neoformans can be considered to belong to the class of the Basidiomycetes on the basis of its sexual reproduction, recently discovered by Kwon-Chung (1975, 1976a, b).

The identification criteria are as follows (Lodder, 1970) : *C. neoformans* like all yeasts belonging to the *Cryptococcus* genus, does not produce filaments nor ferments sugars. It assimilates inositol as a carbon source and hydrolyses urea quickly (1).

Like the majority of yeasts belonging to the same genus, it produces on special media (2) polysaccharides related to starch. Like a large number of strains belonging to certain species of the genus, it is able to form mucous colonies. This mucous aspect is often accompanied by a yellow coloration of the colony and the presence of capsulated cells. The capsule of these cells can be seen under the microscope through addition of a drop of Indian ink diluted with water (1/1).

In the genus *Cryptococcus*, *C. neoformans* is one of the species (there are only two) that grows at 37° C and the only pathogenic one. This leads us to think that the only specific test presently accepted for the determination of *C. neoformans* is its pathogenic character for the white mouse when intracerebrally injected (Vanbreuseghem, 1966).

Another character which is probably specific is the fact that the colonies of *C. neoformans* will become brown after a maximum of 72 hours inoculation on a medium containing an aqueous extract of *Guizotia abyssinica* (Staib, 1962).

The grain of *G. abyssinica*, also called niger grain, is an oleaginous grain which one can find in certain bird mixtures.

In short, for a quick determination of *C. neoformans*, yeasts isolated on ordinary media must be sown on a urea medium and on Sabouraud medium incubated at 37° C.

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(1) Urea agar base medium (Difco).
(2) Aschner medium (Aschner *et. al.*, 1945).

If the two tests become positive after 48 hours, one injects the yeast intracerebrally in the white mouse and eventually inoculates it on a medium that contains *G. abyssinica.*

2) *Agent of human and animal cryptococcosis*

C. neoformans is the agent of cryptococcosis in man and other mammals. Human cryptococcosis is cosmopolite and well known in its meningo-encephalitic form. However, this seems always preceeded by a pulmonary often asymptomatic form (Vanbreuseghem, 1963). Osseous forms are observed in 5 to 10 percent of the cases (Littman and Zimmerman, 1956); cutaneous lesions in 10 percent and mucous lesions in 3 percent of the cases (Drouhet and Segrétain, 1950).

Spontaneous cryptococcosis in animals is similar to human cryptococcosis as far as the clinical or the epidemiological point of view is considered. However, *C. neoformans* is an agent of mastitis in cattle. We know that the number of yeast-mastitis tends to increase these last years, probably because of the abusive use of antibacterial antibiotics (Famerée *et al.*, 1970). We have examined in Belgium 115 samples of milk of healthy cows and 125 samples of milk of cows with mastitis. In 19 cases of mastitis the cause was undetermined and in 106 cases the mastitis seemed to be caused by bacteria. In no case *C. neoformans* was isolated (Swinne-Desgain, 1971).

In the laboratory, the most sensitive animal to *C. neoformans* is apparently the white mouse which can be inoculated by any route (Segrétain and Drouhet, 1947). There are however strains that do not kill 100 percent of the mice or that do not kill any of the mice when inoculated in the peritoneum. The rat, the guinea pig and the hamster are also sensitive to *C. neoformans* but the rabbit always resists to *C. neoformans* if the inoculation is not intracerebral (Smith *et al.*, 1953). This resistance might be due to the high body temperature of the rabbit (± 39,2° C), a temperature at which *C. neoformans* cannot develop (Kuhn, 1939; Bergman, 1966).

3) *Saprophytic life of* Cryptococcus neoformans

In 1951, Emmons isolated four strains of *C. neoformans* out of 716 samples of soil. This constitutes the starting point for many researches that very quickly lead to the isolation of *C. neoformans* out of animal excretions and more specifically bird droppings. In 1954, Emmons isolated twenty strains of *C. neoformans* out of 1,751 soil samples.

Among the 20 positive samples, 8 originated from soil contaminated with pigeon droppings and 5 from soil where pigeons were frequently seen. It was finally found that pigeon droppings are the most important source of *C. neoformans.*

Later on, it was also isolated from canary droppings (Staib, 1961), soil enriched with chicken droppings (Ajello, 1958), turtle-dove (*Streptopelia orientalis*) droppings (Tsubura, 1962) and sparrow droppings (Leone, 1969).

As we have mentioned before, for most of the investigations, *C. neoformans* is exclusively exosaprophytic. However, when one finds a yeast in animal excretions, it is logic to try to isolate it out of the digestive contents of the same animals.

In fact *C. neoformans* is not isolated easily out of the digestive contents of birds.

Emmons (1955) tried this unsuccessfully while carrying out autopsies on 20 pigeons whose droppings were positive. As a conclusion to the negative results of several workers, Emmons wrote (1969) : « The pigeon is not a host and we were not able to isolate the fungus from either the organs or intestinal tracts of birds ».

For Emmons (1955) and others (Staib *et al.*, 1974) birds take up *C. neoformans* with their food and the source of *C. neoformans* must be found in nature.

The epidemiology of cryptococcosis

The main object of our research was to detect *C. neoformans* in pigeon droppings collected in Belgium. We then tried to ascertain which was the source of contamination of these droppings.

A. *Isolation of* Cryptococcus neoformans *from bird droppings*

1) *Isolation methods of* C. neoformans *from pigeon droppings*

Two techniques are available to isolate *C. neoformans* from pigeon's droppings : the indirect and the direct technique.

a) *Indirect technique*

This technique finds its origin in Emmons assays (1949) to isolate *Histoplasma capsulatum* from the soil or animal excreta.

A suspension of 1 ml droppings in 9 ml sterile water is shaked for one minute. After 10 to 15 minutes settling, 1 ml water containing 2 mg of streptomycin and 5 mg of penicillin is added to 4 ml of the supernatant. 0,5 ml of this mixture is intraperitoneally injected in the white mouse.

If *C. neoformans* is present in the suspension, the mouse develops cryptococcosis. The liver and spleen of the inoculated mice collected after variable times according to different writers (4 to 8 weeks) will produce positive cultures when inoculated on Sabouraud medium.

b) *Direct technique*

In this technique, suspension of droppings is inoculated directly on selective media. The medium of choice is the medium of Staib and Seeliger (1968) modified by Swinne-Desgain (1975).

It contains chloramphenicol (0.1 percent), diphenyl (0.1 percent) and a water extract of *G. abyssinica* which gives the specific brown colour to *C. neoformans.*

2) *First assays to isolate* Cryptococcus neoformans *from pigeon droppings in Belgium*

Table 1 gives us the results of the first isolation assays of *C. neoformans* from pigeon droppings collected from private pigeon-houses. We have visited 66 pigeon-houses of this type and have collected 339 samples. We have tried to isolate *C. neoformans* from these dropping samples using 107 times the indirect technique and 232 the direct one.

The indirect technique gave positive results for *C. neoformans* in 8 cases out of 107 (7.5 percent) and the direct one 27 cases out of 232 (11.6 percent). Statistically compared, these two results do not differ significantly ($Z = 0.9783$).

TABLE 1

Isolation of *C. neoformans* from pigeon droppings

Technique	Number of pigeon-houses	Number of droppings	Number of droppings +	%
Indirect	22	107	8	7.5
Direct	44	232	27	11.6
	66	339	35	13.2

But the indirect technique shows some disadvantages :

— some mice died during the first days after the injection from bacterial infection before *C. neoformans* had developed in the mice;

— some strains of *C. neoformans* have a light virulence for the mouse when injected intraperitoneally. These strains were isolated by the direct technique : they could not be isolated by the indirect one. For these reasons we have chosen the direct technique instead of the indirect one.

3) *Positive droppings according to their origin*

We have collected droppings from pigeon-house refuges (*) where pigeons lost in the pigeon-races are collected as long as needed before they are returned home (table 2). The birds are put individually in separate cages with their individual drinking and food containers. During the season of pigeon-racing the refuges are overpopulated and the droppings accumulate in the cages.

Usually the pigeons do not stay more than a fortnight in these refuges, but they have to wait sometimes a month or more. If their owner do not claim them, the birds are killed.

TABLE 2

Number of *C. neoformans* strains isolated from pigeon-droppings from pigeon-breeders sanctuaries

Pigeon-house n°	N° of samples	N° of *C. neoformans* strains	%
45	25	16	64.0
46	25	8	32.0
47 (5/72)	50	46	92.0
47 (4/73)	50	44	88.0
47 (7/73)	50	37	74.0
47 (10/75)	20	16	80.0
	220	167	76.0

Droppings have been collected from three pigeon-house refuges : n^{os} 45, 46 and 47. In the last one, sampling has been repeated four times. 167 samples out of 220 proved to be positive for *C. neoformans* and in each sample, the number of colonies isolated was very high. In the refuge n° 47, air samples were taken with a « casella slit sampler » (**) and they proved to be positive for *C. neoformans.*

(*) They are built by the Pigeon-Breeders Association of Belgium.
(**) C. F. Casella and C^{o} Ltd., Britannia Walk, London.

No relation between the duration of the stay of the pigeon and the positivity of the droppings could be made.

Table 3 compares the results obtained in private with those obtained in refuge pigeon-houses.

TABLE 3

Positivity of droppings according to their origin

Origin	N° of droppings	N° of droppings +	%
Private pigeon-houses	232	27	11.6
Refuges	220	167	76.0

We noticed that in the private pigeon-houses, 27 samples out of 232 had *C. neoformans* while in the refuges 167 out of 220 contained *C. neoformans.*

The difference between these two results is very significant ($Z = 13.7031$) : the droppings taken out of pigeon-house refuges are much more positive than those taken of private houses.

4) *Isolation of* C. neoformans *from the air in a pigeon-house*

In refuge n° 47 where we knew that the air was positive, we placed 6 Petri dishes containing negative droppings, for 8 and 15 days (table 4). As a control we placed the same number of dishes with the same negative droppings in a place where we knew that the air was negative.

TABLE 4

Exposition of Petri dishes containing droppings in pigeon-house n° 47

	Exposure time in days	N° of droppings − before to start	N° of droppings + after exposure
Pigeon-house (air +)	8	6	1
	15	6	1
Control (air −)	8	6	0
	15	6	0

The retrocultures of the droppings that were placed in the refuge were positive.

It can be concluded that the negative pigeon droppings placed in rooms where the air is positive, can become positive themselves.

5) *Positivity of drinking water of pigeons*

In the pigeon-house refuge n° 47 where droppings and air are positive, we took samples of the water given to the pigeons. From the individual drinking troughs we took sterily 2 ml of water and inoculated Petri dishes with an aliquot of 0.5 ml per dish. Table 5 gives the results obtained from 50 individual drinking troughs.

Thirty-seven samples contained *C. neoformans.* Of the 37 samples, 27 were from the cages where the droppings were positive. Statistically there is no relation between the positiveness of the droppings and of the drinking water ($\chi^2 = 0.00778$).

TABLE 5

Simultaneous research of water in drinking troughs and droppings in 50 pigeon-hole refuges

Water + and droppings +	27
Water + and droppings −	10
Water − and droppings +	10
Water − and droppings −	3

We have also tried to isolate *C. neoformans* from the drinking troughs of pigeons with positive droppings in private pigeon-houses and from the water that pigeons are drinking from various containers on public places. The results are mentioned in table 6.

TABLE 6

Presence of *C. neoformans* **in the drinking water of pigeons**

Private pigeon-houses	N° of samples	N° of samples positive
2	26	0
Water containers on public places 13	30	0

Our investigations in both places remained completely negative.

6) *Isolation of* Cryptococcus neoformans *from canary droppings*

Staib (1962b) observed that canary droppings can be positive for *C. neoformans.* We made a similar investigation.

From 14 bird fanciers we got 58 samples of canary droppings (group 1). Group 2 was collected at a canary singing competition. Usually those singing competitions for canaries take place in celebration halls or back halls of pubs.

Hundreds of canaries are locked in individual cages. They have their private drinking and seed troughs. The birds stay locked up for 8 days and the cages are covered with a piece of tissue.

The cages are not cleaned and the droppings accumulate. On the competition day, the tissue is removed and the bird is supposed to sing when seeing light.

TABLE 7

Isolation of *C. neoformans* **from canary droppings**

	N° of samples	N° of samples positive
Group 1		
(14 different places)	58	0
Group 2		
Place 1	100	4
Place 2	100	35

From table 7 it is clear that canary droppings from private bird fanciers do not produce *C. neoformans.* On the contrary the yeast was isolated respectively in 4 and in 35 samples from both singing competitions.

7) *Conclusions*

We may conclude that *C. neoformans* is present in droppings of pigeons and canaries, mainly when these birds live in overcrowded places where the droppings accumulate. There is no doubt that the way of life of the bird influences the positiveness of the droppings. It should also be observed that the drinking water and the air of these overcrowded places can be positive for *C. neoformans* and that negative droppings exposed to the air of a positive place can become positive.

B. *Isolation of* Cryptococcus neoformans *out of nature*

Until now, *C. neoformans*, never isolated from the pigeons digestive tract, was considered to be exclusively exosaprophyte. However in the rare occasions when *C. neoformans* has been isolated out of nature, the possibility of a contamination by pigeon droppings could not be eliminated. Our attempts to isolate *C. neoformans* from two types of samples gave completely negative results.

A first group of samples consisted out of soil collected from cereal and crucifer fields of which pigeons are fond according to Murton and Jones (1973), and open fields where we had seen pigeons looking for food.

The second group of samples was sawdust. We had wondered if *C. neoformans* could not come from wood, having seen birds with positive droppings locked up in wooden cages. Table 8 gives the results.

TABLE 8

Attempts to isolate *C. neoformans* from the outside

Sample	N° of samples	N° of samples positive
Soil	210	0
Sawdust	240	0

These negative results led us to try again to isolate *C. neoformans* from the digestive tract of pigeons.

C. Cryptococcus neoformans *in the digestive tract of pigeons*

1) *Isolation attempts of* C. neoformans *from the intestine of pigeons*

From a poulterer we obtained the offals of 220 pigeons sold from various pigeons breeders.

We cut the digestive tube from the gizzard backwards in three pieces and obtained in this way the duodenum, the intestine and the rectum. The content of each section was squeezed separately into Petri dishes. 5 ml of sterile distilled water were added to each dish. A swab was dropped into the mixture and spread on *G. abyssinica* medium in a Petri dish. In no case was *C. neoformans* isolated from the intestinal content.

2) *Natural positivity of the pigeon crop*

We decided to try our luck on an upper part of the pigeon digestive tract and choose the pigeon crop.

a) *Technique*

Sterile swabs were pushed gently in the pigeons crop, against the coating of the crop and then inoculated on *G. abyssinica* media. Investigations were made as well in pigeon-houses where the droppings were negative as in those where they were positive.

b) *Results*

Results are mentioned in table 9.

TABLE 9

Presence of *C. neoformans* in the crop of pigeons

1. *Pigeon-houses where the droppings are positive (90 % positivity)*	
Number of pigeon-houses	2
Number of swabbed pigeons	114
Number of positive pigeons	57 (50 %)
2. *Pigeon-houses where the droppings are negative*	
Number of pigeon-houses	9
Number of swabbed pigeons	107
Number of positive pigeons	0

It is clear from this research that 50 percent of the crops are positive when the droppings are positive, whereas when the droppings are negative, the crops are also negative.

Statistically the difference is very significant ($Z = 8.17$).

c) *Discussion and conclusions*

Is the crop a better biotope for *C. neoformans* than the rest of the digestive tract ? According to Herpol (1967) the pH in the crop is between 6.3 and 6.8. This indeed is a favourable pH for the maintenance of *C. neoformans.*

On the other hand, the body temperature of the pigeon is around 41/43° C (Littman and Borok, 1968). As we had observed (Swinne, 1978), these temperatures are fungistatic and even fungicidal for *C. neoformans.* According to Borelli (1976, personal communication) the temperature of the crop is lower than the body temperature. From this can be inferred though more investigations are requested, that the crop offers to *C. neoformans* a better abode than the rest of the digestive tract.

3) *Persistence of natural positivity of the pigeon crop*

In november 1974 we have taken 10 pigeons from a private pigeon-house where the droppings were positive. Five of these 10 pigeons had positive crops. The five others were negative. We repeated the examinations of these 10 pigeons five times by swabbing their crops and obtained the following results (table 10).

We have taken dropping samples at the time of every swabbing. The droppings were every time sowed on 10 dishes of *G. abyssinica* medium.

TABLE 10

Persistence of the positivity of the crops of 10 pigeons from a private pigeon-house with positive droppings

Pigeon N°	Nov. 1974	March 1975	April 1975	June 1975	Sept. 1975	Feb. 1976
1	+	+	–	–	–	–
2	+	–	–	+	–	–
3	+	–	–	–	–	+
4	+	–	–	–	–	–
5	+	–	–	–	–	–
6	–	–	–	–	+	–
7	–	–	–	–	+	–
8	–	–	–	–	–	+
9	–	–	–	–	–	–
10	–	–	–	x (1)	–	–

From this table it can be concluded that the positivity is very irregular. As a matter of fact, in March 1975, only one of the five pigeons with positive crops at the beginning, was still positive. In April, at breeding time and production of crop-milk as well by males as by females, all the crops were negative. Later on some crops became again positive in a very irregular manner whereas the droppings maintained their positivity very regularly (table 11). In every case we have isolated *C. neoformans.*

TABLE 11

Persistence of the positivity of pigeon-droppings from a private pigeon-house

	N° of Petri dishes	N° positive
March 1975	10	7
April 1975	10	3
June 1975	10	5
September 1975	10	6
February 1976	10	4

4) *Experimental positivity of pigeon crops* (Swinne-Desgain, 1976)

a) *Technique*

C. neoformans was given orally to the pigeons. This occurred with a syringe containing the yeast in suspension in distilled water. The syringe was pushed gently into the beak up to about the oesophagal opening. The beak was kept closed for a few seconds to assure complete swallowing of the yeast. The pigeons were locked up in individual cages with seed and drinking trough.

During these experiments, samples of air were taken every week with a « Casella slit sampler ». The quantity given per pigeon was : $5,10^6$ *C. neoformans*. The number of pigeons was 10. We used five different strains, one strain for two pigeons : RV.29642, RV.29643, RV.29445, RV.29491, RV.25804. Crop and droppings examinations were made on days 1, 2, 3, 4, 8, 10, 15, 17, 22, 24, 31, 45, 51, 57, 64, 71, 79 and 86.

The 10 pigeons of this experiment were all killed on the 86th day.

(1) : sample not taken.

TABLE 12

Crops and droppings positivity of 10 pigeons given 5 × 10⁶ *C. neoformans* orally

Examination day after ingestion of *C. neoformans* (n° of days)	N° of pigeons crop + and drop. +	N° of pigeons with only crop +	N° of pigeons with only drop. +	N° of positive pigeons
1	9	0	0	9
2	1	5	0	6
3	2	3	1	6
4	2	3	0	5
8	0	3	1	4
10	0	2	0	2
15	0	2	0	2
17	0	1	1	2
22	0	2	1	3
24	0	1	0	1
31	0	1	0	1
37	0	1	0	1
45	0	1	0	1
51	0	1	0	1
57	0	0	0	0
64	0	2	0	2
71	0	1	0	1
79	0	1	0	1
86	0	2	0	2

It is indeed interesting to notice that two pigeon crops were still positive 86 days after being given *C. neoformans.*

On the other hand the droppings of the 10 pigeons were negative after 24 days.

None of the swabs taken post-mortem have given positive cultures, except for those from the two positive crops.

None of the birds had any evidence of infection.

The air of the surroundings was checked and never found positive.

b) *Discussion and conclusion*

At the end of these experiments it is difficult to conclude because the number of pigeons was small. We think however that it adds some information to the epidemiology of cryptococcosis :

— 24 hours after giving $5{,}10^6$ yeasts per os, 9 pigeons out of 10 had positive crops and droppings;

— after 24 days there were no more positive droppings, which is comparable to what Sethi and Randhawa (1968) have observed in similar experiences. On the contrary the positivity of the crop is maintained up to 86 days in two pigeons;

— Littman and Borok (1968) who isolated *C. neoformans* from the beak and the feet of pigeons consider the pigeon as a « mechanical carrier ».

C. neoformans given orally to the pigeon confirms the fact that it would be possible to find *C. neoformans* back in the crop and that it also could be carried in the pigeons crop.

In order to be able to specify this last point better, we have then realized individual examinations of crop and droppings of pigeons.

5) *Individual examination of crop and droppings of pigeons*

In the pigeon-house refuge n° 47, 20 pigeons were hazardously chosen. Crop samples and droppings in their cages were collected. To obtain fresh droppings, the 20 pigeons were locked in sterile cardboard boxes for one night. That gave us droppings that could not have been contamined from the outside. We have also examined fresh droppings of the same pigeons kept for 15 days at room temperature. They were protected from all external contaminations. This was made to enrich the droppings in *C. neoformans* (Swinne, 1978). The results are summarized in table 13.

TABLE 13

Individual examination of crop and droppings of pigeons

N° of pigeons	Crops	Cages	Fresh droppings	Fresh enriched droppings
9	–	+	–	–
4	+	+	–	–
3	–	–	–	–
2	–	+	–	+
1	+	+	+	+
1	+	–	–	–
20	6	16	1	3

The number of fresh positive droppings is smaller than the number of positive crops. Apparently there is no relation between the number of fresh positive droppings and the number of positive droppings collected in the cages. Indeed they could have been contamined by the air, the drinking water on the pigeon itself with the yeasts present in its crop. We have identified the isolated yeasts and found besides *C. neoformans* other yeasts such as *Candida albicans* and *Saccharomyces telluris* which are considered as endosaprophytes by all authors.

6) *Pigeon contamination in a pigeon-house*

In an effort to demonstrate that pigeons can be contamined when brought in a positive pigeon-house we have made the following observations.

Droppings were collected in the original private pigeon-houses of 6 pigeons whose crop was found positive during their stay in the pigeon-house refuge n° 47. We compared the positivity of their crop with the droppings of their original pigeon-house.

Table 14 produces the results.

TABLE 14

Six pigeons from pigeon-house refuge n° 47 N° of colonies isolated from crop	Droppings from the original pigeon-houses N° of + dropping samples
1	2/10
3	0/10
4	3/10
19	0/10
26	0/10
127	0/10

It is clear that dropping from only two pigeon-houses were originally positive. That means that in the four other cases, the pigeons were probably contamined in the pigeon-house refuge. To be more precise, if we introduce a pigeon with negative crop in a pigeon-house with positive droppings, it can become positive.

7) *Conclusions*

C. neoformans is found in crops of pigeons who live in pigeon-houses where the droppings are positive. They can remain positive for a long time. The pigeon crops are more often positive than their fresh droppings. Introduced in a pigeon-house where the droppings are positive a pigeon with negative crop can develop a positive one. Pigeon crops are also a favourable niche for true endosaprophytes such as *C. albicans* and *S. telluris*.

We can conclude that *C. neoformans* is carried by the pigeon in its crop and can remain there for a long time.

General conclusions

C. neoformans has always been considered an exclusively exosaprophyte. Our study proves the existence of an endosaprophytic phase. This phase is in the pigeon crop. The saprophytic life of *C. neoformans* can be resumed as follows. We have shown the frequency of the presence of *C. neoformans* in pigeon droppings as exosaprophyte.

The natural positivity of pigeon crops proves the existence of an endosaprophytic phase. We believe that the contamination of air, water, soil, vegetables, animals and man has only one origin : the pigeon. Nevertheless the droppings and digestive content of other birds cannot be excluded and we may not say that the pigeon is the only reservoir of *C. neoformans*.

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Cryptococcus neoformans **et l'épidémiologie de la cryptococcose.**

Résumé — L'ensemencement direct sur milieu sélectif de fientes de pigeons récoltées en Belgique dans des pigeonniers privés et dans des pigeonniers-refuges, a permis l'isolement de *C. neoformans*.

C. neoformans a aussi été isolé à partir de fientes de canaris.

Il est indubitable que le mode de vie mené par l'oiseau influence la positivité de ses fientes.

Nous avons signalé aussi la positivité de l'eau de boisson des pigeons des pigeonniers-refuges ainsi que la positivité de l'air ambiant. Nous avons constaté que des fientes négatives entreposées dans un local où l'air est positif peuvent devenir positives.

C. neoformans a aussi été retrouvé dans le jabot de pigeons vivant dans des pigeonniers où les fientes sont positives.

Si l'on introduit dans un pigeonnier où les fientes sont positives un pigeon dont le jabot est négatif, il peut devenir positif. *C. neoformans* avait toujours été considéré comme exclusivement exosaprophyte.

Notre étude prouve l'existence d'une phase endosaprophytique dans le jabot du pigeon. Il est possible que cette phase endosaprophytique existe aussi chez d'autres oiseaux.

Cryptococcus neoformans **en de epidemiologie van cryptococcose.**

Samenvatting — Door duivenmest rechtstreeks te enten op selektieve voedingsbodems hebben we in België *C. neoformans* afgezonderd uit meststalen genomen in private duivenhokken en in lokalen waar verdwaalde duiven werden ondergebracht.

Ook bij kanaries is *C. neoformans* kunnen afgezonderd worden uit de mest.

Er is geen twijfel aan dat de levenswijze van de vogel een invloed heeft op de positiviteit van de mest.

Men heeft opgemerkt dat de lucht en het drinkwater van duiven, in overvolle lokalen, positief was, en dat negatieve mest blootgesteld aan de lucht van positieve plaatsen, positief kon worden.

In duivenhokken waar de mest positief was, werd *C. neoformans* ook teruggevonden in de krop van de duiven.

Wordt een duif met negatieve krop in een hok gebracht waar de mest positief is, dan kan deze duif een positieve krop ontwikkelen. *C. neoformans* is altijd beschouwd geweest als een echte exosaprofiet.

Onze studie bewijst het bestaan van een endosaprofietische fase in de duivenkrop. Het is mogelijk dat dezelfde endosaprofietische fase bij andere vogels bestaat.

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