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EPIDEMIOLOGY OF CRYPTOCOCCUS NEOFORMANS

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Abstract

The concept of the epidemiology of *Cryptococcus neoformans* as the causative agent of cryptococcosis and as a basidiomycetous yeast is based on the fact that bird manure has been until now its only known habitat but not plant material which likewise harbours various non-pathogenic *Cryptococcus* species.

It could be shown that the possible influence of nutritional factors on the morphology and morphogenesis earns attention not only in view of the epidemiology of *C. neoformans* but of its perfect states, too.

Introduction

After the detection of cryptococcosis in man by Busse (3) and the first isolation of its etiologic agent, *Cryptococcus neoformans* from fruit juice by Sanfelice (8) in the same year, 1894, it took a relatively long time until the natural habitat of *C. neoformans* was found.

In 1955, Emmons found fecal matter of the pigeon colonized by *C. neoformans* (4). This observation was followed by a worldwide research into the problem of this habitat. So, in many parts of the world this habitat could be confirmed and brought into relation with the worldwide occurrence of cryptococcosis.

Bird manure as nutrient substratum and habitat for C. neoformans

In 1962 it could be shown that the basis of the association of *C. neoformans* with bird excreta was bird urine (11, 13). It was explained that the known urine substances such as purines, urea and creatinine were utilized in highly concentrated form by *C. neoformans* due to its osmophilic capacity. It was furthermore stated that creatinine was exclusively utilized (with a few exceptions) by *C. neoformans* in contrast to the other species of the genus *Cryptococcus* (14). It was also found that bird manure yielded not only *C. neoformans* but also other species of the genus. That were unable to assimilate creatinine (11).

The striking resistance of *C. neoformans* to desiccation was another observation in relation to the epidemiology of cryptococcosis (15). This observation furnished an additional explanation for the association of *C. neoformans* with old dry bird manure.

Occurrence of C. neoformans and other Cryptococcus species in plant material

Because *C. neoformans* could also be isolated from the fecal matter of various pet-birds like canaries, it had to be assumed that by the feed, which mostly consisted of various plant seeds, the intestinal tract of the bird and the cages became infected (10). After having fed birds with infected plant seeds the fungus could be isolated from the fresh fecal matter a few hours later and the cage was infected until the next desinfection. So it seemed clear that by the known resistance to desiccation and osmotolerance, *C. neoformans* had to be found as a kind of spore on single seeds. This assumption was corroborated by the observation that the fungus remained viable on artifically infected seeds for more than 200 days. In addition it has been shown that dried samples of certain selected plant species (leaves and stems) are suitable for the growth of *C. neoformans* under defined laboratory conditions. In this experiment, it was shown that e.g. *C. neoformans* as a budding cell is found around the stomata of the epidermis and growing in the epidermal cells of dried leaves (16).

These results, indicating a possible growth of *C. neoformans* on plants, led to the examination of plant seeds

for *C. neoformans*. The seeds of 117 species of 48 families of plants from the Botanical Gardens of West Berlin, 18 samples of wheat, oats and barley from a big mill in southern Germany and seed mixtures used as bird feed from 4 different pet-shops in West Berlin were examined (17). In 47 out of 117 samples the following species of the genus *Cryptococcus* could be found*: *C. albidus* (30); *C. albidus* together with *C. albidus* var. *diffluens* (5); *C. albidus* together with *C. laurentii* (1); *C. albidus* var. *diffluens* (7); *C. albidus* var. *diffluens* together with *C. laurentii* (1); *C. laurentii* (2); *C. terreus* (1). In 30 different plant species, *C. albidus* could be isolated, but never *C. neoformans* (17). Kurtzman et al. cultured from Canadian wheat, beside a few isolates of *C. laurentii*, *C. albidus* only, and therefore thought this species to be specific for wheat (5). Brandsberg who examined seeds used for feeding pet-birds in the USA, had similar results, *C. albidus*, *C. albidus* var. *diffluens*, *C. laurentii* and *C. luteolus* were isolated but never *C. neoformans* (2).

On the basis of the results obtained by various investigators in various parts of the world it must be assumed that birds do not get into touch with *C. neoformans* by infected plant seeds. After having isolated *C. neoformans* from peaches (21), it could be shown that, contrary to the flesh of peaches, the flesh of all tested varieties of apple did not permit the growth of *C. neoformans* (19). Colonization by *C. neoformans* could be observed only on the cores of the varieties, Jonathan, Red Boskop and Horneburger. In the freshly produced germ-free filtered juice of all tested varieties of apples, no inhibition of growth as on the flesh was found. In spite of initial pH values of 3.0–3.9, *C. neoformans* strain W 71 grew as well as in the Sabouraud's glucose broth (initial pH 6.3) used as control (19). On apple juice agar (initial pH 5.5) all isolates of *C. neoformans*, *C. albidus* and *Candida albicans* showed growth. Four out of five isolates of *C. neoformans* developed a brown pigmentation of the colonies (19).

In connection with these observations the question arose: whether the inhibitory action of the flesh was caused by the high content of polyphenols which is said to be reduced by the pressing of these fruits (Wucherpfennig, personal communication (19). This interpretation is of interest because oxydases specific for *C. neoformans* are held responsible for the brown color effect of *C. neoformans* on agars containing plant seeds as in *Guizotia abyssinica*-creatinine agar which proved to be a quite specific medium for this enzymatic reaction of *C. neoformans* (9, 12, 18). Perhaps further studies on the basis of these observations will help to understand how far a certain inhibitory mechanism for this species exists in plant or plant material.

* Figures given in brackets signify number of isolates.

Isolation of Cryptococcus species from wasps and wasp nests

Because wasps are often found on ripe fruit we examined living wasps and wasp nests for species of *Cryptococcus*.

From one hundred wasps caught in the summer period, *C. neoformans* could not be isolated. The most frequently found yeasts were species of the genera *Saccharomyces* and *Debaryomyces*. From 32 out of 110 wasp nests (*Dolichovespula saxonica*) 40 white to cream-coloured isolates of yeast-like fungi were isolated. Not a single one of these isolates grew at 37 °C. Only one could ferment glucose but 34 hydrolysed urea, all these isolates belonged to the genus *Cryptococcus*. No isolate proved to be *C. neoformans* but 21 were identified as *C. laurentii* and 13 as *C. albidus* (5 *C. albidus* var. *diffluens*). The rest of the isolates belonged to the genera *Debaryomyces* and *Trichosporon*. In the water extract of the wasp nests, nitrogen and carbohydrate and no inhibitory action was present. From these results, namely the impossibility of isolation of *C. neoformans* from plant material unlike bird manure, the latter seems to be, until now, the only known habitat of *C. neoformans* in the free nature. We must assume that the nutrient conditions on ripe fruit, which are limited in terms of time, beside the inhibitory action of plant material and the simultaneous growth of various microbes prevent plant material from serving as a habitat for *C. neoformans*.

Uric acid and its influence on the morphology of C. neoformans

Bird manure is still of greatest interest in the epidemiology of cryptococcosis. For this reason research into the components of bird urine and their significance for *C. neoformans* was continued. The authors' recent study was prompted by observations of the substratum-dependent morphological changes in a rough-looking isolate recovered from the brain of a mouse initially inoculated with a mucoid culture of *C. neoformans*. When the rough-looking colonies were subcultured on synthetic media (Benham's method) containing different nitrogen sources (0.1 %) e.g., urea, creatinine and uric acid, only on the medium containing uric acid did growth take place; this had typical

mucoid appearance and showed only encapsulated round cells (22, 23).

Beside the observations in this not yet classified inositol-positive isolate, the typical *C. neoformans* isolates also showed a noteworthy behaviour in the presence of uric acid as the sole source of nitrogen. Isolates that formed dry colonies on Sabouraud agar, started a mucoid growth on the uric acid agar with capsule formation. Surprisingly, such a mucoid variant showed a loss of virulence for the white mouse (22). In this connection it must be mentioned that the loss of virulence for the white mouse does not exclude a possible selective survival of *C. neoformans* in the brain, a fact which seems to be of significance in further epidemiological studies of *C. neoformans* and cryptococcosis (23).

After having observed the cultural and morphological changes of *C. neoformans* isolates and the rough-looking isolate in the medium containing uric acid as the sole source of nitrogen, the question arises whether it is possible that *C. neoformans* in its widely familiar form is a product of its habitat which is characterised by the highest concentration of substances like uric acid. Because the capsule-formation of *C. neoformans* in man or animal is responsible for the cryptoccoma (20), which is the pathological-anatomical criterion of cryptococcosis, the in vitro production of capsules is of special interest in view of the morphological behaviour of this fungus in man or animal.

Biologic differences between serotypes of C. neoformans

That there are biologic differences between serotypes of *C. neoformans* was found by Bennett (1) who used the quantitative strain-specific creatinine assimilation (13, 14) and the strain specific production of a green color in the *Guizotia abyssinica*-creatinine agar (12, 13, 18). There are strongly and weakly assimilating *C. neoformans* strains (14). Green coloration of *G. abyssinica*-creatinine is due to the high pH of approx. 8.0 caused by the degradation of low-molecular nitrogenous substances like creatinine. This green coloration was found in serotypes B and C but not in types A or D. Bennett found that the isolates of serotypes B and C assimilated creatinine much more rapidly than type A (1). Because there is a certain geographic distribution of serotypes (1) it must be assumed that these biological properties have influenced the antigenicity of this pathogen. In this connection it would be of interest to know how far the serotypes differ in their quantitative assimilation of uric acid.

Finally, Kwon-Chung noticed, in connection with her finding of the perfect state of *C. neoformans* (6) during the course of mating experiments with numerous cultures of *C. neoformans*, that there were differences in the mating of serotypes (A and D) and (B and C) (7).

In our current mating experiments with clinical and natural not yet fully serotyped isolates from Europe we have not been able to obtain the *Filobasidiella neoformans* state in uric acid agar but only on malt and to a lesser degree on Sabouraud's agar. If further experiments would confirm this assumption, it might be concluded that uric acid, accounting for the highest proportion of nitrogenous compounds in bird manure does not permit formation of the perfect state under such nutrient conditions but only the imperfect state: *C. neoformans*. Certainly this concept should not hinder intensified research into the biology and epidemiology of the perfect states of pathogenic and non-pathogenic cryptococci on substrata not related to man or animal, such as plant material.

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