Braz. J. vet. Res. anim. Sci.,
São Paulo, v. 34, n. 5, p. 259-260, 1997

Yeasts in pigeon feacal droppings in Lisbon - Portugal, 1994

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

CORRESPONDENCE TO:
Hermínia Marina Lourdes Martins
Laboratorio Nacional de Veterinaria
Estrada de Benfica 701 - 1500
Lisboa - Portugal

1 - Laboratorio Nacional de Veterinaria - Lisboa, Portugal
2 - Rua Gomes Freire, 1199 - Lisboa, Codex, Portugal

Hermínia Maria Lourdes MARTINS[1]; Fernando Manuel Almeida BERNARDO[2]; Maria Lígia Lourdes MARTINS[1]

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 churchs and monuments where birds nest, rest or eat. From each sample 1 g was weighted and suspensed in 10 ml of destilled sterilized water and consecutive decimal diluitions 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[8] 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[4].

There is no evidence that Candidosis 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[9]. These sources are regarded as major reservoirs of those fungis. 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 substratum. Apparently, creatinine is the source which provides the competitive advantage to *Cryptococcus neoformans*. The present work was undertaken in order to determine the occurrence potencial 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 churchs 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 destiled 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 transfered 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 $8x10^3$ col/g. *Trichosporon cutaneum* was repported in 37.5% of samples, in very high levels (valves greater than 10^8 col/g in one sample).

Collected data are summarized in Tab. 1.

Table 1
Natural occurrence of yeasts on 80 samples of pigeon faecal droppings in Lisbon - Portugal, 1994.

STRAINS	Nº POSITIVE SAMPLES	%	MINIMUM cfu*/g	MAXIMUM cfu*/g
Candida albicans	39	48.7	$1x10^2$	$8.5x10^7$
Candida ciferrii	2	2.5	$2.1x10^2$	$3.2x10^3$
Candida catenulata	2	2.5	$1x10^4$	$2.5x10^4$
Candida humicola	41	51.5	$2x10^2$	$5.8x10^6$
Candida holmii	6	7.5	$1x10^2$	$8x10^3$
Candida krusei	14	17.5	$1.5x10^2$	$1.8x10^6$
Candida lusitaniae	6	7.5	$2.4x10^3$	$13.4x10^5$
Candida parapsilosis	4	5	$32x10^4$	$5.6x10^5$
Cryptococcus laurentii	20	25	$1x10^2$	$1.6x10^6$
Cryptococcus neoformans	4	5	$1x10^2$	$8x10^3$
Trichosporon cutaneum	30	37.5	$2x10^2$	$>10^8$
Trichosporon pullulans	5	6.25	$1x10^2$	$53x10^4$
Saccharomyces cerevisiae	2	2.5	$8x10^3$	$3x10^4$
Rhodotorula rubra	15	18.7	$4x10^2$	$1.2x10^5$
Zygossaccharomyces sp.	2	2.5	$20x10^4$	$7.4x10^5$

*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 *Zygossaccharomyces* ssp. were identified.

Cryptococcus neoformans is commonly refered in soil and pigeon droppings in old nests and under roosting sites. Staib cited by Kreger-Van Rij[6] (1984) isolated *Cryptococcus sp* from 28 samples of 201 excreta of birds; Fragner cited by Kreger-Van Rij[6] (1984) identified *Criptococcus* from different excreta: 48 samples from pigeons, 13 from chickens, 10 from pheasants, 7 from swallows and 4 in jarckdaws. Bernardo Raddei[3]; (1994) on 20 samples of pigeon lungs found *Candida humicola* (41.2%), *Candida pintolopesii* (29.4%), *Trichosporon cutaneum* (11.8%) and *Candida lipolytica* (5.9%). Kamphausen; Raddei[5] (1992) found *Cryptococcus neoformans* in 15% of 800 samples of pigeons faeces. Misawa *et al.*[8] (1993) found 36.7% positive samples for *Cryptococcus neoformans* in 30 small birds and Yasin *et al.*[10] (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 refered 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 potencially 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 pombos 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 pombos 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 leveduras 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 *Zygossaccharomyces* 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.

REFERENCES

1 - AINSWORTH, G.C.; AUSTWICK, P.K.C. **Fungal diseases of animals**. 2. ed. Farnham Royal: Commonwealth Agricultural Bureaux, 1973. p. 469-78.

2 - AJELLO, L.; KAPLAN, W. **Systemic mycoses**. Florida: Boca Raton, 1981. p. 469-85. (Handbook Series in Zoonoses).

3 - BERNARDO, F.M.A.; RODEIA, S.C.; MARTINS, H.M. Criptococose em pombos urbanos: aspectos nosoepidemiológicos. **Revista Portuguesa de Ciências Veterinárias**, v. 89, n. 511, p. 132-38, 1994.

4 - FERREIRA, M.O.; ABREU, J.N.; GONÇALVES, J.A.; COSTA, J.B. **Definição de caso SIDA para fins de vigilância epidemiológica**. Lisboa: Comissão Nacional de Luta Contra a SIDA, 1994. p. 1-21. (Documento, 77)

5 - KAMPHAUSEN, L.; RADDEI, J. B. *Cryptococcus neoformans* in samples of faeces from racing pigeons. **Tagung der Fachgruppe "Geflugelkrankheiten"**, p. 5-6, 1992.

6 - KREGER-VAN RIJ, N. J. W. **The yeasts a taxonomic study**. 3. ed. Amsterdam: Elsevier , 1984. 1082 p.

7 - KWON-CHUNG, K.J.; BERNNETT, J.E. ***Cryptococcosis* - medical mycology**. Philadelphia: Lea & Febiger, 1992. p. 397-446.

8 - MISAWA, N.; NYUTA, S.; UTOH, K.; KONDO, F. Isolation of *Cryptococcus neoformans* from excreta of small birds in pet shops. **Journal of the Japan Veterinary Medical Association**, v. 46, n.5, p. 417-9, 1993.

9 - SHADOMY, H.J.; WARREN, N.G. **Cryptococcosis Laboratory diagnosis of infections diseases. Principles and practice**. New York: Springer, 1998. p. 624-30.

10 - YASIN, M.S.; SAMAD, S.A.; VELLAYAN, S. Isolation of *Cryptococcus neoformans* in the aviaries of the National Zoo, Kuala Lumpur. **Trop. Biomedicine**, v. 8, n. 1, p. 27-32, 1991.

Received: 5/6/95
Accepted: 3/12/96