Occurrence of *Cryptococcus neoformans* and other yeast-like fungi in environmental sources in Bonaire (Dutch Caribbean)

Harish C Guignani^{1,*}, Ferry Hagen², Jacques F Meis³, Arunaloke Chakrabarti⁴

Abstract

Introduction We report here the presence of *Cryptococcus neoformans*, and other potentially pathogenic yeasts and yeast-like fungi in environmental sources in Bonaire.

Methods Seventy environmental samples comprising 40 samples of old pigeon droppings, 18 of woody debris from hollows of living trees of *Caesalpinia* ('Divi Divi'), *Ziziphus jujuba* (Red Indian date), *Tamarindus indica* (Tamarind), *Terminalia catappa* (Tropical almond), *Azadirachta indica* (Neem) and 3 of other unidentified species of trees, 3 of latex from a rubber tree and 6 of coral dust were processed for isolation of pathogenic *Cryptococcus* spp. and other potentially pathogenic yeasts and yeast-like fungi. A variety of mycological media were employed. Identification of the isolates was done with conventional techniques and species identification was done by matrix-assisted laser desorption ionization-time of flight mass spectrometry.

Results Three of the 40 samples from old pigeon droppings yielded *Cryptococcus neoformans*, constituting the first record of environmental occurrence of this important pathogenic yeast in the Dutch Caribbean. Other potentially pathogenic yeasts and yeast-like fungi recovered from these environmental samples included 6 isolates each of *Candida albicans*, 8 of *Candida parapsilosis*, 4 each of *Candida metapsilosis* and *Candida orthopsilosis*, 2 each of *Candida carpophila*, *Candida famata*, *Candida fabianii* and *Candida pelliculosa*, 7 of *Candida* spp., 5 of *Trichosporon* spp. and 2 of *Sporobolomyces* spp.

Conclusions This study has demonstrated for the first time the occurrence of *C. neoformans* in a natural habitat in the Dutch Caribbean. The recovery of many species of potentially pathogenic yeast-like fungi and yeasts from environmental sources is remarkable.

Keywords Environmental yeasts, *Cryptococcus neoformans*, *Candida* spp., *Trichosporon* spp., *Sporobolomyces* spp.

Introduction

Members of the basidiomycetous fungal pathogen *Cryptococcus* genus are known to occur in a variety of ecological niches, such as soil, pigeon droppings, and tree hollows in many countries e.g., Argentina, Brazil, Colombia, India, Kenya, Korea, and South Africa.^{1,2} There

is lack of information on their ecology in the Caribbean region, especially in the Dutch Caribbean. So far there is only a solitary report about the recovery of *C. gattii* from woody debris, collected from inside trunk hollows of a living Divi-Divi tree (*Caesalpinia coriaria*) in Bonaire.³ Occurrence of *C. neoformans* has so far not been

Received: 11 July 2020; revised: 15 August 2020; accepted: 21 August 2020.

¹PhD, FRC. Path. Retd. Professor & Head, Med. Mycology Unit, Department of Microbiology, Vallabhbhai Patel Chest Institute, University of Delhi, J 3/45, Rajouri Garden, New Delhi 110027, India, Emeritus Professor, Saint James School of Medicine, Anguilla, BWI; ²PhD, Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands, Canisius-Wilhelmina Hospital, Weg door Jonkerbos 100, 6532 SZ, Nijmegen, Gelderland, Netherlands; ³MD, PhD, FRC. Path., FIDSA, (F)AAM, (F)ECMM, Consultant, Department of Medical Microbiology and Infectious Diseases Canisius-Wilhelmina Hospital, Weg door

Jonkerbos 100, 6532 SZ, Nijmegen, Gelderland, Netherlands; ⁴MD, DipNB, Professor & Head, Department of Medical Microbiology, Post-graduate Institute of Medical Sciences & Research, Sector 12, Chandigarh-160012, India.

*Corresponding author: Harish C Guignani, harish.guignani@gmail.com

Article downloaded from www.germs.ro

Published September 2020

© GERMS 2020

ISSN 2248 - 2997

ISSN - L = 2248 - 2997

reported from the Dutch Caribbean. This communication reports the isolation of *Cryptococcus neoformans* and other yeast-like fungi from environmental sources in Bonaire.

Methods

Sampling

Forty samples of desiccated pigeon droppings, 21 samples of woody debris from the hollows of different species of trees from several localities, viz. Ricon Village, Hato Village, Kralendijk, Klein Bonaire, and Marine Beach along Leeuwarden, three of latex from a rubber tree and 6 of dust deposit in the corals along the marine coast were collected.

About 5 grams of each sample were suspended in 10 mL of sterile saline and vortexed. One mL quantities of suspension of each sample were spread on Petri plates of tobacco agar and Bird Seed (*Guizotia abyssinica*) Agar (HiMedia Laboratories, Mumbai, India), and incubated at 22-28°C for up to 7 days. There was no facility in our laboratory to incubate the inoculated samples at 37°C. Tobacco agar was prepared as recommended by Tendolkar et al.⁴ Fifty g of tobacco leaves purchased from a local market in Delhi, India were added to one liter of distilled water, boiled for 30 minutes, filtered through gauze and the volume was adjusted to one liter. To this were added 20 g agar, the medium was boiled until the agar dissolved, autoclaved at 121°C for 15 minutes and poured in plates. Chloramphenicol (0.05 mg/mL) and diphenyl (0.1%) were added to these media to cut down contamination due to bacteria and saprophytic molds respectively. We were able to see single brownish colonies suspected to be *Cryptococcus*, not overgrown by other molds in many of the inoculated plates. These and whitish mucoid colonies suggestive of *Candida*, and colonies suggestive of other yeasts were sub-cultured onto slants of Sabouraud dextrose agar supplemented with chloramphenicol (0.05 mg/mL) to get pure cultures. In some of the culture plates saprophytic molds overgrew on colonies of yeasts while in some other plates they appear as discrete colonies. These molds including species of *Aspergillus*, *Penicillium*,

Fusarium, *Cladosporium*, *Alternaria* and *Curvularia* were similarly sub-cultured to obtain pure cultures.

Identification of isolates

The isolates suspected to be *Cryptococcus neoformans* were tentatively identified by study of their morphological characters, and standard biochemical tests, using VITEK2 and API 20C AUX (bioMérieux, Marcy l'Étoile, France), with no change in color to blue on inoculating the plates of canavanine glycine bromothymol blue (CGB) medium (Hardy Diagnostics, Santa Maria, CA, USA). The identity of the isolates as *C. neoformans* was confirmed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) analysis.¹ Cryptococcal mating type and serotype were determined by qPCR using four PCRs that specifically amplify the *STE20a* and *STE20α* alleles that belong to serotype A and D as described by Arsenijevic et al.⁵ The majority of *Candida* isolates were identified by MALDI-TOF at Post-graduate Institute of Medical Sciences & Research, Chandigarh, India.

Some of the isolates of saprophytic molds, which grew on culture plates inoculated with samples were identified as *Aspergillus niger*, *A. fumigatus*, *A. flavus*, *A. candidus*, *Penicillium citrinum*, *Cladosporium cladosporioides*, *Alternaria alternata* and *Curvularia lunata*. Since the aim of our study was to investigate natural occurrence of *Cryptococcus* and other potentially pathogenic yeasts, no attempt was made to identify other isolates of saprophytic molds.

Results

Cryptococcal isolates were identified as *Cryptococcus neoformans* (serotype A, mating-type α) and were recovered from three out of 40 samples of pigeon excreta examined from several locations in Bonaire. The positive samples originated from accumulated excreta under a pigeons' roosting site on the top of a window air conditioner at the back of the Faculty lounge at a medical school in Bonaire. All the remaining 37 samples of pigeon droppings collected from several other locations in Bonaire were negative

for *C. neoformans*. Several isolates of other yeast-like fungi, viz. *Candida albicans*, *Candida parapsilosis*, *Candida orthopsilosis*, *Candida carpophila*, *Candida famata*, *Candida metapsilosis*, *Candida pelliculosa*, *Sporobolomyces* and *Trichosporon* were recovered from different types of environmental samples examined (Table 1).

Discussion

There are only a few reports on the occurrence of pathogenic cryptococci in natural sources in the Caribbean region. Seventeen isolates of *C. gattii* were recovered from cacti and tree detritus in Puerto Rico; 16 of these belonged to VGII/AFLP6 genotype while one isolate was a VGIV/AFLP7 genotype.⁶ Until now only two cases of infections due to *C. gattii* have been reported in Cuba: one from an HIV-negative patient, and the other one from a cheetah at the

National Zoo in Havana.⁷ Eighty-six *C. neoformans* var. *grubii* isolates were recovered from 222 pigeon houses across Cuba;⁷ characterization of these isolates from pigeon excreta, and those from clinical samples by conventional, serological and molecular methods using high-resolution techniques (AFLP, sequencing D1/D2 and ITS1/5.8S/ITS2 fragments and conventional PCR using STE12alpha- and STE12a-specific primers) confirmed the dominance of *C. neoformans* var. *grubii* mating-type MATa.⁷ Vanhove et al.⁸ showed that *C. neoformans* can be divided into three major lineages, with the first two VNI and VNII being globally distributed, whilst the third one VNB seems to be restricted to Southern Africa.

There is a solitary report of recovery of *C. deuterogattii* from 10 samples of woody debris, collected from the inside trunk hollows of a

Table 1. Occurrence of *C. neoformans* and other yeasts in environmental sources in Bonaire (Dutch Caribbean)

Type of sample	Number examined	Species of yeast	Number of isolates
Desiccated pigeon droppings	40	<i>Cryptococcus neoformans</i>	3
		<i>Candida albicans</i>	5
		<i>Candida orthopsilosis</i>	5
		<i>Candida parapsilosis</i>	4
		<i>Candida carpophila</i>	4
		<i>Candida famata</i>	2
		<i>Candida metapsilosis</i>	2
		<i>Candida pelliculosa</i>	2
		<i>Candida</i> spp.	2
		<i>Trichosporon</i> spp.	4
Woody debris from the hollows of Divi tree (<i>Caesalpinia coriaria</i>)	10	<i>Candida metapsilosis</i>	2
		<i>Candida parapsilosis</i>	2
		<i>Candida</i> spp.	2
		<i>Sporobolomyces</i> spp.	2
		<i>Candida</i> spp.	1
Woody debris from the hollows of tropical almond (<i>Terminalia catappa</i>)	2	<i>Trichosporon</i> spp.	1
Woody debris from the hollows of Red date (<i>Ziziphus jujuba</i>)	2	<i>Candida</i> spp.	1
Woody debris from the hollows of Tamarind (<i>Tamarindus indica</i>)	2	<i>Candida</i> spp.	2
Woody debris from the hollows of Neem (<i>Azadirachta indica</i>)	2	<i>Candida</i> spp.	2
Woody debris from the hollows of other trees	3	<i>Candida</i> spp.	2
Dust in corals	6	<i>Candida parapsilosis</i>	2
		<i>Candida</i> spp.	2
Latex from the <i>Hevea brasiliensis</i>	3	<i>Candida fabianii</i>	2
		<i>Candida</i> spp.	1

living Divi-Divi tree (*Caesalpinia coriaria*) in Bonaire.³ Molecular typing of these 10 isolates showed that they all were of mating-type α and genotype AFLP6/VGII, being indistinguishable from each other.

Our study reports for the first time the occurrence of *C. neoformans* from an environmental source in the Dutch Caribbean. Several repeated attempts by the senior author to isolate *Cryptococcus* from woody debris in the hollows of Divi-Divi and other species of trees in several locations in Bonaire were unsuccessful. The current study and the previous report from Bonaire,³ which mentioned an earlier report in 1953 of isolation of *C. gattii* (isolate no CBS1930 in the culture collection) from a child in Aruba indicate possible sources of human cases of cryptococcosis caused by members of the *C. neoformans*/*C. gattii* species complexes in Bonaire and other islands in the Dutch Caribbean. It may be mentioned that common *Eucalyptus* trees, *Eucalyptus camaldulensis* and *E. tereticornis*, known to be frequently associated with *C. neoformans* and *C. gattii*^{9,10} were not seen in the vegetation of Bonaire. A search of literature also did not reveal their presence in other islands in the Dutch Caribbean. The other type of *Eucalyptus* trees, viz. Rainbow *Eucalyptus*, Baby blue *Eucalyptus*, *Eucalyptus pulverulenta* (blue spiral *Eucalyptus*) are seen in Bonaire. These trees do not have hollows; thus, we did not sample any of them for *Cryptococcus* spp. and other yeast-like fungi. Failure to re-isolate *C. gattii* in our study may be due to lack of its persistent occurrence in Divi-Divi trees in Bonaire, thus emphasizing the need to investigate its presence in other natural sources in Bonaire and other countries in the Dutch Caribbean.

It is noteworthy that a variety of other yeast-like fungi including several species of *Candida* were recovered in our study. The importance of pigeon excreta in natural occurrence of *C. neoformans* is well known. Presence of other potentially pathogenic yeasts in pigeon excreta and other environmental sources is often not recognized. In a recent study from Gran Canaria Island,¹² several species of *Candida* and other yeasts, viz. *Candida albicans*, *C. guilliermondii*, *C.*

kefyr, *C. pelliculosa*, *C. parapsilosis*, *C. rugosa*, *Trichosporon* and *T. asahii*, *T. mucoides* were isolated from pigeon excreta. It is noteworthy that in our study isolates of *C. albicans*, *C. parapsilosis*, *C. pelliculosa*, *C. orthopsilosis*, *C. carpophila*, *C. famata*, and *C. metapsilosis* were recovered from samples of pigeon excreta, the association of the latter four species with pigeon excreta being demonstrated for the first time. *C. parapsilosis* is a human commensal¹³ and is known to be associated with nosocomial outbreaks of candidemia.^{14,15} This species is now recognized as an emerging major human pathogen that has drastically increased in importance and prevalence over the past two decades. Infections are particularly associated with solutions for hyperalimentation, prosthetic devices, indwelling catheters, and with the nosocomial spread through the hands of healthcare workers.¹⁶ *C. famata* is echinocandin and azole resistant and has been described as a cause of several types of infections, including bloodstream catheter-related infections, peritonitis, acute zonal occult retinopathy and mediastinitis.^{17,18} Among other species of *Candida* recovered in the study, *C. orthopsilosis* and *C. metapsilosis* have been shown to be virulent in reconstituted human tissue models.²⁰ A study from Brazil reported frequent occurrence of *C. parapsilosis*, occasional presence of *C. famata*, *C. metapsilosis* and *C. orthopsilosis* on the hands of healthcare workers in a neonatal intensive care unit.²⁰ *C. parapsilosis* has also been isolated from pine trees,^{21,22} and from soil.²² Its isolation from the woody debris of the hollows of Divi-Divi trees and from dust in the coral, in our study is noteworthy. *C. parapsilosis* and other *Candida* species recovered here could have possibly transmitted from soil to old pigeon excreta, and woody debris of in the hollows of living trees.

Environmental occurrence of *Histoplasma capsulatum* in the environmental sources, and cases of human infection caused by this fungus in several countries in other parts of the Caribbean have been reviewed by Denning and Gugnani.²³

Conclusions

There is need for comprehensive investigation of the occurrence of *C. neoformans* and *C. gattii* species complexes, as well as that of other pathogenic fungi like *Histoplasma* in the environment and cases of human infections due to these fungi in the Dutch Caribbean.

Authors' contributions statement: HCG did the sampling and preliminary identification of *C. neoformans* isolates and some *Candida* isolates. FH and JFM confirmed the identification of *C. neoformans* isolates and a few *Candida* isolates by MALDI-TOF. They also determined the mating and serotype of the isolates of *C. neoformans* isolates. AC identified the species in most of the *Candida* isolates by MALDI-TOF. All authors read and approved the final version of the manuscript.

Conflicts of interest: All authors – none to declare.

Funding: None to declare.

Acknowledgements: The senior author is grateful to Prof. Kalol Guha, President and CEO of Saint James School of Medicine, Bonaire and Anguilla, for providing the laboratory facilities.

References

- Hagen F, Khayhan K, Theelen B, et al. Recognition of seven species in the *Cryptococcus gattii*/*Cryptococcus neoformans* species complex. *Fungal Genet Biol*. 2015;78:16-48. <https://doi.org/10.1016/j.fgb.2015.02.009>
- Mitchell TG, Castañeda E, Nielsen K, Wanke B, Lazéra MS. Environmental niches for *Cryptococcus neoformans* and *Cryptococcus gattii*. In: Heitman J, Kozel TR, Kwon-Chung KJ, Perfect JR, Casadevall A (eds). *Cryptococcus: from human pathogen to model yeast*. Washington, DC, USA: ASM Press. 2011. pp. 237-59. <https://doi.org/10.1128/9781555816858.ch18>
- Hagen F, Chowdhary A, Prakash A, Yntema JB, Meis JF. Molecular characterization of *Cryptococcus gattii* genotype AFLP6/VGII isolated from woody debris of divi-divi (*Caesalpinia coriaria*), Bonaire, Dutch Caribbean. *Rev Iberoam Micol*. 2014;31:193-6. <https://doi.org/10.1016/j.riam.2013.10.007>
- Tendolkar U, Tainwala S, Jog S, Mathur M. Use of a new medium - tobacco agar, for pigment production of *Cryptococcus neoformans*. *Indian J Med Microbiol*. 2003;21:277-9.
- Arsic Arsenijevic V, Pekmezovic MG, Meis JF, Hagen F. Molecular epidemiology and antifungal susceptibility of Serbian *Cryptococcus neoformans* isolates. *Mycoses*. 2014;57:380-7. <https://doi.org/10.1111/myc.12171>
- Loperena-Alvarez, Y, Ren P, Li X, et al. Genotypic characterization of environmental isolates of *Cryptococcus gattii* from Puerto Rico. *Mycopathologia*. 2010;170:279-85. <https://doi.org/10.1007/s11046-010-9296-3>
- Illnait-Zaragozi MT, Martínez-Machín GF, Fernández-Andreu CM, Perurena-Lancha MR, Hagen F, Meis JF. *Cryptococcus* and cryptococcosis in Cuba. A minireview. *Mycoses*. 2014;57:707-17. <https://doi.org/10.1111/myc.12275>
- Vanhove M, Beale MA, Rohdes J, et al. Genomic epidemiology of *Cryptococcus* yeasts identifies adaptation to environmental niches underpinning infection across an African HIV/AIDS cohort. *Mol Ecol*. 2017;26:1991-2005. <https://doi.org/10.1111/mec.13891>
- Pfeiffer TJ, Ellis DH. Environmental isolation of *Cryptococcus neoformans* var. *gattii* from *Eucalyptus tereticornis*. *J Med Vet Mycol*. 1992;30:407-8. <https://doi.org/10.1080/02681219280000541>
- Gugnani HC, Mitchell TG, Litvintseva AP, Lengeler K, et al. Isolation of *Cryptococcus gattii* and *C. neoformans* var. *grubii* from the flowers and bark of *Eucalyptus* trees in India. *Med Mycol*. 2005;43:565-9. <https://doi.org/10.1080/13693780500160785>
- Rosario Medina I, Román Fuentes L, Batista Arteaga M, et al. Pigeons and their droppings as reservoirs of *Candida* and other zoonotic yeasts. *Rev Iberoam Micol*. 2017;34:211-4. <https://doi.org/10.1016/j.riam.2017.03.001>
- Njoku-Obi AN, Okofor JI, Gugnani HC. Yeast-like fungi recovered from normal human skin in Nuskka (Nigeria). *Antonie van Leeuwenhoek*. 1976;42:101-5. <https://doi.org/10.1007/BF00399453>
- Asadzadeh M, Ahmad S, Al-Sweih N, Hagen F, Meis JF, Khan Z. High-resolution fingerprinting of *Candida parapsilosis* isolates suggests persistence and transmission of infections among neonatal intensive care unit patients in Kuwait. *Sci Rep*. 2019;9:1340. <https://doi.org/10.1038/s41598-018-37855-2>
- Martini C, Torelli R, de Groot T, et al. Prevalence and clonal distribution of azole-resistant *Candida parapsilosis* isolates causing bloodstream infections in a large Italian hospital. *Front Cell Infect Microbiol*. 2020;10:232. <https://doi.org/10.3389/fcimb.2020.00232>
- Trofa D, Gácsér A, Nosanchuk JD. *Candida parapsilosis*, an emerging fungal pathogen. *Clin Microbiol Rev*. 2008;21:606-25. <https://doi.org/10.1128/CMR.00013-08>
- Ahmed IM, Gupta A, Gould K, Clark SC. A fatal fungus. *Ann Thorac Surg*. 2005;80:723-4. <https://doi.org/10.1016/j.athoracsur.2004.02.019>
- Gupta A, Mi H, Wroe C, Jaques B, Talbot D. Fatal *Candida famata* peritonitis complicating sclerosing peritonitis in a peritoneal dialysis patient. *Nephrol Dial Transplant*. 2006;21:2036-7. <https://doi.org/10.1093/ndt/gfl040>
- Pisa D, Ramos M, García P, et al. Fungal infection in patients with serpiginous choroiditis or acute zonal occult outer retinopathy. *J Clin Microbiol*. 2008;46:130-5.

-
- <https://doi.org/10.1128/JCM.02605-06>
19. Gácsér A, Schäfer W, Nosanchuk JS, Salomon S, Nosanchuk JD. Virulence of *Candida parapsilosis*, *Candida orthopsilosis*, and *Candida metapsilosis* in reconstituted human tissue models. Fungal Genet Biol. 2007;44:1336-41.
<https://doi.org/10.1016/j.fgb.2007.02.002>
 20. de Paula Menezes R, Silva FF, Melo SGO, et al. Characterization of *Candida* species isolated from the hands of the healthcare workers in the neonatal intensive care unit. Med Mycol. 2019;57:588-94.
<https://doi.org/10.1093/mmy/myy101>
 21. Maganti HB. Species and genotype diversities of yeasts in the clinical and natural environments in Hamilton. A Thesis submitted to the School of Graduate Studies in partial fulfillment of the requirements for the degree Master of Science, McMaster University, 2011.
 22. Azcón R, Perálvarez Mdel C, Roldán A, Barea JM. Arbuscular mycorrhizal fungi, *Bacillus cereus*, and *Candida parapsilosis* from a multi contaminated soil alleviate metal toxicity in plants. Microb Ecol. 2010;59:668-77.
<https://doi.org/10.1007/s00248-009-9618-5>
 23. Denning DW, Gugnani HC. Burden of serious fungal infections in Trinidad and Tobago. Mycoses. 2015;58(Suppl 5):80-4.
<https://doi.org/10.1111/myc.12394>
 21. Maganti HB. Species and genotype diversities of yeasts in the clinical and natural environments in Hamilton. A Thesis submitted to the School of Graduate Studies in

Please cite this article as:

Gugnani HC, Hagen F, Meis JF, Chakrabarti A. Occurrence of *Cryptococcus neoformans* and other yeast-like fungi in environmental sources in Bonaire (Dutch Caribbean). GERMS. 2020;10(3):195-200. doi: 10.18683/germs.2020.1205