Yeasts in pigeon feecal droppings in Lisbon - Portugal, 1994

Leveduras em fezes de pombos da cidade de Lisboa - Portugal, 1994

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SUMMARY
In this work, the results of a preliminary survey held in city of Lisbon. Eighty faecal samples were examined between Summer and Autumn, 1994, from twelve different urban areas, mainly near churches and monuments where birds nest, rest or eat. From each sample 1 g was weighted and suspended in 10 ml of destilled sterilized water and consecutive decimal dilutions were executed. Yeasts were enumerated and grouped by species, based on morphological types. On eighty faecal samples the most prevalent yeasts identified were: Candida humicola (51.5%), Candida albicans (48.7%), Cryptococcus neoformans (5%) and Trichosporon cutaneum (37.5%).

UNITERMS: Pigeons; Faeces; Yeasts; Contamination.

INTRODUCTION
Cryptococcosis and Candidosis are non-contagious mycoses that presented an increase in importance and incidence the last ten years. They are generally found associated with immunocompromised patients. Infections are more probable to occur on environments were those agents are abundant, and pigeons droppings are considered very relevant sources of city environment contamination.

The soil samples positives for Cryptococcus neoformans were mostly from areas where pigeons, chickens, and infrequently others birds could be found.

In Portugal human Candidosis and Cryptococcosis are considered indicative of AIDS suspicion, and they represent, respectively, 20% and 2% of opportunist illnesses int those patients.

There is no evidence that Candidiasis and Cryptococcosis are transmissible from animal to animal. The consensus is that man and lower animals contract the infection by exposure to sources in nature. These sources are regarded as major reservoirs of those fungi. The association between wild pigeons and Cryptococcus neoformans is considered to be indirect, because pigeons are considered to be resistant to infection. Apparently, their droppings merely provide a suitable substrate for growth of Cryptococcus neoformans to grow. The multiplication of Cryptococcus neoformans in pigeons excreta has been attributed to the fact that creatinine (which is abundant pigeon droppings), is a favorable substrate. Apparently, creatinine is the source which provides the competitive advantage to Cryptococcus neoformans. The present work was undertaken in order to determine the occurrence potential pathogenic yeasts in pigeon droppings.

MATERIAL AND METHOD

Sample sources
During the Summer and the Autumn of 1994, 80 samples of pigeon faeces were collected from twelve different urban areas of Lisbon, mainly near churches and monuments (old town) where these birds rest or eat.

Sample preparation
It was weighted 1g of each sample and diluted in 10 ml of destilled sterilized water (1/10), mixed on vortex and then serially diluted 1/10.

Cultures
An aliquot of 0.1 ml of each dilution was spread on the surface of Sabouraud Dextrose Agar (SDA - DIFCO 0109) with 25 mg/ml of choramphenicol. Plates were incubated at 30°C for 5 days. Yeasts and moulds were enumerated and grouped according to their morphological types. Representative colonies were transferred to SDA with 0.3% of yeast extract and incubated at 30°C for 5 days.

Identification
Yeasts, were identified using ID 32 C (Bio Merieux - Ref 32290), RAT Medium (Rice Tween Agar - Bio Merieux Ref 9003) to observe typical microstructures (pseudomycelium, mycelium, blastospores, chlamydospores, arthrospores) other complementary biochemical tests (urease, nitrates) were performed.

RESULTS
Candida humicola was the most frequently yeast isolated (51.5%), and Candida albicans was present in 48.7% of the samples. Cryptococcus neoformans was only found in four samples, and the maximum level found was 8x10¹⁰ col/g. Trichosporon cutaneum was reported in 37.5% of samples, in very high levels (valves greater than 10¹⁰ col/g in one sample).

Collected data are summarized in Tab. 1.
Natural occurrence of yeasts on 80 samples of pigeon faecal droppings in Lisbon - Portugal, 1994.

<table>
<thead>
<tr>
<th>STRAINS</th>
<th>Nº POSITIVE</th>
<th>%</th>
<th>MINIMUM</th>
<th>MAXIMUM</th>
</tr>
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<tbody>
<tr>
<td>Candida albicans</td>
<td>39</td>
<td>48.7</td>
<td>1x10²</td>
<td>8.5x10⁴</td>
</tr>
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<td>Candida ciferri</td>
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<td>2.5</td>
<td>2.1x10⁴</td>
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<td>2.5</td>
<td>1x10¹</td>
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<tr>
<td>Candida humilica</td>
<td>41</td>
<td>51.5</td>
<td>2x10⁵</td>
<td>5.8x10⁴</td>
</tr>
<tr>
<td>Candida holmii</td>
<td>6</td>
<td>7.5</td>
<td>1x10⁹</td>
<td>8x10¹</td>
</tr>
<tr>
<td>Candida krusei</td>
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<td>17.5</td>
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<td>1.8x10⁴</td>
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<tr>
<td>Candida lusitaniae</td>
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<td>7.5</td>
<td>2.4x10⁴</td>
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<td>4</td>
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<tr>
<td>Cryptococcus laurentii</td>
<td>20</td>
<td>25</td>
<td>1x10⁹</td>
<td>1.6x10⁴</td>
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<tr>
<td>Cryptococcus neoformans</td>
<td>4</td>
<td>5</td>
<td>1x10⁹</td>
<td>8x10⁴</td>
</tr>
<tr>
<td>Trichosporon cutaneum</td>
<td>30</td>
<td>37.5</td>
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<td>&gt;10⁸</td>
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<tr>
<td>Trichosporon pullulans</td>
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<td>6.25</td>
<td>1x10⁶</td>
<td>53x10³</td>
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<td>Saccharomyces cerevisiae</td>
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<td>Rhodotorula rubra</td>
<td>15</td>
<td>18.7</td>
<td>4x10⁴</td>
<td>1.2x10⁵</td>
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<tr>
<td>Zygosaccharomyces sp.</td>
<td>2</td>
<td>2</td>
<td>2x10⁹</td>
<td>7.4x10⁴</td>
</tr>
</tbody>
</table>

*cfu - colony forming unities*

Note: Moulds also grew in 7.5% of samples (Aspergillus flavus, Aspergillus niger, Mucor spp. Penicillium spp. and Cladosporium sp.).

**DISCUSSION AND CONCLUSIONS**

On 80 samples of pigeon excreta 8 species of Candida, 2 species of Cryptococcus, and one of Saccharomyces cerevisiae, Rhodotorula and Zygosaccharomyces sp. were identified.

**REFERENCES**


Cryptococcus neoformans is commonly referred to in soil and pigeon droppings in old nests and under roosting sites. Staib cited by Kreger Van Rij* (1984) isolated Cryptococcus sp from 28 samples of 201 excreta of birds; Fragner cited by Kreger-Van Rij* (1984) identified Cryptococcus from different excreta: 48 samples from pigeons, 13 from chickens, 10 from pheasants, 7 from mallards and 4 in jarekdaws. Bernardo Raddei; (1994) on 20 samples of pigeon lungs found Candida humicola (41.2%), Candida pintoletesii (29.4%), Trichosporon cutaneum (11.8%) and Candida lipolytica (5.9%). Kamphausen; Raddei; (1992) found Cryptococcus neoformans in 15% of 800 samples of pigeons faeces. Misawa et al.* (1993) found 36.7% positive samples for Cryptococcus neoformans in 30 small birds and Yasin et al.* (1991) isolated 2.4% of Cryptococcus neoformans in 82 birds excreta from aviaries of Kuala Lumpur Zoo. These figures are quite superior to those found in this study. The differences are probably due to some peculiar climatic characteristic, namely: sun hours exposure and ambient temperature.

From the fifteen species of yeasts isolated in this survey, only six of them have been referred in human pathologic conditions and Candida albicans and Cryptococcus neoformans are undoubtedly relevant int terms of Public and Animal health, although some complementary studies with epidemiological markers should be undertaken.

This survey suggests the importance of pigeon droppings as a natural source of environmental contamination of urban sites by potentially pathogenic yeasts.

**Acknowledgments**

The authors are specially grateful to Anabela Ramos and Candida Camejo for their commitment on the technical assistance.

**RESUMO**

Este trabalho apresenta o levantamento da ocorrência natural de levedura em fezes de pomos de cidade. Oitenta amostras de fezes foram colhidas, durante o verão e o outono de 1994, em 12 diferentes pontos de cidade de Lisboa, próximos dos locais onde os pomos se nidificam, se alimentam e se abrigam. As amostras foram suspensas em água destilada (1g em 10ml) e efetuaram-se diluições decimais; para efeito de contagem foi utilizado Agar Sabouraud Dextrose. A identificação das espécies de levedura foi baseada nas características macro e microscópicas típicas e testes bioquímicos complementares. Foram identificadas oito espécies de Candida, duas de Cryptococcus e Trichosporon e uma de Saccharomyces, Rhodotorula rubra e Zygosaccharomyces respectivamente. As de maior prevalência foram: Candida humicola (51.5%), Candida albicans (48.7%), Cryptococcus neoformans (5%) e Trichosporon cutaneum (37.5%). Os dados confirmam a importância das fezes de pombo como uma fonte natural de contaminação ambiental dos locais públicos por leveduras potencialmente patogênicas.

**UNITERMOS:** Pombos; Fezes; Leveduras; Contaminação.