Multilocus sequence typing of the agents of cryptococcosis in the Rio Negro river basin, Brazilian Amazon region

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INTRODUCTION

Cryptococcosis is a life-threatening systemic mycosis affecting humans and animals worldwide. The disease is acquired by inhalation of infectious propagules of the *Cryptococcus neoformans*/*C. gattii* species complexes from the environment (1,2). Pioneering environmental studies in connection with cryptococcosis in AIDS in Central Africa and in Brazil demonstrated that individuals living in contaminated dwellings are at risk to acquire cryptococcosis from indoor contamination (3,4). In the heart of the Amazon rainforest, a study revealed the occurrence of *C. gattii* VGII in dust samples from dwellings and wood poles in a remote human settlement in the Rio Negro Microregion, Amazonia, Brazil. The high density of cryptococcal colonies suggested that the local inhabitants are daily exposed to the yeast (5).

METHODS

Figure 1: Study area. The study was conducted in the Micro region of Rio Negro that composed of four municipalities (Barcelos, Novo Airão, Santa Isabel do Rio Negro and São Gabriel da Cachoeira), this Micro region has a population of 110,602 inhabitants (according to IBGE 2015 estimates) and an area of 332.278,183 km².

Figure 2: Methods applied to study the 89 samples resulting in 9 isolates.

RESULTS

Table 1: List of environmental samples collected and identified the according to the phenotypic identification (BSA, Urea and CGB) and molecular characterization (type URA5-RFLP, Mating type and MLST type).

Municipalities	Environmental sample	Sample collected	Sample positive	Frequency of positivity (%)	Molecular type isolate	MLST
Santa Isabel do Rio Negro	Dust indoor	51	3	5.8	VGII	VGIIa, VGIIb, VGIISt5 and News ST's (264,265,266,267, and 268)
Barcelos	Wood Poles	10	2	20	VNI	VNI ST5 and VNI ST93
Novo Airao	Dust indoor	12	2	16	VNI	VNI ST5 and VNI ST93
	Dust indoor	16	2	12.5	VGII	VGIIb and New ST (VGII ST445)
					VNI	VNI ST5
All Municipalities	N/A	89	9	10.1	N/A	N/A

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Figure 3: *C. gattii* isolates after inoculation of *G. mellonella* larvae

Figure 4: SplitsTree analysis of the combined MLST loci showing the placement of the 9 isolated strain (numbers in bold) from the Micro Region of Rio Negro in context with the sequence types obtained form the global *C. gattii* VGII population.

Figure 5: Survival curves of with *C. neoformans* and *C. gattii* infected *Galleria mellonella* larvae. *G. mellonella* larvae infected with *C. gattii* VGII resulted in a more rapid death compared to the ones infected with *C. neoformans* VNI.

CONCLUSION

Analysis of indoor dust/wood pole samples revealed the presence of VGII and VNI. Population genetics analysis showed the presence of the Vancouver Island outbreak genotypes ST20 (VGIIa) and ST7 (VGIIb) in the Amazon region, and identified additional genetic diversity, including the most common global *C. neoformans* genotypes ST5 and ST93. Preliminary virulence studies in the *G. mellonella* model showed that environmental isolates of *C. gattii* VGII have a higher death rate then *C. neoformans*, and a such could present a major risk of infection.