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REVIEW ARTICLE

Cryptococcosis in the Democratic Republic of Congo from 1953 to 2021: A systematic review and meta-analysis

Bive Bive Zono[1,2] | Dacquin Muhandwa Kasumba[1] | Hippolyte Situakibanza Nani-Tuma[3] | Ben Bepouka Izizag[3] | Marc Yambayamba Kapenga[4] | Ruth Nsuka Yanga[1] | Tshimy Tshimanga Yona[5] | Erick Kamangu Ntambwe[1] | Marie-Pierre Hayette[2] | Georges Mvumbi Lelo[1]

[1]Molecular Biology Service, Department of Basic Sciences, Faculty of Medicine, University of Kinshasa, Kinshasa, the Democratic Republic of Congo

[2]Department of Clinical Microbiology, National Reference Center for Mycosis, Center for Interdisciplinary Research on Medicines, University of Liege, Liege, Belgium

[3]Department of Internal Medicine/ Department of Tropical Medicine, Faculty of Medicine, University of Kinshasa, Kinshasa, the Democratic Republic of Congo

[4]Department of Epidemiology and Biostatistics, Kinshasa School of Public Health, University of Kinshasa, Kinshasa, the Democratic Republic of Congo

[5]Department of Medical Biology, Higher Institute of Medical Techniques-Kinshasa, Kinshasa, the Democratic Republic of Congo

Correspondence
Bive Bive Zono, Molecular Biology Service, Department of Basic Sciences, Faculty of Medicine, University of Kinshasa, Kinshasa, the Democratic Republic of Congo.
Email: bive.zono@unikin.ac.cd

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Abstract

Cryptococcosis is a common opportunistic infection associated with HIV/AIDS. The present review systematically describes the clinical and biological aspects of cryptococcosis in the Democratic Republic of Congo (DRC) and estimates its 2020 burden in people living with HIV (PLHIV). Following PRISMA guidelines, we searched online databases for records of cryptococcosis/*Cryptococcus* spp. in the DRC. Meta-analysis was then performed to estimate summary statistics and the corresponding 95% confidence intervals (CI). A total of 30 studies were included. These included 1,018 cryptococcosis patients, including 80.8% with neuromeningeal cryptococcosis (NMC) and predominantly immunocompromised due to HIV/AIDS (97.6%). The NMC mean prevalence was estimated at 9.63% (95% CI: 5.99–14.07). More than one in two patients (52.7%) under treatment died. Monotherapy with fluconazole was the main treatment administered (80.6%). Furthermore, we estimate that about 9,265 (95% CI: 5,763–13,537) PLHIV had cryptococcosis in 2020, in DRC; of which about 4,883 (95% CI: 3,037–7,134) would have died in the same year. Among isolates in all included studies, 74 strains have been characterised. Of these, 82.4% concerned *Cryptococcus neoformans* sensu lato (s.l) (exclusively of serotype A and mostly of molecular types VNI and VNII) and 17.6% concerned *Cryptotoccus gattii* s.l (belonging to serotype B/molecular type VGI). Cryptococcosis remains common with an unacceptably high mortality rate. A large number of PLHIV affected by and dying from cryptococcosis in 2020 demonstrates its heavy burden among the Congolese PLHIV. To mitigate this burden, it is important to improve the quality and accessibility of care for all PLHIV.

KEYWORDS
burden, cryptococcosis, *Cryptococcus neoformans/Cryptococcus gattii*, DRC, meta-analysis, systematic review

1 | INTRODUCTION

In the 1980s, the global emergence of the human immunodeficiency virus (HIV) with its associated acquired immunodeficiency syndrome (AIDS), profoundly altered the epidemiology of many infectious diseases.[1] Cryptococcosis, one of these infectious diseases, is an opportunistic invasive mycosis caused by encapsulated yeasts of the genus *Cryptococcus*, harbouring seven distinct

haploid species (*Cryptococcus neoformans*, *Cryptococcus deneoformans*, *Cryptococcus gattii*, *Cryptococcus bacillisporus*, *Cryptococcus deuterogattii*, *Cryptococcus tetragattii*, and *Cryptococcus decagattii*) in the *Cryptococcus neoformans/Cryptococcus gattii* species complexes, and three non-pathogenic species (*C. amylolentus*, *C. depauparatus and C. luteus*).[2,3] Transmission occurs by inhalation of propagules, after which the fungus may establish itself in the pulmonary tract. In immunocompetent individuals, infection is often quickly resolved. In immunosuppressed people, to the contrary, the yeast may disseminate to the central nervous system, causing meningoencephalitis (the so-called neuromeningeal form of the disease).[4] HIV/AIDS infection is considered to be the most important risk factor of cryptococcosis. Other known risk factors include organ transplantation, long-term corticosteroid therapy and some chronic illnesses, such as autoimmune diseases.[5] In 2014, the global incidence of cryptococcal meningitis was estimated at 223,100, with a case-mortality rate of over 80%, resulting in over 181,100 deaths. About three-fourths of this burden falls on sub-Saharan Africa.[6]

According to the 2021 United Nations Programme on AIDS (UNAIDS) in DRC, in 2020, 74.5% of the 510,000 people living with HIV (PLHIV) were on antiretrovirals (ARV). Despite ARV treatment, 4.5% (17,000) of PLHIV yet succumbed to HIV infection.[7] Incomplete ARV coverage and the challenges of comprehensive HIV management as routinely deplored in clinics, have favoured the occurrence of multiple opportunistic infections in the DRC. Thus, 63% of PLHIV have suffered from at least one opportunistic infection. Twelve per cent of PLHIV are in a bedridden state on their first visit to a health facility. In Kinshasa, the capital city of the DRC, most PLHIV (70%) admitted to the hospital have advanced HIV disease with a low median CD_4 count of 84 cells/μl. The resulting immunocompromised state increases their risk of developing opportunistic infections.[8]

There exists notable experience in DRC with the various microbiological and clinical presentations of *Cryptococcus* spp. and cryptococcal disease, both classical and atypical. Indeed, while the *Cryptococcus neoformans* spherical shape by microscopy was considered as the only morphology reference, the first observations of elongated cryptococcal yeasts in DRC led to a better description of the *Cryptococcus neoformans* variety *gattii* species.[9] This was followed by the description of the *Cryptococcus neoformans* variety *gattii* meningitis in Congolese PLHIV whilst this species had exclusively infected only immunocompetent patients at that time.[10-12]

In many countries, data on the distribution of *Cryptococcus* species and antifungal susceptibility profiles are available and regularly updated, both in clinical and environmental settings. In the DRC, epidemiological surveillance is still underdeveloped, despite the growing magnitude of the HIV pandemic, the increasing number of a socio-economically vulnerable population, and the precariousness of the healthcare system.[13] Therefore, this systematic review is conducted to collect and analyse available clinical and biological data on cryptococcosis in DRC, and to estimate its burden in the high-risk HIV population. Doing so will help to understand the need, from a public health perspective, to raise awareness about this mycosis and will serve as a reference for further research on the topic.

2 | MATERIALS AND METHODS

This review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines (PRISMA) and analysed published papers on human cryptococcosis/*Cryptococcus* spp. from 1953 to 2021 in DRC (previously called Zaire).[14]

2.1 | Search strategy

We searched Google Scholar, PubMed and African Online (from the oldest paper to the last online search date, 30 September 2021) and grey literature without any language restrictions using the terms "*Cryptococcus* OR *Cryptococcus neoformans* OR *Cryptococcus gattii* OR cryptococcosis OR torulopsis OR torulosis"; "Democratic Republic of Congo OR Zaire OR Zairean OR Congolese OR Kinshasa OR Lubumbashi OR Bukavu OR Kimpese."

2.2 | Inclusion and exclusion criteria

Research articles and case reports were included if they involved Congolese (Zairian) subjects or isolates. Reviews articles were not included.

2.3 | Study selection and data extraction

Three authors (BZB, RNY and TTY) independently reviewed the studies and selected them for consideration in this review. The hierarchical approach based on title, abstract and full text was used to assess the relevance of reports. Additional results were obtained from article references identified during the searches. Cryptococcosis prevalence, epidemiological and clinical (demographic characteristics, sex, age, marital status and clinical signs), biological and therapeutic data, as well as isolates characterisation, were then extracted from each study as available.

2.4 | Data analysis

Data were collated in a Microsoft Excel sheet (Microsoft, Redmond, MA, USA). Meta-analysis was performed using SPSS 26.0 software (IBM, Armonk, NY, USA), to estimate the pooled summary statistics and the corresponding 95% confidence intervals (CI). Descriptive statistics, such as simple counts, ranges and percentages were used to describe data.

2.5 | Estimates of the 2020 annual burden of the neuromeningeal cryptococcosis (NMC) among PLHIV in the DRC

The category of PLHIV who are at high risk to develop NMC are deduced from the UNAIDS 2020 DRC estimates and are adapted to local data. More specifically this category includes adults (over 15 years of age) with HIV (430,000) who know their HIV status (75%) and have an LT CD_4 count of fewer than 200 cells/μl (58.9% average) and were not under ARV (25%).[7,8,15] In addition, we considered cohorts of those on ARV in treatment failure (21.6% average) and the lost to follow-up patients proportion (12.6%).[16–18]

Thus, the number of PLHIV at cryptococcosis risk was estimated as follows: [Adults living with HIV (430,000) X proportion with known serostatus (0.75) X proportion with CD4 < 200 cells/μl (0.589) X proportion of untreated patients (0.25)] + proportion of patients on ARV who are in treatment failure (0.216) + proportion of patients lost to follow-up (0.126)]. This procedure for estimating the at-risk HIV population and the cryptococcosis burden was adapted from earlier literature.[6,19]

Moreover, mean NMC prevalence and the mortality rate as estimated in this manuscript were considered to estimate the cryptococcosis burden among PLHIV in DRC.

3 | RESULTS

A total of 57 papers were retrieved (46 from database search and 11 from grey literature). After deduplication, 54 were retained for abstract review. Twenty-three studies were excluded due to the lack of specific data on cryptococcosis in DRC (Zaire). Out of 31 full-text articles examined, one additional study was excluded as it was a review article. Of 30 studies that were included in the present systematic review (Figure 1), 25 studies were conducted in the clinical setting,[9,11,12,15,20-40] one was an environmental study,[10] and four studies specifically focused on the laboratory characterisation of Congolese isolates.[41-44] The basic characteristics of the clinical studies are summarised in Table 1.

From 1953 to 2021, 1,018 cryptococcosis patients have been reported from DRC (Zaire), including 823 (80.84%) with neuromeningeal forms, 125 (12.28%) with bloodstream forms, 47 (4.62%) with ocular forms, 19 (1.87%) with neuromeningeal and cutaneous-associated forms, three (0.29%) with neuromeningeal and bone-associated forms and one (0.09%) with the digestive form. HIV infection was the underlying risk factor in 97.6% of the cases (994 of 1,018 included patients). The remaining cases concerned patients whose HIV status was undetermined (because these cryptococcal infection occurred before the first characterisation of HIV/AIDS in the 1980s; this concerned 23 of 1,018 patients) and one HIV-negative and apparently immunocompetent patient (1 of 1,018). Based on the included prevalence studies, mean neuromeningeal cryptococcosis (NMC) prevalence was found to be 9.63% (95% CI: 5.99–14.07) among PLHIV, while the bloodstream

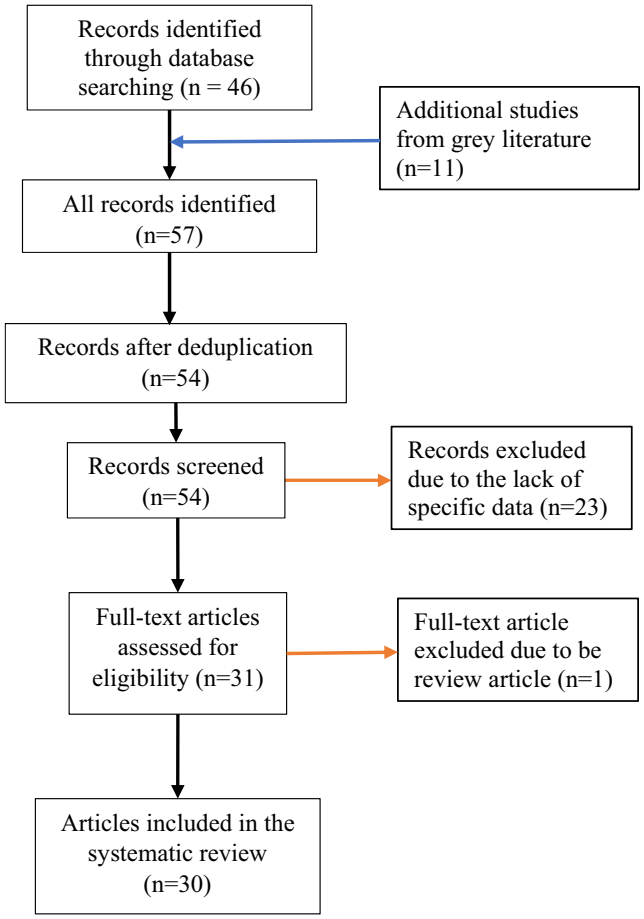

FIGURE 1 Flowchart of the article selection process

cryptococcosis mean prevalence was 14.75% (95% CI: 12.2–17.3) in the same population.

All published data on cryptococcosis originated from medical centres in DRC provinces sharing borders with neighbouring countries (hence having higher border trade activities). The provincial cities concerned are as follows: (1) Kinshasa (10.47% prevalence, 95% CI: 6.27–16.17), (2) Lubumbashi (7.65% prevalence, 95% CI: 2.5–17.29), (3) Bukavu (10.9% prevalence) and (4) Kimpese (11.3% prevalence) as illustrated in Figure 2.

The patients included in this review were mostly women (51.7%, 213 out of 412), with a median age of 35 (28–41) years old and married (52%, 13 out of 25). Overall, the clinical presentation was mainly marked by headache (71.5%, 261 of 365), fever (69.9%, 255 of 365) and meningeal signs (36.9%, 135 of 365; Table 2). The intestinal cryptococcosis was characterised by chronic diarrhoea. Ocular involvement caused by *Cryptococcus* spp. was characterised by visual field defects, abnormal saccades, abnormal eye pursuits, ocular motor paralysis, papilledema and optic atrophy.[32]

Biologically, the median LT CD4 count was 161.8 (98.0–499.6) cells/μL, with a wide distribution ranging from 79 to 610 cells/μl. The cerebral spinal fluid (CSF) was mostly clear (18/30), exhibiting elevated protein levels and lowered glucose levels in 96.5% (111/115) and 93% (107/115) of cases, respectively. The median CSF white cell

TABLE 1 Summary of studies included in the review

N°	Authors	Last collection year	Publication year	Type of study	City	Underlying disease	General population (*n*)	Study population (*n*)	Clinical presentation	Prevalence (%)
1	Stijns J et al	1951	1953	Case report	Kinshasa	HIV	1	1	NMC	-
2	Michaux JL et al	1962	1963	Cases report	Kinshasa	ND[a]	3	3	NMC + bone	-
3	Gatti F et al	1966	1970	Case report	Kinshasa	ND[a]	1	1	NMC	-
4	Vandepitte J et al	1977	1983	Case report	Kinshasa	HIV	1	1	NMC	-
5	Lamey B	1982	1982	Research article	Kinshasa	HIV	-	15	NMC	-
6	Kornaszewski W et al	1984	1986	Research article	Kinshasa	HIV	78	19	NMC + cutaneous	24.3
7	Odio W et al	1985	1985	Research article	Kinshasa	HIV	181	11	NMC	6.0
8	Colebunders R et al	1986	1988	Research article	Kinshasa	HIV	28	1	Digestive	3.6
9	Kapend K et al	1986	1987	Case report	Lubumbashi	HIV	1	1	NMC	-
10	Masengo-Bwanga et al	1987	1988	Research article	Lubumbashi	HIV	26	1	NMC	3.8
11	Patrick Desmet et al	1988	1989	Research article	Kinshasa	HIV	450	55	Bloodstream	12.2
12	Muyembe JJ et al	1990	1992	Case report	Kinshasa	HIV	1	1	NMC	-
13	Perriëns JH et al	-	1992	Research article	Kinshasa	HIV	104	6	NMC	5.6
14	Cheesbrough J. S. et al	1995	1995	Research article	Kimpese	ND[a]	299	19	NMC	11.3
15	Situakibanza H et al	1996	1996	Research article	Kinshasa	HIV	64	8	NMC	12.5
16	Mwanza J-C et al	2002	2004	Research article	Kinshasa	HIV	166	47	Ocular	28.3
17	J. Kivukuto Mutendela et al	2011	2013	Research article	Bukavu	HIV	514	56	NMC	10.9
18	Zono Bive et al	2014	2020	Research article	Kinshasa	HIV	261	23	NMC	8.8
19	Mbayo Lukasu et al	2014	2016	Research article	Lubumbashi	HIV	2100	52	NMC	2.5
20	Mwamba Claude, thèse	2014	2014	Research article	Lubumbashi	HIV	405	70	Bloodstream	17.3
21	Mbula MMK et al	2014	2021	Research article	Kinshasa	HIV	270	15	NMC	5.6
22	Joe Kabongo Katabwa et al	2017	2021	Research article	Lubumbashi	HIV	4283	108	NMC	2.5
23	Dams K. Ngoy et al	2018	2021	Research article	Lubumbashi	HIV	1877	409	NMC	21.8
24	Georges Yumba Numbi	2019	2020	Case report	Lubumbashi	IC[b]	1	1	NMC	-
25	Rose Manga et al	2019	2021	Research article	Kinshasa	HIV		94	NMC	-

[a]Not defined.

[b]Immunocompetent patient.

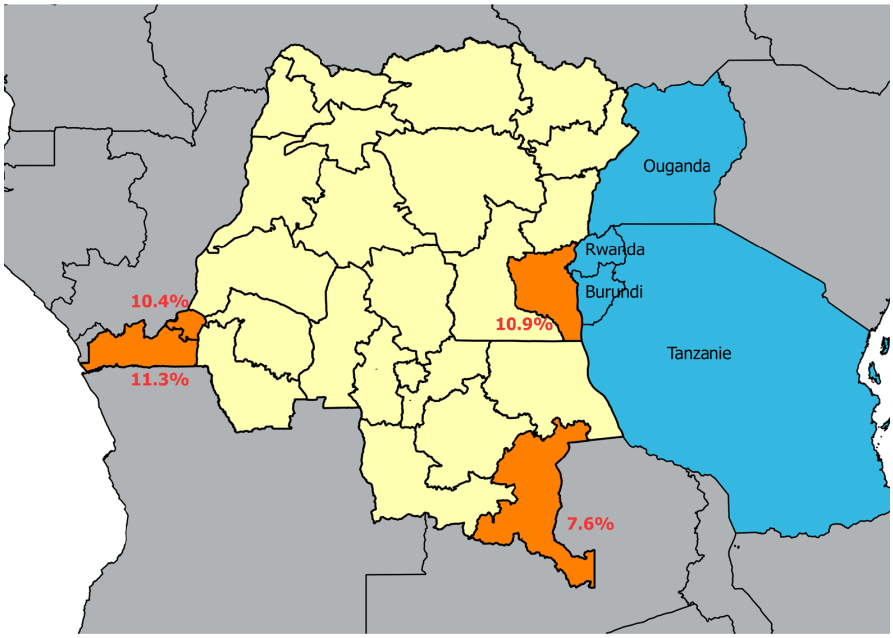

FIGURE 2 Map focusing on DRC and its neighbouring countries, displaying the average prevalence of neuromeningeal cryptococcosis in provinces coloured orange and neighbouring countries where similar isolates to those isolated in DRC have been reported (in blue)

TABLE 2 Clinical data

Parameter[a]	*n* (%)
Demographic characteristics	
Female sex, *n* = 412	213 (51.7)
Median age (P25–P75) (year)	35 (28–41)
Marital status, *n* = 25	
Single	6 (24)
Married	13 (52)
Divorced	3 (12)
Widower	3 (12)
Clinical signs, *n* = 365	
Headaches	261 (71.5)
Fever	255 (69.8)
Coma	52 (14.2)
Weight loss	47 (12.8)
Cough	22 (6.0)
Convulsions	32 (8.7)
Confusions	42 (11.5)
Dizziness	2 (0.5)
Vomiting	54 (14.8)
Meningeal signs	135 (36.9)
Balance disorder	16 (4.4)
Facial paralysis	3 (0.8)
Visual disorders	11 (3.0)
Skin abscess	2 (0.5)
Bone pain	3 (0.8)
Motor deficit	37 (10.1)
Clear CSF appearance, *n* = 30	18 (60)

[a]Based on available data for each parameter.

count was 80 cells/μL (range 42.5–291.9) with neutrophil predominance in 67.9% of cases (19/28).

Although more than one diagnostic test was used for NMC diagnosis, direct India ink staining was the reference test used in most studies (264/357), followed by the cryptococcal antigen test (210/357) and culture (116/357).

Among the patients with severe cryptococcosis (including the neuromeningeal form, the ocular form, the neuromeningeal and cutaneous-associated form, the neuromeningeal and bone-associated form and the digestive form), 319 (out of 893) were reported to have been treated with antifungal drugs. This consisted of fluconazole monotherapy in 80.6% (257/319) of cases. Overall, the death rate in this category of patients was close to half the number of cases (49.3%). Of note, in the cohort with neuromeningeal cryptococcosis death rate was slightly higher (52.7%).

Furthermore, 74 *Cryptococcus neoformans* sensu lato (s.l) and *C gattii* s.l isolates were identified and reported in the DRC over the period covered by this systematic review. Among these isolates, 20.3% were recovered from environmental surveys, potentially implying that the DRC environment contains *Cryptococcus* spp.[10] In clinical and environmental samples, biovar characterisation allowed identification of 13 isolates of *C gattii* s.l isolates (out of 74; 17.6%) and 61 isolates of *C neoformans* s.l (of 74; 82.4%). These isolates were serotyped as follows: 11 of serotype A (out of 15; 73.3%) and 4 of serotype B (out of 15; 26.7%). In all reports included in this review, only 16 isolates underwent molecular characterisation. Among these isolates, eight were characterised as molecular type VNI (50.0%), one as VNII (6.25%), and four of VGI (25.0%). In each study, molecular characterisation was performed according to molecular typing methods established by the International Society of Human and Animal Mycology (ISHAM) *Cryptococcus neoformans/C gattii* complexes working group. On

the contrary, one of each of the recognised DNA fingerprints patterns were identified using the DNA probe UT-4p: pattern II, V and VI (6.25% for each; Figure 3).[41-44]

Based on available published data meeting our inclusion criteria, and considering the DRC mean NMC prevalence as calculated and previously presented in this manuscript (9.63%), as well as the number of PLHIV at greatest risk of NMC (approximately calculated at 96,211), we estimate that 9,265 (95% CI: 5,763–13,537) PLHIV suffered from cryptococcosis in 2020. We also estimate that 4,883 (95% CI: 3037–7134) of these patients died the same year.

4 | DISCUSSION

About 1,018 cryptococcosis cases have been reported in the DRC from 1953 to 2021. The neuromeningeal form was the main clinical presentation. About half of the patients died from this infection. Although the global number of patients newly infected with HIV has decreased from 2.1 million PLHIV in 2015 to 1.5 million in 2020, and the access to ARV has significantly improved from 23.3 million PLHIV in 2018 to 27.5 million in 2020, the HIV epidemiological situation in the clinics remains worrisome, mainly in the advanced HIV management clinics.[45-47] In Kinshasa (DRC), at the Centre Hospitalier Kabinda (CHK), about 70% of PLHIV hospitalised for the first time for AIDS complications exhibit a CD_4 counts below 200 cells/µl. Among these hospitalised PLHIV, a monthly average of 12% suffer from the cryptococcal disease.[8] Although these are data from a single hospital, it is likely that other institutions hospitalising PLHIV for AIDS-associated complications are dealing with similar or even higher figures. This suggests that the incidence data obtained in this study may yet be underestimated. Nevertheless, this review can serve as a starting point for larger epidemiological studies and development of a much-needed surveillance system.

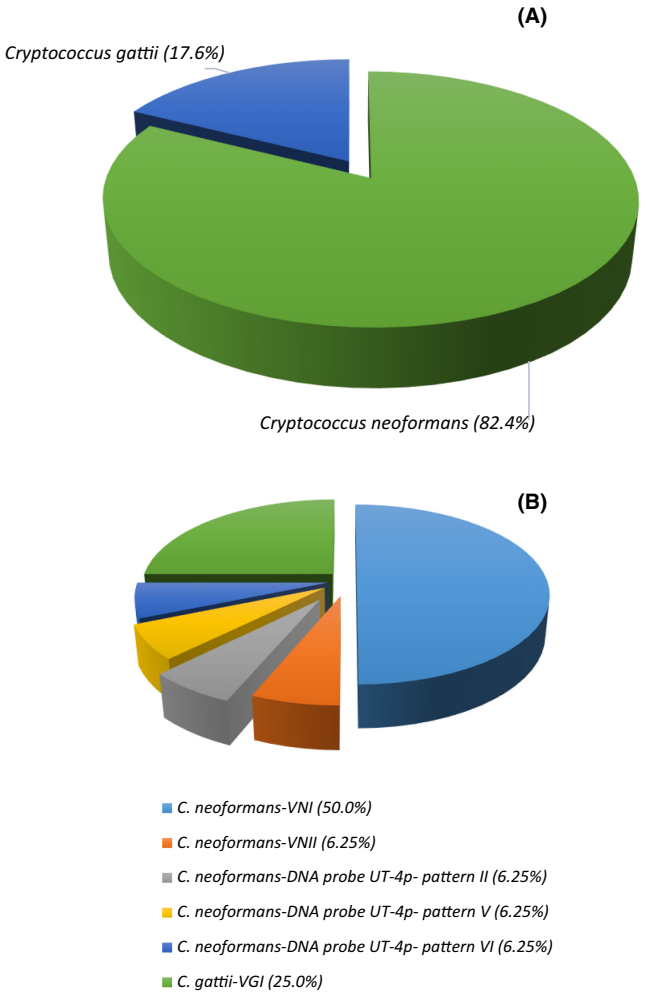

FIGURE 3 Percentage of *Cryptococcus neoformans* s.l and *C gattii* s.l isolates ($n = 74$) identified in the DRC at species or biovar (a) and molecular type level (b)

Overall, cryptococcosis was mainly detected in females with an average of 32.6 ± 10.6 years old. Without being too disproportionate to the men infected proportion, the predominantly female distribution was in line with the general HIV sex distribution in the word as well as in the DRC (310,000 versus 120,000, HIV-positive women and men, respectively). Also in DRC, it appears that the HIV-positive men proportion who die each year (5.4%, 6500/120,000) is currently higher than the HIV-positive women proportion who succumb (3.1%, 9800/310,000), further increasing the HIV-positive women number at high risk of cryptococcosis.[7,46] Paradoxically, in vitro studies suggest that macrophages offer better protection against *Cryptococcus* species in the presence of oestrogen, potentially explaining a decreased susceptibility for cryptococcal infections in females.[48] On the contrary, in the mouse model of disseminated cryptococcosis, describes by Lortholary et al, it is shown that the expression of all cytokines in plasma and of tumour necrosis factor-α and interferon-γ in the spleen are significantly increased in female mice compared with male mice, but survival and fungal load were quite similar in both sex-groups.[49] Interestingly, exposure to *Cryptococcus* ecological niches, once thought to be more pronounced in males than females, is outdated in the African context, especially in rural areas where field and other activities are no longer considered the preserve of males. Here, we observed that females living with HIV could still be the main group affected by cryptococcosis. This warrants further epidemiological and biological investigations. For example, a comparative study of the occurrence of cryptococcosis in each sex-proportion, in a single population controlled for confounding factors, could improve knowledge about the clinical presentation of this deadly disease. The interplay of possible oestrogen-mediated protection, with immunological aspects of HIV infection, would also need to be taken into consideration; especially since most of the above-mentioned experiments were not conducted in an environment of permanent HIV-*Cryptococcus* co-infection.

Cryptococcosis develops mainly in the T-cell immunosuppression context, which may be caused by HIV/AIDS or other risk factors such as solid organ transplantation, long-term corticosteroid therapy, malignancy, diabetes and cirrhosis. It can also occur in patients without identified immunosuppression, whose confirmation is highly dependent on the capacity of the available diagnostic equipment.[50]

Nineteen of the 23 cases described in this manuscript without any specification of underlying risk factors occurred in the HIV/AIDS era (1995s). We assume that they would have been developed in HIV patients but this would just not have been mentioned in the paper. The remaining four cases were described before the 1980s. Interestingly, Numbi et al described a seemingly immunocompetent patient (610 CD_4 cells per µl) who developed *Cryptococcus neoformans* s.l meningitis, based on the diagnostic tools available in the region.[35] However, it cannot be excluded that in similar cases there may be other underlying factors, not necessarily related to HIV/AIDS, which may similarly undermine immunity against infection by these yeasts. For example, the presence of auto-antibodies against GM-CSF (granulocyte-macrophage colony-stimulating factor) was reported to predispose otherwise immunocompetent patients to infections caused by certain species of *Cryptococcus* spp.[51,52] These observations invite further research in factors that may predispose to infection by each of the various *Cryptococcus* species. Within the *Cryptococcus gattii* species complex, for example, *C. gattii* sensu stricto and *C. deuterogattii* are major cause of infections encountered among immunocompetent patients, whereas *C. bacillisporus*, *C. tetragattii* and *C. decagattii* are commonly associated with immunocompromised subjects. This tropism is also described for the *C. neoformans* species complex.[53,54] To clear it up, some studies have compared virulence factors of *C. gattii* species complex to those found in other cryptococcal species, such as *C. neoformans* s.l. Indeed, while *C. neoformans* s.l mutants who lack the calcineurin gene (a gene involved in yeast resistance to high-temperatures conditions) become avirulent, *Cryptococcus deuterogattii* (VGIIa) strains remain viable at high temperatures, suggesting the involvement of other important thermal regulatory gene factors.[55] In addition, the enzymatic activity of laccases in melanin production was found to be higher in serotype AD hybrids, followed by *C. neoformans*, *C. bacillisporus*, *C. deuterogattii*, *C. deneoformans* and *C. gattii*. On the contrary, isolates of serotype AD hybrids had the thinnest capsules compared with *C. gattii*, followed by *C. neoformans*, *C. deneoformans*, *C. bacillisporus* and *C. deuterogattii* which were thicker.[2] The aforementioned case report of an immunocompetent individual infection could harbour features that have not been elucidated.

The occurrence of CSF increased leukocyte counts with neutrophil predominance is another interesting observation that calls for further studies. A bacterial aetiology of meningitis may be part of the differential diagnosis of meningitis in PLHIV. Therefore, to understand the phenomenon of neutrophil predominance in cryptococcal meningitis, studies would be needed that systematically exclude a probable bacterial aetiology or co-infection.[56] Moussa Togola et al described a similar profile in the hospital setting in Togo.[57]

In the DRC context, the diagnosis of cryptococcal meningitis was mainly based on direct microscopic analysis of CSF after India ink staining. However, CSF direct microscopic analysis has been poorly rated than cryptococcal antigen tests and culture.[58] Treatment regimens consisted mainly of monotherapy with fluconazole. Worryingly, in recent years, the efficacy of this azole has been decreasing due to rising drug resistance.[59] This may have contributed to the stark case-fatality ratio of cryptococcal infections observed in this study, which approximates 50%.

Of the 30 included studies, only 4 were conducted before 1980, which is considered the year marking the dramatic worldwide spread of HIV/AIDS to reach pandemic status or the quick rise of identified cases in the world. Although PLHIV already existed as early as 1920 in DRC, the later rise of AIDS cases in the world, particularly in western parts of the world, certainly raised the attention toward opportunistic infections in PLHIV such as cryptococcosis, which has already been described in DRC. We believe that this may explain the quick post-1980 rise of reports and data related to this opportunistic fungal disease in the world, particularly in the DRC where HIV is believed to have originated.[60]

Additionally, it is believed that an increase in the vulnerable population, due to the HIV/AIDS pandemic in the 1980s, had a major influence on epidemiological changes in the prevalence of *Cryptococcus* species globally. Before 1969, most isolates in DRC were of the *gattii* biovar, known to thrive in immunocompetent hosts. The post-1970s period, which saw the dramatic spread of HIV/AIDS, also saw the emergence of the *neoformans* biovar.[11,61] Evidence suggests that the emergence of the *neoformans* biovar was the result of the HIV/AIDS pandemic.[41] Although other immunocompromising factors, not necessarily related to HIV, could be influencing host susceptibility to *C. gattii*, epidemiological evidence supports the existence of a link between AIDS-related immune status and *Cryptococcus* species in patients.[51,52] Despite the heterogeneity in cryptococcal species distribution in DRC at that time, molecular characterisation of isolates has not been completed with new characterisation methods, let alone for newly isolated strains.

Such characterisation could, for example, provide a better understanding of the epidemiological, clinical and biological situation, while measuring the impact of circulating cryptococcal species in the burden of this deadly disease, as well as the link with environmental strains.

Whether a biological and epidemiological link between environmental and clinical isolates exist remains speculative. Indeed, *Cryptococcus* spp. in the Congolese environments have been reported both in the PLHIV homes, domestic cockroaches, pigeon and chicken droppings, as well as in the air and dust.

In order to determine the significance of environmental reservoirs, Zairian isolates from PLHIV were subject to DNA fingerprinting. Surprisingly, environmental isolates were shown to have different DNA fingerprints compared with isolates recovered from patients.[62] Large-scale causality studies will be of great importance for clarifying the relationship between environmental reservoirs and strains involved in clinical infections.

In DRC, the majority (82.4%) of strains are *C. neoformans* s.l, exclusively from serotype A, and most of the molecular types VNI and VNII. The remaining 17.6% are *C. gattii* s.l, serotype B, only molecular type VGI. Notably, *C. neoformans* type VNI and VNII have also been isolated in the DRC neighbouring countries such as Rwanda, Burundi, Tanzania and Uganda, hence raising questions on the possibility of fungal spread through birds and wind.[42,63] Similarly, using

multilocus sequence typing (MLST), characterisation of strains of the *C. neoformans/C. gattii* complexes, the only sequence type ST32 strain previously isolated in Zaire (DRC) and recorded in the MLST ISHAM Fungal Database, was also identified in Tanzania and Uganda (https://mlst.mycologylab.org/).

We estimate that 9,265 (95% CI: 5,763-13,537) PLHIV suffered from cryptococcosis and 4,883 (95% CI: 3,037-7,134) died in 2020, in DRC. The number of deaths among PLHIV due to cryptococcosis approximately represents 28.7% of all PLHIV who died in the same year (17,000).[7] While this annual incidence is not statistically different to the DRC incident numbers previously reported,[13] these data are slightly higher than the incident cases reported across the borders in the Republic of Congo[64] and Uganda.[65] These numbers are much higher than those reported in Europe[66,67] and Asia.[68] Apart from differences in HIV prevalence in different countries, disproportionate results could perhaps also be attributed to differences in the HIV population at high risk of cryptococcosis considered in each study.

To relieve this humanitarian and public health burden, a Congolese national mycosis control programme should (1) consider the identification of high-risk people to develop mycosis, (2) focus on active screening of fungal diseases among this high-risk population, (3) improve the capacity and conditions of the diagnostic and therapeutic technical platform by increasing availability of adequate tests and therapeutic resources, (4) incite local research and (5) encourage continuous training of service providers.

5 | CONCLUSIONS

Data on cryptococcosis/*Cryptococcus* spp. in the DRC are historically rich but outdated and incomplete. While the cryptococcosis burden has been heavy over the years, clinical and biological presentations of the disease have mostly remained classic, apart from certain atypical situations. Because of the growing scale of HIV in DRC and the burden of cryptococcosis in this population, it is important to improve the quality and accessibility of care for all PLHIV. The establishment of a national fungal disease control programme can support these efforts.

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CONFLICT OF INTEREST

The authors report nothing to disclose.

AUTHOR CONTRIBUTION

Bive BIVE ZONO: Conceptualization (equal); Formal analysis (equal); Investigation (lead); Methodology (lead); Project administration (lead); Resources (equal); Software (lead); Visualization (lead); Writing – original draft (lead). **Dacquin Kasumba:** Writing – review & editing (equal). **Hippolyte Situakibanza:** Supervision (equal). **Ben Bepouka:** Conceptualization (equal). **Marc Yambayamba:** Formal analysis (equal). **Ruth Nsuka:** Resources (equal). **Tshimy Tshimanga:** Resources (equal). **Erick Kamangu:** Writing – review & editing (equal). **Marie-Pierre Hayette:** Funding acquisition (equal); Supervision (equal); Validation (equal); Writing – review & editing (equal). **Georges Mvumbi:** Funding acquisition (equal); Supervision (equal); Validation (equal); Writing – review & editing (equal).

ORCID

Bive Bive Zono https://orcid.org/0000-0002-0084-5068

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