

The status of cryptococcosis in Latin America

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Cryptococcosis is a life-threatening fungal infection caused by the encapsulated yeasts *Cryptococcus neoformans* and *C. gattii*, acquired from the environment. In Latin America, as occurring worldwide, *C. neoformans* causes more than 90% of the cases of cryptococcosis, affecting predominantly patients with HIV, while *C. gattii* generally affects otherwise healthy individuals. In this region, cryptococcal meningitis is the most common presentation, with amphotericin B and fluconazole being the antifungal drugs of choice. Avian droppings are the predominant environmental reservoir of *C. neoformans*, while *C. gattii* is associated with several arboreal species. Importantly, *C. gattii* has a high prevalence in Latin America and has been proposed to be the likely origin of some *C. gattii* populations in North America. Thus, in the recent years, significant progress has been made with the study of the basic biology and laboratory identification of cryptococcal strains, in understanding their ecology, population genetics, host-pathogen interactions, and the clinical epidemiology of this important mycosis in Latin America.

Key words: *Cryptococcus neoformans* - *Cryptococcus gattii* - Latin America - cryptococcosis

In Latin America, the study of cryptococcosis and its etiological agents has become increasingly important, as this mycosis has significant morbidity and mortality, with more than 5,000 individuals affected with cryptococcal meningitis each year, and 2,400 attributable annual deaths in Latin America alone. The main goal of this review is to present recent and relevant data regarding the studies done in Latin American countries on cryptococcosis and the etiological agents *Cryptococcus neoformans*, and *C. gattii*. Topics related to epidemiology, clinical data and treatment, molecular studies on clinical and environmental isolates, host-pathogen interactions, laboratory data on antifungal susceptibility, preliminary data detecting antigenemia in patients, and studies to search for the environmental niche of the fungus are presented here.

Epidemiology - *C. neoformans* is the most important cause of fungal meningitis in the world, and in sub-Saharan Africa, meningeal cryptococcosis is the most common type of meningitis in adults infected with HIV (Rajasingham et al. 2017). From a total of 223,100 cases of meningeal cryptococcosis that were estimated to have occurred globally in people living with HIV in 2014, the third largest number of cases in the world were from Latin America, with an estimated incidence of 5,300 cases per year. From those, Brazil and Colombia were the countries

with the highest incidence, between 1,001 to 2,500 cases, followed by Argentina and Mexico with an incidence of 501 to 1,000 cases (Rajasingham et al. 2017). In particular, an average annual incidence of 4.5 cases of meningeal cryptococcosis per 10⁶ inhabitants was reported in the general population of the state of Rio de Janeiro, Brazil (Leimann and Koifman 2008). In Colombia, the average annual incidence of cryptococcosis in the general population has been estimated to be 2.4 cases per 10⁶ inhabitants, while in the HIV-infected population this value has been estimated to be 3,000-3,300 cases per 10⁶ people (Lizarazo et al. 2007, Escandón et al. 2012). In addition, Lizarazo et al. (2014a) reported an average annual incidence of 0.0017 cases per 10⁶ in Colombian children.

There is little data regarding the prevalence of cryptococcosis in Latin America. In Mexico, a study of the etiological agents of meningoencephalitis found that the most common mycosis was cryptococcosis, with a prevalence of 10% (Barriga et al. 2005). In Venezuela, a study of systemic mycoses performed at a national reference centre found that cryptococcosis ranked third with a prevalence of 19%, following histoplasmosis and paracoccidioidomycosis; however, in the population with HIV, *C. neoformans* was isolated in 27% of the cases, following the etiological agent of histoplasmosis (Reviákina et al. 2007). A national survey conducted in Argentina identified cryptococcosis as the second most frequent deep fungal infection with a prevalence of 20%, following that of yeast fungemia (Davel and Canteros 2007). In Mexico, *C. neoformans* had a prevalence of 21% causing fungemia in patients with different types of immunosuppression, only following *Histoplasma capsulatum* (Gaona-Flores et al. 2016). In the same country, cryptococcosis was the third most common invasive fungal

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infection (13%) found in a highly specialised hospital, following candidiasis and mucormycosis (Méndez-Tovar et al. 2016). In Colombia, Castro-Jiménez et al. (2011) reported cryptococcosis as the most common opportunistic mycosis in patients with HIV/AIDS, with a prevalence of 76%. In the same population in Guatemala, it was estimated that meningeal cryptococcosis is the fourth most common opportunistic fungal infection following candidiasis (esophageal), *Pneumocystis jirovecii* pneumonia, and disseminated histoplasmosis (Medina et al. 2017).

In Colombia, among opportunistic infections of the central nervous system (CNS) in patients with HIV, cryptococcosis is the second most common, after toxoplasmosis, which has been demonstrated in clinical studies (Lizarazo et al. 2006, Ávila and González 2007), and autopsies (Mantilla and Cárdenas 2009). The same findings have been described in Cuba (Lamotte 2014).

Most cases of cryptococcosis are reported in young HIV positive male patients. Among the HIV negative patients, males are also the predominant sex, although in a smaller proportion, and patients also tend, on average, to be slightly older (Dromer et al. 1996). This epidemiological distribution has also been found in Latin America (Table I). Globally, as in many developing countries, many cases of cryptococcosis in Latin America may not be reported or be confirmed by diagnosis, which in part is reflected by the number of people living with AIDS not in care, undiagnosed, and lost to follow-up, or living in resource-limited areas, which does not allow for a prompt and correct diagnosis (Rajasingham et al. 2017).

Clinical aspects - Cryptococcosis mainly affects the CNS, causing meningitis. In a lesser proportion it affects the lungs, and organs such as skin, eyes, prostate, and bone, among others (Maziarz and Perfect 2016). Classically, patients with meningeal cryptococcosis present a clinical picture consisting of headache and fever, lasting approximately two weeks. Many of these patients also present with nausea, vomiting, cranial nerve involvement, and decreased visual acuity due to intracranial hypertension. If the disease progresses without treatment, mental changes, seizures, and a decreased state of consciousness leading to coma are observed (Limper et al. 2017, Williamson et al. 2017). In Latin America, meningeal cryptococcosis is also the main form of clinical presentation, with headache as the cardinal symptom (Table I). Intracranial hypertension, one of the most feared complications, has been reported in several Latin American studies, in more than 50% of the cases (Table I), which suggests a high percentage of patients with advanced forms of the disease. In autopsy studies conducted in Latin America, patients with neurocryptococcosis, most of whom were infected with HIV, predominantly had disseminated forms of the disease, with multiple organ involvement. Pure meningoencephalitic forms are less frequent, and the presence of cryptococcomas very rare (Reséndiz et al. 2008, Klock et al. 2009, Mantilla and Cárdenas 2009, Torres et al. 2016).

Treatment - For meningeal cryptococcosis, the treatment of choice recommended by the guidelines of the Infectious Diseases Society of America (IDSA), consists of

a combination of amphotericin B deoxycholate (AmBd) 0.7 mg/kg/day, and 5-fluorocytosine (5FC) 100 mg/kg/day for two weeks as induction phase, followed by fluconazole (FCZ) 400 mg daily for eight weeks as consolidation phase, and FCZ 200 mg daily as the maintenance phase. In the induction phase it is recommended to use, as an alternative, the use of liposomal AmB (LAmB) associated (or in combination) with 5FC (Perfect et al. 2010). In Latin America, due to the unavailability of 5FC and the high cost of LAmB preparations, an alternative regimen recommended by the IDSA for countries with limited resources, with a good level of evidence (AI), consists of AmBd 0.7 mg/kg/day, plus FCZ 800 mg/day for the induction phase of two weeks; followed by FCZ 800 mg per day for eight weeks for the consolidation phase (Table II). An alternative scheme for the induction phase is the use of AmBd alone, at a dose greater than 1 mg/kg/day (Perfect et al. 2010). Similar considerations have been made in the Brazilian (Moretti et al. 2008), and Colombian (Castañeda and Lizarazo 2012) management guidelines.

The lethality of meningeal cryptococcosis in Latin America has been reported to range from 13% to 73%, with many cases ranging between 30% and 60% (Table I), which are very high figures compared to that of the industrialised countries (van der Horst et al. 1997), but similar to those recorded in sub-Saharan Africa (Jarvis and Harrison 2016). In Brazil, among the systemic mycoses, cryptococcosis causes the highest number of deaths in patients with HIV (Prado et al. 2009). In this country, the mortality rate of cryptococcal infections was 0.47 per million inhabitants, and was the thirteenth cause of death (Soares 2015). In Colombia, however, the survival rate of patients with meningogenic cryptococcosis has been reported to be 82% for HIV negative patients, and only 46% for HIV positive patients, with a survival time of only four months after diagnosis (Lizarazo et al. 2012).

Molecular studies on C. neoformans and C. gattii - Several broadly used molecular techniques have been used to determine the genotype of individual isolates, and compare groups of isolates among *C. neoformans* and *C. gattii* species, to carry out molecular epidemiology and population genetics studies. Among these techniques, polymerase chain reaction (PCR) fingerprinting (Meyer and Mitchell 1995), amplified fragment length polymorphisms (AFLP) (Boekhout et al. 2001, Hagen et al. 2010), microsatellite typing (Illnait-Zaragozi et al. 2010a), restriction fragment length polymorphisms (RFLP) (Meyer et al. 2003), multilocus sequence typing (MLST) (Meyer et al. 2009), and more recently, whole genome sequencing (WGS) (Loftus et al. 2005, D'Souza et al. 2011), have allowed for the identification of eight major molecular types among the cryptococcal species in the world: VNI (AFLP1) and VNII (AFLP1A/B) for *C. neoformans* var. *grubii* serotype A isolates, VNIV (AFLP2) for *C. neoformans* var. *neoformans* serotype D isolates, VNIII (AFLP3) for hybrids between the serotypes A and D, and VGI (AFLP4), VGII (AFLP6), VGIII (AFLP5) and VGIV (AFLP7) for *C. gattii* serotype B, and C isolates.

Although in most of the studies on *Cryptococcus* and cryptococcosis reported from Latin America, the isolates

TABLE I
Cryptococcosis in Latin America. Epidemiological and clinical data

Country	Percentage of cases										Reference	
	Number of cases	Male	HIV+	Clinical presentation (%)	Headache	High ICP > 25 cm H ₂ O	<i>C. gattii</i>	Lethality				
								global	HIV+	HIV-		
Argentina												
Buenos Aires	58	100	100	Meningitis (100)	88			21	21			Cangelosi et al. (2009)
Buenos Aires	2041	75	98	Meningitis (90)	70	79	0.3		40			Arechavala et al. (2017)
Buenos Aires	58	88	100	Meningitis (100)				19	19			Ramirez et al. (2017)
Buenos Aires Province	106	71	100	Neuro (91)				36	36			Mónaco and Antabak (2008)
Chaco (Corrientes)	26	69	69	Meningitis (96)			0					Cattana et al. (2015)
Córdoba (Córdoba)	53	66	100									Luque et al. (2017)
Brazil												
Bahia	104	62	12		93			43	12			Darzé et al. (2000)
Bahia	50	70	52	Meningitis (100)	92	77 (> 20 cmH ₂ O)		22				Mascarenhas-Batista et al. (2013)
Goiás (Goiânia)	62	74	85	Meningitis (100)	76		6	48	47	56		Hasimoto e Souza et al. (2013)
Mato Grosso do Sul (Campo Grande)	123	68	84	Neuro (84)	76		10	50	51	41		Lindenberg et al. (2008)
Mina Gerais (Uberlandia)	96	80	81	Meningitis (56)			7	73	77			Moreira et al. (2006)
Mina Gerais (Uberlandia)	41	83	85	Meningitis (75)			0	58	60	25		Aguiar et al. (2017)
Mina Gerais (Uberaba)	131	69	91	Meningitis (79)	60		53	51				Mora et al. (2012)
Río de Janeiro (Rio de Janeiro)	696	73	61	Meningitis (96)				52	61			Leimann and Koifman (2008)
Río de Janeiro (Rio de Janeiro)	138	76	100	Meningitis (100)				62	62			Ramirez et al. (2017)
Río Grande do Sul (Porto Alegre)	126	77	95	Meningitis (100)	80		5					Leal et al. (2008)
São Paulo (São Paulo)	98	78	97	Meningitis (100)	91	55		30				Vidal et al. (2012)
São Paulo (São Paulo)	29	55	0	Neuro (86)	96	76 (≥ 20 cmH ₂ O)	64	21		21		Lomes et al. (2016)
National, children	53	53	24	Neuro (96)	85		30	41				Severo et al. (2009)
Bolivia												
Cochabamba	31	83	100	Meningitis (100)	23			67	67			Castro and Córdoba (2014)
Chile												
Santiago	28	93	100	Meningitis (100)				21	21			Ramirez et al. (2017)



Country	Percentage of cases										Reference	
	Number of cases			Clinical presentation (%)	Headache	High ICP > 25 cmH ₂ O	<i>C. gattii</i>	Lethality				
	Male	HIV+	HIV-					global	HIV+	HIV-		
Colombia												
National 1997-2005	931	83	78	Neuro (96)	85		4					Lizarazo et al. (2007)
National 2006-2010	526	77	83	Neuro (81)	84	20	3	32				Escandón et al. (2012)
Norte de Santander (Cúcuta)	90	77	70	Meningitis (100)	90	57	22	34	49	16		Lizarazo et al. (2012)
Atlántico (Barranquilla)	41	76	78	Meningitis (66)			1					Noguera et al. (2015)
National, children	41	58	24	Meningitis (88)	78		6					Lizarazo et al. (2014a)
Costa Rica												
San José	71	79	73	Neuro (73)	100		0	46	46	0		Ávila and Villalobos (2016)
Cuba												
La Habana	16	82	0	Neuro (100)	81			31		31		Fernández-Concepción et al. (2003)
Ecuador												
Guayaquil	82	73	100	Meningitis (100)	78							Sánchez et al. (2016)
Guatemala												
Guatemala	110	73	100	Meningitis (100)	80			14	14			Romero et al. (2005)
Guatemala	60	80	100	Meningitis (100)				43	43			Mejía and del Valle (2012)
Guatemala	28	72	100	Meningitis (100)	93			21	21			Herrera et al. (2014)
Honduras												
Tegucigalpa	27	59	100	Meningitis (100)				33	33			Ramírez et al. (2017)
Mexico												
Ciudad de Mexico	34	85	100	Meningitis (100)				38	38			Ramírez et al. (2017)
Panama												
Panama	28	61	100	Meningitis (100)	96	57 (≥ 20 cmH ₂ O)		50	50			González et al. (2013)
Paraguay												
Itauguá	13	76	62	Meningitis (100)	90			46	83	20		Sierra (2013)
Peru												
Lima	47	87	100	Meningitis (100)	100	64 (≥ 20 cmH ₂ O)	0	19	19			Dammert et al. (2008)
Lima	90	77	100	Meningitis (100)				41	41			Canessa et al. (2011)
Lima	234		100	Meningitis (100)	86			20	20			Concha-Velasco et al. (2017)
Uruguay												
Montevideo	147		92				0.7					Carbia et al. (2017)
Venezuela												
Caracas	110	83	83	Meningitis (97)				30	32	21		Pérez et al. (2009)

ICP: intracranial pressure; Neuro: meningitis, meningoencephalitis and cryptococcoma.

TABLE II
Cryptococcosis in Latin America. Clinical presentation and treatment

Country	Number of cases	Clinical presentation (%)	Percentage of cases					Reference
			AmB (1996-2009)	AmB + FCZ (2010)	AmB + 5FC (1986-1995)	AmB + FCZ or 5FC	FCZ Other	
Argentina								
Buenos Aires	2041	Meningitis (90)	100 (1996-2009)	100 from 2010	100 (1986-1995)			Arechavala et al. (2017)
Provincia de Buenos Aires	106	Neuro (91)		100				Mónaco and Antabak (2008)
Brazil								
Bahia (Salvador)	50	Meningitis (100)	62	32	0		4	Mascarenhas-Batista et al. (2013); Lindenbergh et al. (2008)
Mato Grosso do Sul (Campo Grande)	123	Neuro (84)	86				60 ^a	
Mina Gerais (Uberlandia)	96	Meningitis (56)	54	19	0		3	Moreira et al. (2006)
Mina Gerais (Uberlandia)	41	Meningitis (75)	85				75 ^a	Aguiar et al. (2017)
São Paulo (São Paulo)	29	Neuro (86)				100 including LAmB	3	Lomes et al. (2016)
National, children	32		28			66		
Bolivia								
Cochabamba	31	Meningitis (100)	100					Castro and Córdoba (2014)
Colombia								
National 1997-2005	931	Neuro (96)	73	21			6	Lizarazo et al. (2007)
National 2006-2010	526	Neuro (81)	72	28				Escandón et al. (2012)
Norte de Santander (Cúcuta)	90	Meningitis (100)		93				Lizarazo et al. (2012)
Atlántico	41	Meningitis (66)	29	5			17	Noguera et al. (2015)
National, children	41	Meningitis (88)	61	16	6	3	3	Lizarazo et al. (2014a)
Costa Rica								
San José	71	Cerebral (73)	79	21				Ávila and Villalobos (2016)
Cuba								
La Habana	16	Neuro (100)	69	23		8		Fernández-Concepción et al. (2003)
Guatemala								
Guatemala	110	Meningitis (100)	78 (15) *				6	Romero et al. (2005)
Panamá								
Panamá	28	Meningitis (100)	100					González et al. (2013)



Country	Number of cases	Clinical presentation (%)	Percentage of cases					Reference
			AmB	AmB + FCZ	AmB + 5FC	AmB + FCZ or 5FC	FCZ Other	
Paraguay Itauguá	13	Meningitis (100)	85	7				Sierra (2013)
Peru Lima Lima	47 234	Meningitis (100) Meningitis (100)	62	100			13	Dammert et al. (2008) Concha-Velasco et al. (2017)

α: consolidation phase; AmB: amphotericin B; *LAmB: liposomal amphotericin B; FCZ: fluconazole; 5FC: 5 fluocytosine; neuro: meningitis, meningoencephalitis and cryptococcoma.

have been identified only at species level, variety, or serotype; it is now possible to determine the major molecular type of the cryptococcal isolates recovered not only from clinical, but also from environmental sources, and much less frequently from veterinary samples, by using one or more of the above mentioned molecular techniques.

Using these techniques, the major molecular type of 3,486 isolates in Latin America is presently known, with most of the isolates reported from Brazil (45.87%), followed by Colombia (24.99%), Mexico (9.21%), Argentina (8.09%), Cuba (6.05%), Venezuela (2.90%), Peru (1.06%), Ecuador (0.77%), Chile (0.55%), Guatemala (0.43%), as well as Honduras, Paraguay, and Uruguay with one isolate each (Table III). From Bolivia, Costa Rica, Dominican Republic, El Salvador, Haiti, Nicaragua, and Panama, there are not data reported about the molecular type of cryptococcal strains.

From these 3,486 isolates, 67.7%, and 32.2% were recovered from clinical, and environmental sources, respectively. In concordance with global data, the molecular type VNI (AFLP1), corresponding to *C. neoformans* var. *grubii*, serotype A is the predominant molecular type (72.8%) causing cryptococcosis in Latin America, followed by *C. gattii* molecular type VGII (AFLP6) (14.4%) (Fig. 1). Interestingly, in the environment these two molecular types also predominate with slightly different proportions (57.0% for *C. neoformans* VNI, and 28.7% for *C. gattii* VGII) (Fig. 1), although this distribution may be biased by the high prevalence of *C. gattii* VGII in Brazil and Colombia, which are the countries with the largest number of studied isolates (Fig. 1, Table III). Of all these studies, only six *C. gattii* isolates (0.2%) have been reported from veterinary cases: one molecular type VGI from Cuba (Illnait-Zaragozi et al. 2011b) and five molecular type VGII from Brazil (Cardoso et al. 2013, Headley et al. 2015, da Silva et al. 2017) (Fig. 1). Interestingly, in Brazil an outbreak of infection occurred in a breeding aviary in São Paulo, where seven parrot-like birds died of disseminated cryptococcosis. Although the molecular type of the causative agent was not identified, all cases were caused by *C. gattii* serotype B, resistant to FCZ, as determined by biochemical, physiological, and serological testing (Raso et al. 2004). To date, veterinary cases of *C. neoformans* have not yet been reported in Latin American countries.

Clinical cases of cryptococcosis from which the major molecular type of the isolates has been identified, date back to 1961 (Meyer et al. 2003), with most of the studies being retrospective. While Argentina, Brazil, Colombia, and Cuba have described isolates from the 1980s (Igreja et al. 2004, Escandón et al. 2006, Illnait-Zaragozi et al. 2010a, Arechavala et al. 2017), most of the isolates recovered in Latin America are from the mid-1990s, to date. Case reports, in which *C. gattii* has been isolated as etiological agent, have been documented much more recently (Illnait-Zaragozi et al. 2013, Nascimento et al. 2014, Cicora et al. 2015, Solar et al. 2015, Noguera et al. 2017), with the earliest occurring in 2005 in Brazil (Pinto Jr et al. 2010). The major molecular type of the environmental isolates was first reported about two decades ago in Cuba (Illnait-Zaragozi et al. 2010a), although most of the

TABLE III

Distribution of *Cryptococcus neoformans* and *C. gattii* isolates recovered in Latin America and identified at molecular type level

Country*	n	Source	<i>C. neoformans</i>				<i>C. gattii</i>				Total
			VNI	VNII	VNIII	VNIV	VGI	VGII	VGIII	VGIV	
Brazil	1599	Clinical	695	38	-	-	19	269	18	-	1039
		Environmental	301	8	1	23	7	215	-	-	555
		Veterinary	-	-	-	-	-	5	-	-	5
Colombia	871	Clinical	317	17	-	6	10	54	39	2	445
		Environmental	229	1	-	-	3	106	76	11	426
Mexico	321	Clinical	209	21	12	7	11	5	26	7	298
		Environmental	18	-	-	-	-	-	5	-	23
Argentina	282	Clinical	209	13	9	2	3	1	1	-	238
		Environmental	19	-	-	-	23	-	2	-	44
Cuba	211	Clinical	141	-	-	-	-	-	1	-	142
		Environmental	68	-	-	-	-	-	-	-	68
		Veterinary	-	-	-	-	1	-	-	-	1
Venezuela	101	Clinical	76	6	1	-	5	10	3	-	101
Peru	37	Clinical	25	9	-	2	1	-	-	-	37
Ecuador	27	Clinical	27	-	-	-	-	-	-	-	27
Chile	19	Clinical	4	3	3	5	-	-	-	-	15
		Environmental	4	-	-	-	-	-	-	-	4
Guatemala	15	Clinical	14	-	-	-	-	-	1	-	15
Honduras	1	Clinical	-	-	-	-	1	-	-	-	1
Paraguay	1	Clinical	-	-	-	-	-	-	1	-	1
Uruguay	1	Environmental	-	-	-	-	-	1	-	-	1
Total	3,486	Clinical	1,717	107	25	22	50	339	90	9	2,359
		Environmental	639	9	1	23	33	322	83	11	1,121
		Veterinary	-	-	-	-	1	5	-	-	6

Molecular data have been combined from studies reported in **Argentina** (Meyer et al. 2003, Refojo et al. 2009, Cattana et al. 2013, Mazza et al. 2013, Cattana et al. 2014, Cattana et al. 2015, Cicora et al. 2015, Arechavala et al. 2017), **Brazil** (Casali et al. 2003, Trilles et al. 2003, Igreja et al. 2004, Abegg et al. 2006, Ribeiro et al. 2006, Matsumoto et al. 2007, Lugarini et al. 2008, Ribeiro & Ngamskulrungronj 2008, dos Santos et al. 2008, Trilles et al. 2008, Costa et al. 2009, Andrade-Silva et al. 2010, Pinto Jr et al. 2010, Souza et al. 2010, Ferreira-Paim et al. 2011, da Silva et al. 2012, Freire et al. 2012, Silva et al. 2012, Cardoso et al. 2013, Tsujisaki et al. 2013, Anzai et al. 2014, Nascimento et al. 2014, Brito-Santos et al. 2015, Headley et al. 2015, Castro e Silva et al. 2016, Figueiredo et al. 2016, Lomes et al. 2016, Souto et al. 2016, Aguiar et al. 2017, da Silva et al. 2017), **Chile** (Calvo et al. 2001, Meyer et al. 2003, Solar et al. 2015), **Colombia** (Meyer et al. 2003, Escandón et al. 2006, Escandón et al. 2010, Firacative et al. 2011, Lizarazo et al. 2012, Lizarazo et al. 2014a, Lizarazo et al. 2014b, Noguera et al. 2015, Cogliati et al. 2016, Firacative et al. 2016, Noguera et al. 2017, Vélez and Escandón 2017), **Cuba** (Illnait-Zaragozi et al. 2010a, Illnait-Zaragozi et al. 2010b, Illnait-Zaragozi et al. 2011b, Illnait-Zaragozi et al. 2013), **Ecuador** (Sánchez et al. 2017), **Guatemala** (Meyer et al. 2003), **Honduras** (Boekhout et al. 2001), **Mexico** (Meyer et al. 2003, Olivares et al. 2009, Firacative et al. 2016, González et al. 2016), **Paraguay** (Firacative et al. 2016), **Peru** (Meyer et al. 2003, Bejar et al. 2015), **Uruguay** (Engelthaler et al. 2014) and **Venezuela** (Calvo et al. 2001, Meyer et al. 2003, Firacative et al. 2016, Ferrara et al. 2017). *From Bolivia, Costa Rica, Dominican Republic, El Salvador, Haiti, Nicaragua, and Panama there is not data reported about the molecular type of cryptococcal strains.

reports on the molecular types of *C. neoformans* and *C. gattii* from the environment are from after the year 2000.

The combined analysis of the molecular data also showed that in Colombia, the prevalence of the *C. gattii* VGIII is very similar to that of *C. gattii* VGII and that in Mexico, the molecular type VGIII is the most common among *C. gattii* isolates and the second most common after *C. neoformans* VNI. In Argentina however, the most common molecular type among *C. gattii* isolates is

VGI (Fig. 2). Mexico is the only country where all major molecular types have been reported, while in Brazil and Argentina only *C. gattii* VGIV seems to be absent and in Colombia only *C. neoformans* VNIII has not been recognised. However, apart from VNIII, the hybrid most commonly found in *C. neoformans*, inter- and intra-specific hybrids have been reportedly for Latin America. One isolate described as the VNII/VNIV hybrid was reported from Argentina, another isolate with the same genotype

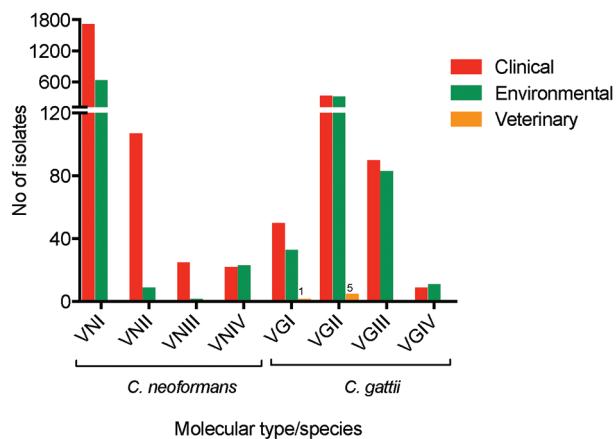


Fig. 1: distribution of *Cryptococcus neoformans* and *C. gattii* identified at molecular type level (n = 3486), recovered from clinical, environmental^a or veterinary sources in Latin America. Molecular data have been combined from studies reported in Argentina, Brazil, Chile, Colombia, Cuba, Ecuador, Guatemala, Honduras, Mexico, Paraguay, Peru, Uruguay, and Venezuela (Table III). Data from Bolivia, Costa Rica, Dominican Republic, El Salvador, Haiti, Nicaragua, and Panama about the molecular typing of cryptococcal strains has not been reported. *a*: *C. neoformans* has been mostly recovered from avian droppings, decaying organic matter, and soil (Casali et al. 2003, Ribeiro et al. 2006, Lugarini et al. 2008, Ribeiro and Ngamskulrungrong 2008, Andrade-Silva et al. 2010, Illnait-Zaragozi et al. 2010a, Souza et al. 2010, Ferreira-Paim et al. 2011, Canónico-González et al. 2013, Castro e Silva et al. 2016). *C. gattii* is associated with several tree species (Escandón et al. 2006, Trilles et al. 2008, Costa et al. 2009, Refojo et al. 2009, Escandón et al. 2010, Firacative et al. 2011, Mazza et al. 2013, Anzai et al. 2014, Cattana et al. 2014, Brito-Santos et al. 2015, Vélez and Escandón 2017).

VNII/VNIV and two isolates VNI/VGII from Brazil, and five isolates VNI/VNII and one isolate VNI/VGII from Colombia (Aminnejad et al. 2012, Aminnejad et al. 2016).

When the mating type of the isolates was identified by specific PCR, the mating alpha was the most common in both *C. neoformans* and *C. gattii* isolates recovered in Latin America, as noted globally (Kwon-Chung et al. 2014). However, in Colombia all *C. gattii* VGIII isolates recovered from *Corymbia ficifolia* detritus, and one VGI isolate recovered from *Ficus* spp. were mating type a (Escandón et al. 2010, Firacative et al. 2011), as well as 13 clinical and three environmental VGII isolates from Brazil (Brito-Santos et al. 2015, Souto et al. 2016), and two VGIII clinical isolates recovered in Mexico (Firacative et al. 2016). Interestingly, there are no reports on *C. neoformans* mating type a in the region.

The use of MLST and WGS to further advance the understanding of *C. neoformans* and *C. gattii* populations has also been achieved in Latin America. Firstly, a highly clonal population structure of clinical and environmental isolates of *C. neoformans* var. *grubii* was revealed in Brazil (Ferreira-Paim et al. 2017), which is in agreement with the clonal evolution and dispersion of *C. neoformans* that has been reported worldwide, these results detail the absence or restriction of genetic recombination, and the persistence of widespread clones. Opposing this, the genetic population structure of many isolates of the less common *C. neoformans* var. *neofor-*

mans, from different geographical origin, including Colombia, and from different sources, showed that this is a highly recombinant population, with the isolates being strictly correlated to each other, and characterised by high variability (Cogliati et al. 2016).

The global diversity of a number of *C. gattii* VGII isolates was also studied, which provided evidence on the evolution of this pathogen in North America and gave support to the extensive evolution in, and dispersal from, South America, most likely from the Amazonia and the Northeastern regions of Brazil, where *C. gattii* VGII strains have shown to have the most genetic variability, compared to isolates from the rest of the world (Engelthaler et al. 2014, Souto et al. 2016).

Additionally, Mexico and Colombia have been proposed to be the likely origins of the VGIII *C. gattii* population, as isolates from these countries are the basal group of isolates recovered worldwide; they are very variable genetically, and have both mating types, which may lead to sexual reproduction and recombination events (Firacative et al. 2016).

Other molecular techniques employed in Latin American countries have allowed the identification and characterisation of cryptococcal isolates, although without determining the major molecular types. PCR fingerprinting with GACA₄ primers was used for the genotyping of the first environmental isolate of *C. gattii* recovered in Argentina (Meyer and Mitchell 1995, Davel et al. 2003). Electrophoretic karyotyping of 40 *C. neoformans*, and 11 *C. gattii* clinical isolates from Brazil, Chile and Venezuela showed a broad genotypic diversity among the isolates (Calvo et al. 2001). Sequencing of the internal transcribed spacer (ITS) identified one *C. gattii* isolate recovered from an immunocompetent patient in Chile (Solar et al. 2015), and one and five *C. neoformans* environmental isolates recovered from an almond tree in Cuba (Illnait-Zaragozi et al. 2012), and derived from pigeon excreta in Mexico (Canónico-González et al. 2013), respectively. In addition, ITS sequencing allowed for the discrimination of an uncommon species causing cryptococcosis, *C. liquefaciens*, in a HIV positive patient from Guatemala (Conde-Pereira et al. 2015). Lastly, using comparative genome hybridisation, the genome content of a clinical *C. neoformans* VNI strain from Argentina was examined, which showed that this strain shared most, if not all, of its genome with H99, the reference strain of *C. neoformans* var. *grubii* (Hu et al. 2008).

Diagnostic and identification of cryptococcal species from clinical and environmental samples has also been possible by using molecular methodologies, some of which have been developed in Latin America. PCR of the capsular gene *CAP59*, followed by RFLP was used in Argentina to determine the serotypes of cryptococcal strains directly from a yeast suspension, thus avoiding DNA extraction and making serotype identification quick, simple and inexpensive (Siachoque et al. 2010). In Colombia, a nested PCR targeting the ITS region was developed for the diagnosis of *C. neoformans* and *C. gattii* from human samples, including bronchoalveolar lavage (BAL), bronchial lavage (BL), biopsy, and, cerebrospinal fluid (CSF), showing a sensitivity and specificity of

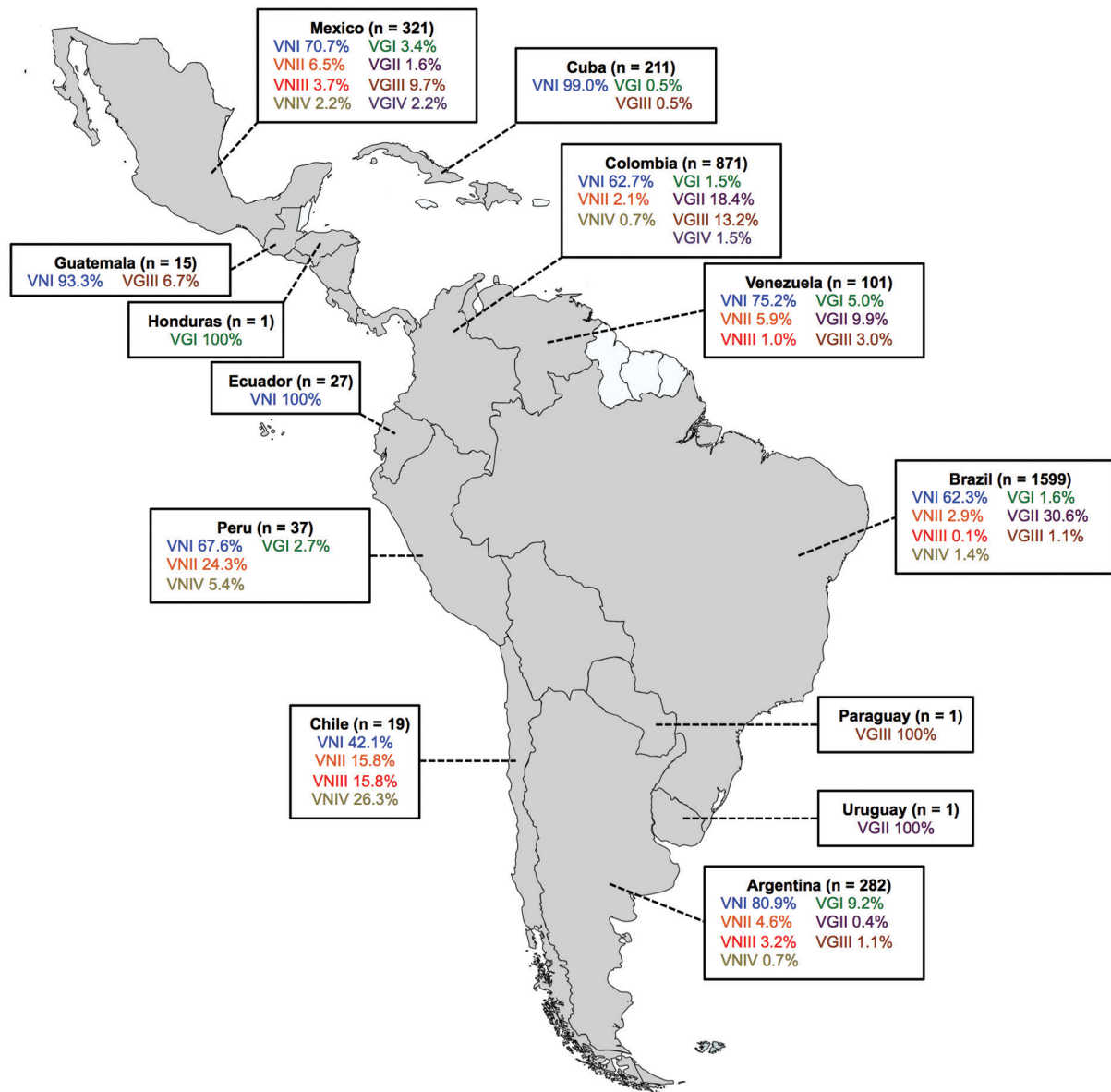


Fig. 2: geographic distribution of *Cryptococcus neoformans* and *C. gattii* isolates from Latin America, identified at the molecular type level (n = 3486). Molecular data have been combined from studies reported in Argentina, Brazil, Chile, Colombia, Cuba, Ecuador, Guatemala, Honduras, Mexico, Paraguay, Peru, Uruguay, and Venezuela (Table III). Data from Bolivia, Costa Rica, Dominican Republic, El Salvador, Haiti, Nicaragua, and Panama regarding the molecular typing of cryptococcal strains have not been reported.

100% (Rivera et al. 2015). In addition, in Colombia, two techniques to extract cryptococcal DNA from contaminated soil were standardised, namely glass beads with agarose blocks and an ultra-clean DNA soil kit (MoBio, Solano Beach, CA, USA), which allowed a faster yet specific detection of cryptococcal isolates from soil samples through the amplification of species-specific DNA using the primers CN4 and CN5 (Castañeda et al. 2004). The rapid, sensitive and specific identification of the eight major molecular types, including hybrid strains, from culture and directly from clinical samples is also now possible after the development of a hyperbranched rolling

circle amplification of the phospholipase gene *PLBI* in combination with a semi-nested PCR (Trilles et al. 2014).

Thus, the use of molecular techniques for the study of the etiological agents of cryptococcosis in Latin America, as elsewhere, shows an undoubted value especially for rapid detection and genetic epidemiology studies, and at the same time shows that these methodologies are complementary to traditional techniques.

Host-Cryptococcus - The role of *C. neoformans* and *C. gattii* as the major causative agents of fungal meningoencephalitis in humans and animals worldwide (Rajasingham et al. 2017) has stimulated more than half-century's

worth of investigations on these fascinating and versatile fungi. Nevertheless, many questions about this species and the interactions with the host are not yet fully elucidated, including: (1) whether reactivation of long-term latent infection is a more important cause of cryptococcosis in patients than *de novo* acquisition from the environment; (2) the bases of the preference of some genotypes for certain hosts (i.e. immunosuppressed vs. immunocompetent) and target organs (i.e. brain vs. lung); (3) the mechanism by which the infection becomes persistent and/or recurrent despite that *in vitro* resistance to antifungal drugs does not seem to be a problem for cryptococcal treatment; and (4) the diverse pathways related to sexual reproduction, among many others (Rodríguez et al. 2007).

In Latin American, specifically in Argentina, Brazil and Cuba, different investigations related with these topics have been conducted mostly in order to reveal and comprehend host-pathogen interactions, to investigate the role of interactions between the yeasts and other microorganisms, and to review alternative approaches to antifungal treatments against cryptococcosis and other mycosis. A clear majority of the authors have attempted then to understand the role of the immune cells and the cytokines/antibodies induction not only from human clinical samples but also by using *in vitro* and *in vivo* models.

In AIDS patients with cryptococcal meningitis, cytokine patterns in the CSF and sera were shown to be related to fungal burden and clinical outcome. CSF levels of IL-8, IL-12p40, IL-17A, TNF- α , INF- γ and sera TNF- α were significantly higher among survivors, which indicates that this progressive shift in cytokine expression favouring a Th1 pattern is crucial in controlling cryptococcal infection and would be a prognostic marker in cryptococcal meningitis (Mora et al. 2015, Mora et al. 2017). In addition, the study of the cytokine profile of human peripheral blood mononuclear cells (PBMCs) of healthy individuals, after *in vitro* stimulation with heat-killed *C. gattii* and *C. neoformans* (40 different strains), demonstrated that clinical heat-killed *C. gattii* isolates induced a more pronounced inflammatory response with higher concentrations of pro-inflammatory cytokines (IL-1 β , TNF- α and IL-6 and the Th17/22 cytokine IL-17 and IL-22) compared to other *Cryptococcus* species and non-clinical *C. gattii*, which is dependent on TLR4 and TLR9 as cellular receptors (Schoffelen et al. 2013).

By using vertebrate models, Garro et al. (2011a), Garro et al. (2011b) showed that in rats, peritoneal eosinophils can migrate into lymphoid organs to act as antigen-presenting cells of *C. neoformans* (strain 102/85) antigens, priming naïve and re-stimulating infected animals to induce T-cell and B-cell responses, and the development of a Th1 microenvironment with increased levels of nitric oxide (NO) synthesis and *C. neoformans*-specific immunoglobulin production, which all lead to a protective immune response against subsequent infection with fungus. Also in rats, the mechanisms involved in macrophage apoptosis promoted by cryptococcal glucuronoxylomannan (GXM) through NO generation showed to be novel immunomodulatory mechanisms that could contribute to limit inflammation during infec-

tion in these group of rodents (Chiapello 2017). Obtained from mice, the monoclonal antibodies (mAb) 4B3 were shown to stimulate phagocytosis of the *C. neoformans* (strain 028 LMIPK) by macrophages without fungicidal effect, thus favouring yeast dissemination and decreasing the survival of mice due to cryptococcal infection (Gato-Armas et al. 2006, Illnait-Zaragozi et al. 2011a).

In addition to cryptococcosis alone, mouse models have been also used to study the influence of or interaction between other fungi, bacterial and viral agents, and *C. neoformans* and *C. gattii* infections. Fungal-fungal specific interactions between *C. neoformans* and *H. capsulatum*, which co-exist in the environment, that can impact in virulence and disease were investigated. Co-infected mice showed to have significantly higher mortality than infection with *C. neoformans* (strain H99) or *H. capsulatum* alone, or acapsulated strains, as shown by higher pulmonary fungal burden in co-infected animals. Enhanced pellicle formation with a hybrid polysaccharide matrix with higher reactivity to GXM mAbs and increased resistance to phagocytosis and killing by macrophages was also observed, which together indicate that a microbial interaction involving the transfer of virulence traits may translate into enhanced lethality during mixed fungal infections (Cordero et al. 2016). Secondly, the influence of microbiota in the host response against *C. gattii* (strain L27/01) was demonstrated. Germ-free (GF) mice were more susceptible to infection, showing lower survival, higher fungal burden in the lungs and brain, increased behavioural changes, reduced levels of inflammatory cytokines (IFN- γ , IL-1 β and IL-17), and lower NF κ Bp65 phosphorylation compared to conventional mice, which were associated with smaller yeast cells and polysaccharide capsules in the lungs, and less tissue damage. Moreover, macrophages from GF mice showed reduced ability to engulf, produce reactive oxygen species (ROS), and kill *C. gattii*. Restoration of microbiota in mice that received feces from conventional animals or administration with *Escherichia coli*, made mice more responsive to infection, which was associated with increased survival and higher levels of inflammatory mediators (Costa et al. 2016). More recently, the interaction between *C. gattii* infection (strain L27/01) and the concurrent infection with influenza A virus (IAV) strain H1N1 was studied. This co-infection resulted in a major increase in morbidity and mortality, with severe lung damage and a high brain fungal burden when mice were infected in the acute phase of influenza multiplication, indicating that IAV infection is a predisposing factor for severe disease and adverse outcomes in mice co-infected with *C. gattii*. IAV not only alters the host response to the yeast, leading to recruitment of significantly more neutrophils and macrophages into the lungs, but also induces the production of type I interferons (IFN- α 4/ β) and significantly reduces the levels of IFN- γ , which is associated with an impaired immune response. Reduced phagocytosis, killing of cryptococci and production of ROS by IAV-infected macrophages were also shown, leading to increased proliferation of the fungus within macrophages (Oliveira et al. 2017).

In vitro, the interaction between antibodies and the cryptococcal capsule, and the effect of protective and non-protective mAbs on *C. neoformans* H99 replication and the capsule's physical properties have been also examined. Protective mAbs were shown to directly alter cell division by trapping and preventing the full release of newly budded cells. This effect is caused by an Ab-mediated increase in capsule stiffness, involving cross-linking of GXM molecules. The ability of mAbs to impair *C. neoformans* budding through changes in the capsule's mechanical properties indicates a new, non-classical mechanism of Ab function, and presents important implications for understanding Ab-mediated immunity (Cordero et al. 2013). As the interaction between *C. neoformans* H99 and the amoebae is similar to that of the yeast-macrophage association, the participation of fungal virulence-associated structures with *Acanthamoeba castellanii* was evaluated by Rizzo et al. (2017). Fungal extracellular vesicles (EVs) and the GXM were shown to be internalised by *A. castellanii* with no impact on the viability of amoebal cells. However, EVs, but not free GXM, modulate antifungal properties of *A. castellanii* by inducing enhanced yeast survival. Phagocytosis of *C. neoformans* by amoebal cells and the pathogenic potential in a *Galleria mellonella* model are not affected by EVs, but previous interactions with *A. castellanii* rendered fungal cells more efficient in killing this invertebrate host, which all support the notion that interaction of *C. neoformans* with environmental predators results in enhanced virulence (Rizzo et al. 2017).

Both *in vivo* and *in vitro*, the role of mammalian β -galactoside-binding protein Galectin-3 (Gal-3) in *C. neoformans* H99 infection was also revealed. Gal-3 levels not only augment in human sera but also in spleen, lung, and brain tissues of infected mice. Gal-3-deficient mice are more susceptible to cryptococcosis than wild-type animals, as the first group present higher fungal burden and lower animal survival. *In vitro* experiments showed that Gal-3 also inhibits fungal growth and exerts a direct lytic effect on *C. neoformans* EVs, which could benefit the host (Almeida et al. 2017).

Regarding the research efforts targeting alternative antifungal strategies, Baronetti et al. (2006), Baronetti et al. (2011) showed that subcutaneous pretreatment of rats with heat killed cells of the *C. neoformans* strain 102/85 (HKC) emulsified in complete Freund adjuvant (CFA), promoted protection against an intraperitoneal challenge with viable *C. neoformans* var. *grubii* with significantly better clearance of yeasts from tissues, a lower concentration of GXM in serum and tissues, and better histopathological parameters compared to unpretreated infected rats. Passive immunisation with a mAb to glucosylceramides (GlcCer), a lipid that is present at the fungal plasma membrane, cell wall, and secretory vesicles, and which induces antifungal antibodies and regulates the virulence of *C. neoformans* in animal infections, significantly reduced host inflammation and prolonged the survival of mice lethally infected with *C. neoformans* strain 24067 (Serotype D), revealing a potential therapeutic strategy to control cryptococcosis (Rodrigues et al. 2007). *In vitro*, mAb-based drugs

have shown to be potentially a powerful alternative to standard antifungals, and although *C. neoformans* H99 was reported to be the least susceptible among other fungal species, it was found that wheat germ agglutinin (WGA), linked to the effector Fc region of murine IgG2a (WGA-Fc), was able to inhibit *in vitro* fungal growth, to increase phagocytosis by macrophages, as well as their antifungal functions (Liedke et al. 2017). Lastly, treatment of *C. neoformans* H99 cells with WGA followed by infection of mice also delayed mortality relative to animals infected with untreated fungal cells. Reduced brain colonisation by lectin-treated cryptococci was also observed. Blocking chitin-like oligomers also rendered yeast cells less efficient in their ability to associate with phagocytes. WGA did not affect fungal viability, but inhibited GXM release to the extracellular space and capsule formation. In WGA-treated yeast cells, genes that are involved in capsule formation and GXM traffic had their transcription levels decreased in comparison with untreated cells (Fonseca et al. 2013).

In this way, the wide variety of approaches and methodologies used in different laboratories from Argentina, Brazil and Cuba have globally contributed to understand particular issues related to the host-pathogen interactions in cryptococcosis.

Laboratory data: Antimicrobial susceptibility of clinical and environmental isolates - Most of the data in this topic have been generated on the numerous studies carried out in Brazil and Argentina. The studies have been focused on the methodology and interpretation of the phenotypic tests employed, the susceptibility data generated with clinical and environmental isolates and the study of resistance to some antifungals (Table IV).

Methodology and interpretation of results - Two reference methods have been used in Latin American studies to determine the minimal inhibitory concentration (MIC), the Clinical Laboratory Standard Institute (CLSI) microdilution method from M27-A3, and the EDef 7.2 from the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Several additional techniques have been developed to simplify the standard ones, such as, time kill curves (TKC), E-Test strips, and a disk diffusion method (Ochiuzzi et al. 2010, Córdoba et al. 2015, de Oliveira et al. 2017). Recently, the use of flow cytometry (FC) was evaluated in two publications, which both showed a positive significant correlation (Benaducci et al. 2015, Morales et al. 2015). In one of them, the conclusion was that FC has an excellent correlation with the standard methods, and it is a reliable, fast, and safe method to test the susceptibility to AmB (Benaducci et al. 2015). In the other study, the antifungal susceptibility of 17 clinical isolates of *C. neoformans* and 18 *C. gattii* VGII, was analysed by both the microdilution method (CLSI M27-A3) and FC, showing that FC allowed to determine MIC for AmB in a 2 h incubation time, with MICs data obtained on the same day (Morales et al. 2015).

In three additional studies, the epidemiological cut-off values (ECV) for AmB, 5FC, FCZ, itraconazole (ITZ), posaconazole (PCZ) and voriconazole (VCZ), were established (Espinel-Ingroff et al. 2012a, Espinel-

TABLE IV
Latin American studies on the antifungal susceptibility of *Cryptococcus neoformans* and *C. gattii* clinical isolates: methodology, epidemiological cut-off values (ECV), susceptibility patterns, resistance and heteroresistance results

Country	Comment	Reference
Argentina	Susceptibility data for FCZ and AmB for <i>C. neoformans</i> isolates from HIV-positive patients	Arechavala et al. (2009)
	Correlation of ETest and Neo-Sensitabs diffusion assays with broth microdilution reference method (CLSI-M27-A2) for testing susceptibility of <i>C. neoformans</i> to AmB and FCZ	Ochiuzzi et al. (2010)
	<i>In vitro</i> activity of AmB B by time-kill curve methodology	Córdoba et al. (2011)
	International study of wild-type susceptibility endpoint distributions and epidemiological cutoff values for FCZ, ITZ, PCZ, and VCZ. Argentina, Brazil, Cuba, Mexico	Espinel-Ingroff et al. (2012a)
	Comparison of different <i>in vitro</i> tests to detect <i>C. neoformans</i> not susceptible to AmB	Córdoba et al. (2015)
	Susceptibility profile and epidemiological cut-off values of <i>C. neoformans</i> species complex from Argentina	Córdoba et al. (2016)
	<i>C. neoformans</i> lanosterol 14- α -demethylase involved in fluconazole resistance in clinical isolates	Bosco-Borgeat et al. (2016)
	An international study of wild-type susceptibility endpoint distributions and epidemiological cutoff values for AmB and 5FC in <i>C. neoformans</i> and <i>C. gattii</i>	Espinel-Ingroff et al. (2012a)
	Susceptibility to antifungal agents and genotypes of Brazilian clinical and environmental <i>C. gattii</i> strains	Silva et al. (2012)
	Microbiological characteristics of clinical isolates of <i>Cryptococcus</i> spp. in Bahia, Brazil: molecular types and antifungal susceptibilities	Matos et al. (2012)
Brazil	Environmental isolation, biochemical identification, and antifungal drug susceptibility of <i>Cryptococcus</i> spp.	Teodoro et al. (2013)
	Susceptibility profile of clinical and environmental isolates of <i>C. neoformans</i> and <i>C. gattii</i>	Andrade-Silva et al. (2013)
	A flow cytometry method for testing the susceptibility of <i>Cryptococcus</i> spp. to AmB	Benaducci et al. (2015)
	<i>In vitro</i> susceptibility testing of AmB for <i>C. neoformans</i> variety <i>grubii</i> AFLP1/VNI and <i>C. gattii</i> AFLP6/VGII by CLSI and flow cytometry	Morales et al. (2015)
	Heteroresistance to ITZ and changes in the morphology and virulence of <i>C. gattii</i>	Ferreira et al. (2015)
	Antifungal susceptibility of clinical <i>C. deuterogattii</i> (AFLP6/VGII) isolates from Southern Brazil	Herkert et al. (2016)
	Resistance to FCZ and changes on virulence and morphological aspects of <i>C. neoformans</i> and <i>C. gattii</i>	Rossi et al. (2016)
	Antifungal susceptibility testing and genotyping characterization of <i>C. neoformans</i> and <i>C. gattii</i> isolates from HIV-infected patients	Figueiredo et al. (2016)
	Evaluation of antifungal combination against <i>Cryptococcus</i> spp.	Reichert-Lima et al. (2016)
	<i>C. neoformans</i> and <i>C. gattii</i> isolates from both HIV-infected and uninfected patients: antifungal susceptibility and outcome of cryptococcal disease	Nascimento et al. (2017)
Cuba	FCZ non-susceptible <i>C. neoformans</i> . Use of LAmB in AIDS patient	Santana et al. (2017)
	FCZ levels in serum and cerebrospinal fluid	Schiave et al. (2018)
	Time-kill curves studies with AmB against <i>C. neoformans</i> / <i>C. gattii</i> species complex clinical isolates	de Oliveira et al. (2017)
Heteroresistance to FCZ in clinical and environmental strains of <i>C. neoformans</i> and <i>C. gattii</i>	Feliciano et al. (2017)	
Venezuela	<i>C. neoformans</i> , FCZ susceptibility	Fernández-Andreu et al. (1999)
	New azoles and <i>C. neoformans</i> Cuban clinical isolates	Illmait-Zaragozi et al. (2008)
	FCZ, VCZ susceptibility of Cuban isolates	Illmait-Zaragozi et al. (2009)
A review of <i>C. neoformans</i> ECVs	Ferrara et al. (2017)	

AmB: amphotericin B, FCZ: fluconazole; ITZ: itraconazole; PCZ: posaconazole; VCZ: voriconazole.

Ingroff et al. 2012b, Córdoba et al. 2016) and recently, a review done by the Venezuelan group was reported (Ferrara et al. 2017). The first two studies were conducted as a part of a collaborative international effort with the participation of Argentina, Brazil, Cuba, and Mexico (Espinel-Ingroff et al. 2012a, Espinel-Ingroff et al. 2012b). The rationale of this work was to propose clinical breakpoints for *C. neoformans* and *C. gattii*, according to the molecular types, which was not available, as such ECVs for AmB and 5FC, on basis of MICs (CLSI methodology) were determined. ECVs are commonly used to separate wild-type (WT) isolates from isolates with reduced susceptibility to antifungal drugs with probable acquired resistance mechanisms, are useful to monitor the emergence of strains with mutations that could lead to reduced antifungal susceptibility to antifungal drugs, however they should not be used as predictor of clinical outcome. In the study by Córdoba et al. (2016) with Argentinian isolates, the highest ECVs, which included $\geq 95\%$ of the WT population studied, was observed for 5FC and FCZ (32 $\mu\text{g}/\text{mL}$ each), values that were higher than those previously proposed by Espinel-Ingroff et al. (2012a), Espinel-Ingroff et al. (2012b).

Clinical and environmental data - The studies are presented in Table IV and the data is variable; hence, it is important to analyse each one of the articles' data, separately (Arechavala et al. 2009, Illnait-Zaragozí et al. 2009, Matos et al. 2012, Silva et al. 2012, Andrade-Silva et al. 2013, Teodoro et al. 2013, Figueiredo et al. 2016, Herkert et al. 2016, Reichert-Lima et al. 2016, Nascimento et al. 2017).

As a general observation, clinical *C. gattii* strains are more resistant to FCZ in comparison to clinical *C. neoformans* strains. Otherwise, for AmB and 5FC no difference has been reported. Another general observation is that environmental isolates are more susceptible to antifungal drugs than clinical isolates.

It is important to mention the work by de Oliveira et al. (2017) describing the phenomenon of tolerance to AmB in some low-MIC strains of *C. neoformans* and *C. gattii*. Clinical studies are warranted to ascertain the relevance of this occurrence as a tool for guide treatment. Additionally, resistance to FCZ was studied by Bosco-Borgeat et al. (2016), in which they found that lanosterol 14- α -demethylase was involved in FCZ resistance in *C. neoformans* clinical isolates. The sequencing revealed the G1855A mutation in three isolates, resulting in the enzyme amino acid substitution G484S. These strains were isolated from two fluconazole-treated patients. Importantly, this mutation would not intervene in the susceptibility to the other widely use azoles, ITZ and VCZ.

Novel compounds for treatment - The synthesis of novel compounds has been explored by one research group in Colombia (Acosta et al. 2015, Ramírez et al. 2015, Montoya et al. 2016, Ramírez-Prada et al. 2017) and by two Brazilian groups (da Silva et al. 2016, Palanco et al. 2017) and their preliminary results look promising. Pyrazolonaphthyridines (Acosta et al. 2015), thiazole-based pyrimido[4,5-b] [1,4] diazepines (Ramírez et al. 2015), chalcones and N-aryl-2-pyrazolines (Montoya et al.

2016), and quinolone-based pyrazoles (Ramírez-Prada et al. 2017) have shown antifungal activity not only against *C. neoformans* but also against *Candida albicans*, with some of them showing fungicidal, rather than fungistatic activity, which makes them good antifungal candidates.

Laboratory data: Early diagnosis in HIV patients - Early diagnosis of cryptococcosis in HIV infected patients has been a topic of interest in Latin America since 1995 (Negroni et al. 1995, Lizarazo et al. 2002, Costa et al. 2013). In all, diagnosis was established with the determination of circulating antigen (CrAg) with the Latex test. Results of the studies were dissimilar but the importance of the CD4+ cells count to increase the sensitivity of the test was established. In 2011, a new CrAg lateral flow assay (CrAg LFA) was developed and preliminary experience in Colombia, Brazil, and Argentina (Escandón et al. 2013, Vidal et al. 2016, Frola et al. 2017) showed comparable results to that demonstrated globally (Huang et al. 2015, Wake et al. 2018). That is, a Point-of-Care Test (POCT) like CrAg LFA plays an important role in the early diagnosis of cryptococcosis in HIV patients, especially in resource limited settings. Inclusion of the CrAgLFA in the National Guides for managements of the patients with AIDS must be a compromise of the countries in the region without delay.

Searching the environmental niche: From avian droppings to tree hollows - The saprobic nature of the *Cryptococcus* spp. is a well-established fact. Infection with *Cryptococcus* spp. is invariably acquired by exposure to blastoconidia or basidiospores from an exogenous source and the host becomes an accident in the life cycle of the yeast. Consequently, studies on the natural reservoir of *C. neoformans* and *C. gattii* hold paramount interest even though they require a very extensive, programmed, coordinated and sequential field-work. In some Latin-American countries, this search has been conducted for many years. For this review, we will focus on the most recent studies (Table V).

The search for the habitat of these yeasts have been successfully achieved in many parts of the world, including Latin America, following Emmons' findings (Emmons 1960) on the strong association of cryptococci with soil enriched with dry pigeon excreta. Following this, studies on the recovery of the yeasts, not only from pigeon droppings but also from other bird faeces, especially from captive birds, have been successful good in Latin America (Álvarez et al. 2010, González-Hein et al. 2010, Ferreira-Paim et al. 2011, Andrade-Silva et al. 2012, Takahara et al. 2013, Teodoro et al. 2013). Although most of the isolates therein recovered were identified as *C. neoformans*, VNI, mating type alpha, it is worth mentioning that in one study (Teodoro et al. 2013) *C. gattii* was recovered in 5.2% of samples. Globally, it is known that most of the isolates recovered from bird droppings are *C. neoformans* (Kwon-Chung et al. 2014), with the percentage of recovery varying from place to place from 2% up to 70%.

From the numerous studies done globally on the search for the fungus in avian droppings, it has been very well established that bird droppings shielded from direct sun and ultraviolet light are an excellent substrate

TABLE V
Findings from recent Latin American environmental studies searching for the niche of *Cryptococcus neoformans* and *C. gattii*

Country	Sample			Species recovered			Studies			Reference
	Bird excreta	Tree detritus		<i>C. neoformans</i>	<i>C. gattii</i>		Phenotypic	Molecular		
Argentina		X		X	X		X	VNI, VGI	Refojo et al. (2009)	
	X			X			X		Álvarez et al. (2010)	
Brazil		X			X			VGI, VGIII	Mazza et al. (2013)	
		X		X	X			VNI, VGI	Cattana et al. (2014)	
		X		X	X		X	VNI, VGII	Costa et al. (2009)	
	X			X			X	VNI, VNII, MATα	Ferreira-Paim et al. (2011)	
	X	X		X	X		X	VNI, VNII	Andrade-Silva et al. (2012)	
	X	X		X	X		X		Takahara et al. (2013)	
Chile				X			AS		Andrade-Silva et al. (2013)	
	X			X			AS		Teodoro et al. (2013)	
		X			X		X	VGII	Anzai et al. (2014)	
	X	X			X		X	VGII	Brito-Santos et al. (2015)	
Colombia		X		X			X	VNI, MATα	Castro e Silva et al. (2016)	
	X			X	X		AS	VNI, VGII	Alves et al. (2016)	
	X			X			AS		González-Hein et al. (2010)	
		X		X	X		X	VGIII	Toro-Zúñiga et al. (2015)	
Cuba		X		X	X		X	VGIII	Escandón et al. 2005	
	X				X		X	VGIII	Escandón et al. (2010)	
		X			X		X	VNI,	Firacative et al. (2011)	
	X	X		X	X		X	B, VGI, VGIII	Mak et al. (2015)	
Mexico		X		X	X		X	VNI, VGIII	Noguera et al. (2015)	
	X			X	X		X	Satellites	Vélez and Escandón (2017)	
	X			X	X		X		Illnait-Zaragozi et al. (2010a)	
				<i>Cryptococcus</i> spp.					Illnait-Zaragozi et al. (2012)	
	X		X	X	X			X	Canónico-González et al. (2013)	

AS: antifungal susceptibility.

for the growth of the yeasts because of their high urea concentrations that is converted to ammonium, and carbamate by the urease enzyme, which is one of the landmark enzymes of the genus.

On the other hand, by the end of the 80s, the natural habitat of *C. gattii* was still unknown, until 1990, when David Ellis and Tania Pfeiffer (Ellis and Pfeiffer 1990) published their seminal paper revealing that the habitat of *C. gattii* in Australia was related to *Eucalyptus camaldulensis* trees.

Following the Australian report, recovery of *C. gattii* from *E. camaldulensis* detritus was achieved initially in Mexico (Licea et al. 1999) and later in other Latin American countries. However, it is of utmost importance that the work done by Marcia Lazera and her group in Fiocruz, Rio de Janeiro, Brazil, as they demonstrated that the niche of *C. gattii* is not only related with *Eucalyptus* spp. but with an uncountable number of tree species, with isolates recovered especially from tree hollows and decayed woods (Lazera et al. 1998, Fortes et al. 2001). Importantly, their studies revealed as well that trees are also a habitat for *C. neoformans* (Lazera et al. 1996), and that sometimes both species are found in the same tree. Those findings encouraged a group in Colombia to search for the habitat of *C. gattii* in a variety of trees and, described, for the first-time, almond trees (*Terminalia catappa*) as the natural habitat of *C. gattii*, serotype C, VGIII (Callejas et al. 1998). Recently the serotype and molecular type of this species has been isolated from *Tipuana tipu* a native tree from Argentina (Mazza et al. 2013).

Following Lazera's findings, tree hollows, decayed wood and soil samples have been described as the habitat of *C. gattii*, and *C. neoformans* in a plethora of indigenous and imported trees in some Latin-American countries, including: Argentina (Refojo et al. 2009, Mazza et al. 2013, Cattana et al. 2014), Brazil (Costa et al. 2009, Andrade-Silva et al. 2012, Anzai et al. 2014, Brito-Santos et al. 2015, Castro e Silva et al. 2016), Chile (Toro-Zúñiga et al. 2015), Colombia (Escandón et al. 2005, Escandón et al. 2010, Firacative et al. 2011, Mak et al. 2015, Noguera et al. 2015, Vélez and Escandón 2017), and Cuba (Illnait-Zaragozí et al. 2010a, Illnait-Zaragozí et al. 2012) (Table V). Some of the trees genus and species that are the reservoir of *C. gattii* as well as *C. neoformans* are: *E. camaldulensis*, *E. tereticornis*, *Eucalyptus* spp., *Acacia* spp., *Corymbia ficifolia*, *Phoenix* spp., *Tipuana tipui*, *Moquilea tomentosa*, *Cassia grandis*, *Ficus* spp., *Croton* spp., *Cedrus* spp., *T. catappa* and *Tabebuia rosea*.

The rationale for the association of *C. neoformans* and *C. gattii* with decayed wood as a primary ecological niche for both species is that they are lignicolous, the so-called wood decay fungi, due to laccase (diphenoloxidase), an enzyme that oxidizes diphenolic substrates to form long polymers of melanin. Laccase provides protection to the fungus in the environment, and it is one of the most important and most studied virulence factors in *Cryptococcus* (Kwon-Chung et al. 2014).

Environmental isolates have been analysed with some phenotypic and molecular techniques. Antifungal susceptibility has been determined in some of them (Andrade-Silva et al. 2013, Teodoro et al. 2013, Firacative et al.

2016) and the results are quite variable. Andrade-Silva et al. (2013) reported that 6.2% of the environmental *C. neoformans* isolates were resistant to FCZ. Although in general, clinical isolates have lower susceptibility than environmental isolates to AmB and ITZ and environmental isolates have had lower susceptibility than clinical isolates to FCZ, VCZ, and KCZ, Teodoro et al. (2013) reported that all environmental *C. neoformans* isolates were susceptible to the antifungals tested. In addition, Firacative et al. (2016) reported that *C. gattii* VGIII environmental isolates were less susceptible to 5FC, PCZ, VCZ, ITZ and FCZ, and more susceptible to AmB, compared with clinical and veterinary isolates. Clinical isolates of *C. neoformans* were reported to be less susceptible to antifungal drugs than environmental isolates (Souza et al. 2010). Although the contribution of these studies is of great importance, more work needs to be done to have enough data for an analysis, especially for the correlation between the source of the isolates, clinical and environmental, and their antifungal susceptibility profiles.

As it was mentioned earlier, molecular studies have been done with some of the environmental isolates, indicated on Table III and Table V, and the importance of the studies done in Latin America was revealed when the global diversity of the *C. gattii* VGII isolates was studied, and the results provided evidence on the evolution of this pathogen in North American Pacific Northwest and the dispersal from South America, most likely from the Amazonia and the Northeast of Brazil, where *C. gattii* VGII strains have shown to be the most variable genetically (Engelthaler et al. 2014).

Many questions about the presence of the fungus in the environment were still without a complete answer, and one of them was related to the survival time of the blastoconidia in the samples. In this way, a couple of studies were carried out in Latin America, and the findings indicated that blastoconidia could remain viable from 45 days up to ten years (Álvarez et al. 2013, Escandón and Castañeda 2015). Efforts have been also made in the design of a culture medium, which use results appropriate for environmental studies to recover the fungus with a major efficiency from the environment (Silva et al. 2012, Álvarez et al. 2013). It is worth mentioning that the Staib culture medium supplemented with seeds of *Guizotia abyssinica* (niger seeds) is the medium of choice for environmental studies. These seeds provide the substrate for the laccase and the end product is melanin, which is associated with the cell walls and gives the colonies a characteristic brown pigment allowing for the differentiation from other yeast colonies.

Related to *C. gattii*, the pioneer environmental studies that have been done in Latin America and the ongoing ones with the assistance of molecular tools will allow to understand more clearly the expansion of the ecological niche of this species.

Particularly in Colombia, a study on ecological niche modelling was also used to forecast the distribution of cryptococcal species and to produce risk area maps for cryptococcal disease in the country (Mak et al. 2015). This study was conducted following the success of accurately identifying ecological niche areas of human and an-

imal cases of *C. gattii* in British Columbia, Canada (Mak et al. 2010) and also complements the report on niche prediction of *C. neoformans* and *C. gattii* in Europe (Cogliati et al. 2017). As ecological niche modelling is a great tool to very likely predict where these pathogenic yeasts survive in the environment and therefore to identify areas with high risk of infection, it would be relevant to conduct such studies in all Latin-American countries, in order to increase awareness of this disease in regions at risk of environmental colonisation of *C. neoformans* and *C. gattii*.

Concluding remarks - The study of cryptococcosis and its etiological agents have been taking place in several Latin-American countries for several years and because of this, significant progress has been made on topics such as clinical epidemiology, laboratory identification and typing of cryptococcal strains, searching, and understanding the environmental niche, and recently, on the basic biology and population genetics of *C. neoformans* and *C. gattii*.

Key points - (i) globally, *C. neoformans* molecular type VNI, causes more than 90% of the cases of cryptococcosis in Latin America and the main risk factor for acquiring it (70-90%) is to be infected with HIV. *C. gattii*, VGII and VGIII, are also recovered, affecting otherwise healthy individuals; (ii) AmB and FCZ are the antifungal drugs of choice, since 5FC is unavailable in the region; (iii) laboratory diagnoses and species or molecular type identification are performed with the standardised protocols; (iv) determination of the antifungal susceptibility shows that in 90% of cases, both species are susceptible to the antifungals tested, namely: AmB, 5FC, FCZ, PCZ, VCZ, and ITZ. Particularly, *C. gattii* VGIII, serotype C isolates have shown decreased susceptibility to azoles, especially to FCZ; (v) molecular studies of clinical and environmental isolates show that in Latin America, *C. neoformans*, molecular type VNI, is not only predominant among the clinical cases, but also in the environment, followed by *C. gattii* molecular type VGII, with similar proportions in both clinical-, and environmental sources; (vi) a great amount of information is from the Latin American studies on the environmental niche, especially for *C. gattii*; (vii) the increasing application of genotyping methods in Latin America, has also greatly contributed to the global study of *C. neoformans* and *C. gattii*, to the recognition of their great inter-, and intra-species genetic diversity, to understand how these pathogenic yeasts have spread around the world and what is their population structure, and to better define some disease aspects of cryptococcosis, an important mycosis in the region.

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CF, JL, MTIZ and EC wrote the manuscript. The Latin American Cryptococcal Study Group contributed with papers, summary tables per country and approved the final version of the manuscript.

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