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Epidemiology of adult meningitis during antiretroviral therapy scale-up in southern Africa: Results from the Botswana national meningitis survey

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SUMMARY

Objectives: Data on meningitis epidemiology in high HIV-prevalence African settings following antiretroviral therapy scale-up are lacking. We described epidemiology of adult meningitis in Botswana over a 16-year period.
Methods: Laboratory records for adults undergoing lumbar puncture (LP) 2000–2015 were collected, with complete national data 2013–2014. Cerebrospinal fluid (CSF) findings and linked HIV-data were described, and national incidence figures estimated for 2013–2014. Temporal trends in meningitis were evaluated.
Results: Of 21,560 adults evaluated, 41% (8759/21,560) had abnormal CSF findings with positive microbiological testing and/or pleocytosis; 43% (3755/8759) of these had no confirmed microbiological diagnosis. Of the 5004 microbiologically-confirmed meningitis cases, 89% (4432/5004) were cryptococcal (CM) and 8% (382/5004) pneumococcal (PM). Seventy-three percent (9525/13,033) of individuals undergoing LP with identifiers for HIV registry linkage had documented HIV-infection. Incidence of LP for meningitis evaluation in Botswana 2013–2014 was 142.6/100,000 person-years (95%CI:138.3–147.1); incidence of CM was 25.0/100,000 (95%CI:23.2–26.9), and incidence of PM was 2.7/100,000 (95%CI:2.4–3.1). In contrast to previously reported declines in CM incidence with ART roll-out, no significant temporal decline in pneumococcal or culture-negative meningitis was observed.

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Conclusions: CM remained the predominant identified aetiology of meningitis despite ART scale-up. A high proportion of cases had abnormal CSF with negative microbiological evaluation.

Introduction

Central nervous system (CNS) infections are a major cause of morbidity and mortality in people living with HIV (PLWH).[1] Studies in high HIV prevalence regions of central, east, and southern Africa have shown cryptococcal, TB, and pneumococcal meningitis to be the most common microbiologically-confirmed etiologies of meningitis.[2–5] However, these studies have generally been limited to single referral centres,[2,3,5] or have used sub-national surveillance data,[4] and data regarding temporal trends in meningitis with ART scale-up and vaccine introduction are limited. Nationally-representative longitudinal data of meningitis under routine care conditions can provide important insights into the shifting epidemiology of CNS infections in sub-Saharan Africa (SSA), highlight limitations of current diagnostic strategies, and inform research on approaches to improve management for CNS infections.

Botswana, a middle-income country in southern Africa with a 23% adult HIV prevalence in 2017,[6] established the first antiretroviral therapy (ART) program in Africa in early 2002 with public sector care delivered free-of-charge to citizens. Expanded access to ART has led to high coverage, with more than 70% of PLWH estimated to have been diagnosed, started on ART, and virologically suppressed.[7] Despite progress, late presentation with advanced HIV/AIDS,[8,9] as well as care default,[10] are common. We previously showed ongoing high incidence of HIV-associated cryptococcal meningitis in Botswana despite excellent ART coverage.[11] Incidence and temporal trends for other HIV-associated meningitides have not been described, including potential changes in pneumococcal meningitis following the 2012 introduction of childhood 13-valent pneumococcal conjugate vaccine (PCV13).[12]

In a nationwide meningitis study, we collected laboratory records at all facilities that performed microbiological testing of cerebrospinal fluid (CSF). We characterized cases over a 16-year period (2000–2015) in adults (≥15 years old), determined national incidence of meningitis during 2013–2014 when full national laboratory data were available, and examined temporal trends over an 11-year period at Botswana's two referral hospitals (2004–2014) when full records were available at these centres.

Methods

Study design and data collection

A cross-sectional study was performed using data from the Botswana National Meningitis Survey (BNMS), as described previously but extended through 2015.[11] BNMS included records from laboratory facilities that perform cerebrospinal fluid (CSF) testing for meningitis evaluation. Data sources included paper-based records collected during in-person laboratory visits and records from electronic medical records (EMRs) used during the study period. Records were merged and de-duplicated.

Standard CSF evaluation, following national laboratory protocols, remained unchanged during the study period and consisting of white cell count (WCC) and differential (lymphocytes, neutrophils) when WCC ≥10 cells/μL, protein and glucose, microscopy (gram stain, India ink), and bacterial and fungal culture.[13] The full work-up was incomplete for some patients (e.g. missing WCC, protein, or glucose), due to reagent stockout or incomplete laboratory recording. CSF latex agglutination cryptococcal antigen (CrAg) testing was performed infrequently. Tuberculous meningitis evaluation by acid-fast bacillus (AFB) [Ziehl-Neelsen] smear was performed in some laboratories, on request, with TB culture available as a send-out to a national reference laboratory (National Tuberculosis Reference Laboratory). XPert MTB/RIF was introduced in 2012 but was not used on CSF samples. Other molecular diagnostic testing (e.g. herpes simplex viruses or *Toxoplasma gondii* PCR) and syphilis testing have not been introduced in public-sector laboratories.

HIV-related data (HIV status, CD4 count, ART use) were obtained from EMR records and from a national HIV registry through deterministic linkage of laboratory and HIV records using a unique 9-digit national identification number ("Omang"). As Omang was not documented in paper records, linkage was restricted to individuals undergoing evaluations at centres using the EMR, Integrated Patient Management System (IPMS). For CD4 T-cell count, the closest value within a 6-month period of lumbar puncture (LP) was used. Prior ART use was defined as documented prescription of ART or any HIV viral load testing within 3 months of CSF evaluation (as initial viral load testing only occurs after at least 3 months on ART[14]).

The study was approved by the University of Botswana, the Botswana Ministry of Health and Wellness' Health Research Development Committee, Botswana referral and district hospitals with research ethics committees, the University of Pennsylvania, and the University of Washington.

Case definitions

A "case," or episode, was defined as receipt of a LP with CSF analysis at any lab in Botswana from 2000 to 2015. We restricted our analysis to adults (≥15-years) because of differences in the HIV prevalence and epidemiology of CNS infections in adults and children, and for consistency with age strata in UNAIDS denominator estimates. Cases were characterized by microbiological diagnosis: Cryptococcal meningitis was defined as positive CSF microscopy (India ink), culture, and/or CrAg. TB meningitis was defined as positive CSF culture for *Mycobacterium tuberculosis* or AFB smear. Two definitions were used for pneumococcal meningitis: A restrictive definition limited to individuals with CSF culture growth of *S. pneumoniae*, and a second including positive CSF culture or gram stain showing gram-positive cocci. This definition was used due to imperfect sensitivity of culture and added diagnostic yield of gram stain,[15,16] particularly in patients receiving pre-LP antibiotics. Other meningitides were defined by positive CSF culture.

Cases without positive microbiological test results (microscopy, culture, and/or CrAg) were defined as *normal* (CSF WCC 0–5 cells/μL), with *minor abnormalities* (WCC 6–20 cells/μL), or *markedly abnormal* (WCC >20 cells/μL), the latter stratified as lymphocytic (≥50% lymphocytes) or neutrophilic (<50% lymphocytes). CSF protein and glucose analyses were not performed in a sizeable proportion of samples, thus additional classifications of culture negative-disease were not explored. To account for repeated LPs (e.g. therapeutic LPs for cryptococcal meningitis), all CSF evaluations within $a \leq 14$-day window from a previous LP were defined as part of the same episode, and results from the first LP used in

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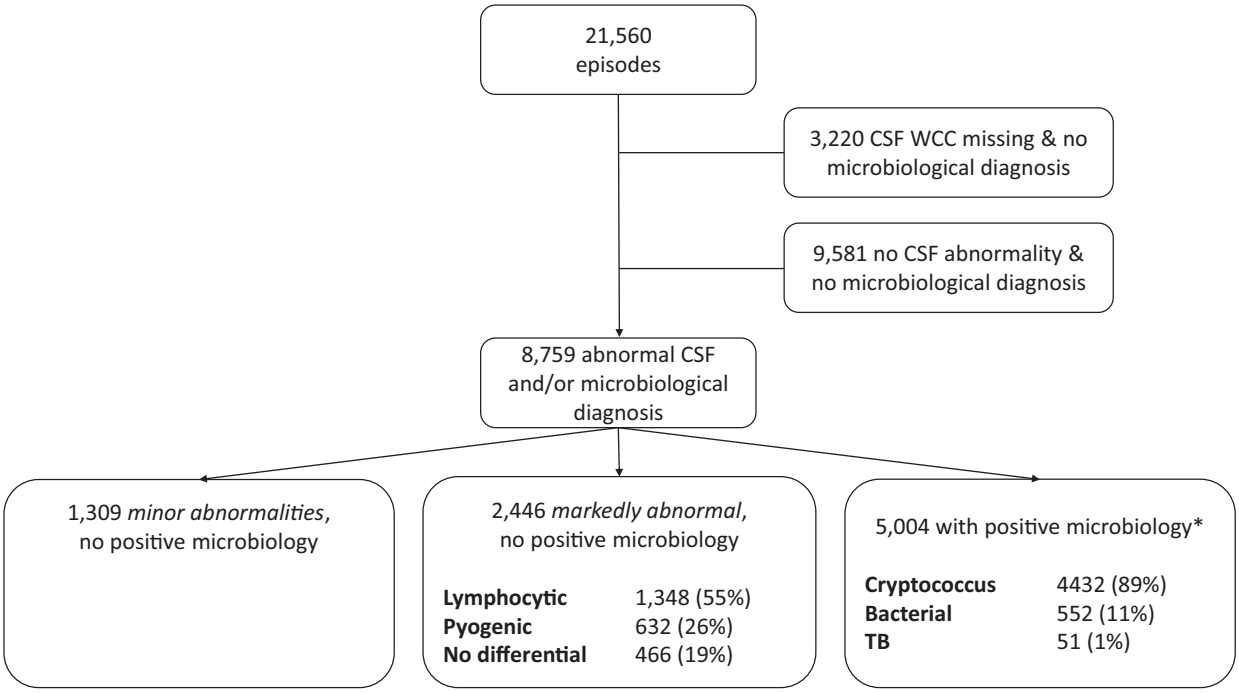

Fig. 1. Overall description of 21,560 adult cases. Of 34,505 overall lumbar puncture assessments, 7238 samples from children, 4014 with undocumented age, and 1693 repeat lumbar punctures excluded. Having minor abnormalities was defined as a CSF WCC 6–20 cells/μl; markedly abnormal CSF was defined as a WCC >20 cells/μL.
CSF - cerebrospinal fluid; TB - tuberculosis; WCC - white cell count
*>100 percent due to 31 mixed cryptococcal and bacterial meningitis cases and 2 mixed cryptococcal and TB meningitis; 1 additional case of *Candida spp* and 1 non-tuberculous Mycobacterium without further speciation also found.

the analysis. For TB meningitis, LP evaluations within a 6-month window of diagnosis were considered part of a single episode.

Data analysis

Findings from all adult cases 2000–2015 were described, including proportion of cases with positive microbiological findings and classification of cases without positive microbiological findings by WCC strata. Patient demographic, HIV clinical characteristics, and CSF findings were evaluated using descriptive statistics (number and percentage or median and interquartile range [IQR]).

Complete CSF records were obtained from all laboratories in Botswana 2013–2014. During this two-year period the overall and HIV-specific incidence of meningitis cases in adults (≥15 years) was determined by category (any LP, cryptococcal meningitis, pneumococcal meningitis, or markedly abnormal CSF [WCC >20 cells/μL] with or without positive bacterial gram stain or culture). TB meningitis incidence was not evaluated due to the small number of confirmed cases resulting from limitations in TB diagnostics. UNAIDS Spectrum data were used for national population denominator estimates.[11] Incidence estimates with 95% confidence intervals (95% CIs) were calculated using a Poisson distribution.

To account for missing age in a small proportion of cases (3%), when deriving incidence estimates it was assumed that the age distribution (adult vs. child) was the same in cases without documented age as in those with age data for each type of meningitis. The total number of HIV-associated cases for each meningitis type was estimated by applying the HIV prevalence data derived from the cases registered in IPMS and linked the HIV database to the entire cohort. This provides a conservative estimate of HIV-associated cases as some PLWH may have been undiagnosed.

At referral hospitals with complete longitudinal records 2004–2014 the number of cases were plotted by year to evaluate trends in cryptococcal, pneumococcal, and culture-negative meningitis with pleocytosis (including CSF WCC 6–20/μL and >20/μL). Due to the lack of defined catchment populations for the two referral hospitals, it was not possible to statistically evaluate incidence trends. Rather, given consistent clinical care provision and referral pathways over the full study duration (as evidenced by a consistent proportion of cases seen at the two referral hospitals), univariate Poisson regression was used to assess trends in case numbers over time. Finally, seasonality of culture-confirmed pneumococcal meningitis cases was assessed by plotting number of cases by calendar month (January, February, etc.), along with average Botswana temperature and rainfall by month 2000–2015.[17] Both adult and paediatric cases were included for seasonality evaluation as similar findings were observed for both groups. We did not perform a time-series analysis for seasonality due to the small number of cases per month. Analyses were performed in Stata (Version 13, College Station, TX) and figures generated using Stata and the ggplot2 package in R.[18] Statistical significance was defined as a p-value <0.05.

Results

Overall description of cases

A total of 34,505 LP samples were documented from 2000 to 2015. After restricting to adults (≥15 years), and excluding repeat samples, 21,560 unique cases were observed (Fig. 1). Fifty-six percent (12,143/21,560) were from referral hospitals. Median age was 36 years (IQR 30–45) and 52% (11,108/21,488) were male. Forty-one percent (8759/21,560) had at least minor CSF WCC abnormalities and/or positive microbiological findings. A microbiological diagnosis was made in 23% (5004/21,560) of cases. Of those with HIV-related information, 73% (9525/13,033) were known to be HIV-infected. Median CD4 count was 91 cells/μL (IQR 37–216) with 47% (4474/9525) initiated on ART prior to the date of LP.

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Table 1
Clinical and laboratory characteristics of patients evaluated for meningitis.

Pathogen	Age (years), Median (IQR)	Sex,% (n) male	CSF WCC (/μL), median (IQR)*	Lymphocyte% (IQR)	Protein (g/dL), median (IQR)	Glucose, mmol/L, median (IQR)	HIV +ve,% (n/N)†	CD4 count (cells/μL) if HIV +ve, median (IQR)	On ART if HIV +ve,% (n)
With microbiological diagnosis									
Cryptococcus (*n* = 4432)	36 (31–43)	61.5% (2720/4421)	5 (2–34)	90% (60–95)	0.91 (0.49–1.75)	2.10 (1.23–2.86)	99.9% (2772/2775)	42 (19–89)	37.5% (1039/2772)
TB (*n* = 51)	36 (31–45)	54.9% (28/51)	48 (8–150)	90% (20–96)	2.56 (0.93–3.27)	0.83 (0.61–1.36)	82.8% (24/29)	104 (27–165)	20.8% (5/24)
Pneumococcus (*n* = 382)	36 (30–45)	43.2% (164/380)	250 (40–946)	10% (5–24)	3.66 (2.42–6.66)	0.07 (0.01–0.90)	61.5% (150/244)	196 (127–379)	39.3% (59/150)
Without microbiological diagnosis									
CSF WCC 0–5 (*n* = 9581)	36 (30–46)	47.6% (4546/9557)	–-	–-	0.47 (0.26–0.92)	3.20 (2.60–3.85)	66.2% (4052/6119)	113 (44–268)	54.5% (2207/4052)
CSF WCC 6–20 (*n* = 1309)	36 (30–45)	50.2% (654/1302)	–-	–-	0.84 (0.40–1.80)	2.84 (2.00–3.72)	66.8% (514/770)	129 (55–254)	52.0% (267/514)
CSF WCC >20, no differential (*n* = 466)	36 (30–45)	53.7% (249/464)	70 (32–230)	–-	1.58 (0.80–3.18)	2.10 (1.12–3.17)	64.8% (136/210)	138 (69–317)	46.3% (63/136)
CSF WCC >20, lymphocytic (*n* = 1348)	36 (29–43)	52.6% (708/1345)	123 (54–312)	90% (80–96)	1.83 (1.00–3.53)	1.92 (1.10–2.89)	70.7% (662/936)	156 (77–284)	48.0% (318/662)
CSF WCC >20, pyogenic (*n* = 632)	36 (30–44)	57.1% (358/627)	244 (75–810)	15% (9–30)	2.37 (1.18–4.21)	1.46 (0.90–2.43)	60.1% (217/361)	116 (53–214)	43.3% (94/217)

+ve - positive; ART - antiretroviral therapy; IQR - interquartile range; WCC - white cell count.
* Upper limit recorded for WCC 2000/μL.
† Among patients with documented HIV status.

Characteristics by pathogen

Cryptococcal meningitis (CM) accounted for the majority of cases with positive microbiological findings (89%, 4432/5004). Although India ink and culture was performed routinely on all CSF samples, CrAg testing was performed in only 9% (1844/21,560) of episodes. Most CM patients were males (62%, 2720/4421) (Table 1), with mild CSF inflammation (median WCC 5/μL [IQR 2–34]). Nearly all with HIV registry linkage were known HIV-infected (>99.9%, 2772/2775), with a median CD4 count of 42 cells/μL (IQR 19–89) and 38% (1039/2772) already taking ART.

TB meningitis (TBM) was diagnosed in 1% (51/5004) of cases with positive microbiological findings; 36 by culture, 14 by AFB smear, and 1 by both. This was in the context of infrequent evaluation for TB, with smear performed in 22% (4755/21,560) and culture in 7% (1517/21,560) of cases. CSF was characterized by moderate lymphocytic pleocytosis but significant spread (median WCC 48/μL [IQR 8–150]). Most patients with available HIV-related data were known HIV-infected (83%, 24/29), with a median CD4 count of 104 cells/μL (IQR 27–165).

Cases with confirmatory bacterial culture and/or gram stain accounted for 11% (552/5004) of those with positive microbiological findings; 72% (395/552) of these were culture-positive and 28% (157/552) had a positive gram stain alone. Pneumococcal meningitis was identified in 69% (382/552), 73% (278/382) of whom were *S. pneumoniae* culture-positive. Pneumococcal meningitis cases showed highly inflammatory neutrophil-predominant pleocytosis and hypoglycorrhachia; 62% with HIV-related data (150/244) were known HIV-infected with a median CD4 count of 196 cells/μL (IQR 127–379). CSF and clinical characteristics were similar when we restricted to culture-confirmed cases alone (**Supplemental Table 1**).

Other typical bacterial pathogens were uncommon: 11 *Haemophilus influenzae*, 6 *Group B Streptococcus*, 1 *Neisseria meningitidis*, and 1 *Listeria monocytogenes* cases. Other cultured isolates included 34 *Enterobacteriaceae* (14 *Klebsiella pneumoniae*, 13 *Escherichia coli*, 4 *Salmonella spp*, 2 Enterobacter spp, 1 *Proteus mirabilis*), 32 *Staphylococcus spp* (20 coagulase-negative *Staphylococcus*, 12 *Staphylococcus aureus*), 15 non-pneumococcal and non-group B *Streptococcus spp*, 3 *Pseudomonas aeruginosa* cases, and 12 with other pathogens. Clinical data to distinguish likely contamination versus clinical disease for atypical pathogens, e.g. coagulase-negative *Staphylococci*, were unavailable.

Cases with negative microbiological evaluation

Seventy-seven percent (16,556/21,560) of LP evaluations for meningitis had no positive microbiological findings (Fig. 1), of which 19% (3220/16,556) had no CSF WCC recorded. Of cases with WCC, 28% (3755/13,336) showed elevated WCC; 10% (1309/13,336) mildly abnormal (6–20 cells/μL) and 18% (2446/13,336) markedly abnormal (>20 cells/μL), the majority with lymphocyte predominance (55% [1348/2446]) in those with a WCC differential. Demographic and HIV-related characteristics were similar between WCC categories. Of all cases with negative evaluation, 66% (6526/9897) with HIV registry linkage were known HIV-infected with a median CD4 count of 120 cells/mL (IQR 49–269) and 51% (3343/6526) were on ART before evaluation.

National incidence and temporal trends

Over the two-year period with complete national data (2013–2014), an estimated 142.6 LPs / 100,000 person-years (95%CI: 138.3–147.1) were performed in adults overall, and 405.7 LPs /100,000 person-years (95%CI: 391.6–420.1) among those known to be HIV-infected (Table 2). Using the combined definition for pneumococcal meningitis (positive CSF culture and/or gram stain), the estimated overall incidence of pneumococcal meningitis was 2.7/100,000 person-years (95%CI: 2.4–3.1) and 5.9 /100,000 person-years (95%CI: 4.3–7.9) in the HIV-infected population. Restricted to culture-confirmed pneumococcal meningitis, incidence was 1.8 /100,000 person-years (95%CI: 1.4–2.4) overall and 4.3/100,000 person-years (95%CI: 2.9–6.0) among those known to be HIV-infected. Incidence of cryptococcal meningitis was 25.0/100,000 person-years (95%CI: 23.2–26.9) overall and 92.9/100,000 person-years (95%CI: 86.2–99.9) in HIV-infected adults.

As previously described,[11] cases of cryptococcal meningitis declined from 2004 to 2014 at referral hospitals (Fig. 2).

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Table 2
Botswana national incidence of adult (≥15 years) meningitis, 2013–2014.

Strata	Category	Number of cases	Person-years	Incidence (per 100,000 PYO)	95% confidence interval (per 100,000 PYO)
Underwent lumbar puncture	Overall population	4114	2,884,278	142.6	138.3 - 147.1
	Known HIV-infected	3150	776,523	405.7	391.6 - 420.1
Cryptococcal meningitis	Overall population	721	2,884,278	25.0	23.2 - 26.9
	Known HIV-infected	721	776,523	92.9	86.2 - 99.9
Pneumococcal meningitis	Overall population	73	2,884,278	2.7	2.4 - 3.1
(+ve culture or GPCs)	Known HIV-infected	46	776,523	5.9	4.3 - 7.9
Pneumococcal meningitis	Overall population	53	2,884,278	1.8	1.4 - 2.4
(+ve culture)	Known HIV-infected	33	776,523	4.3	2.9 - 6.0
Any abnormal CSF (WCC >20 or	Overall population	313	2,884,278	10.9	9.7 - 12.1
non-*Cryptococcus* pathogen)	Known HIV-infected	213	776,523	27.4	23.9 - 31.4

+ve - positive; CSF - cerebrospinal fluid; GPC - gram positive cocci; WCC - white cell count.

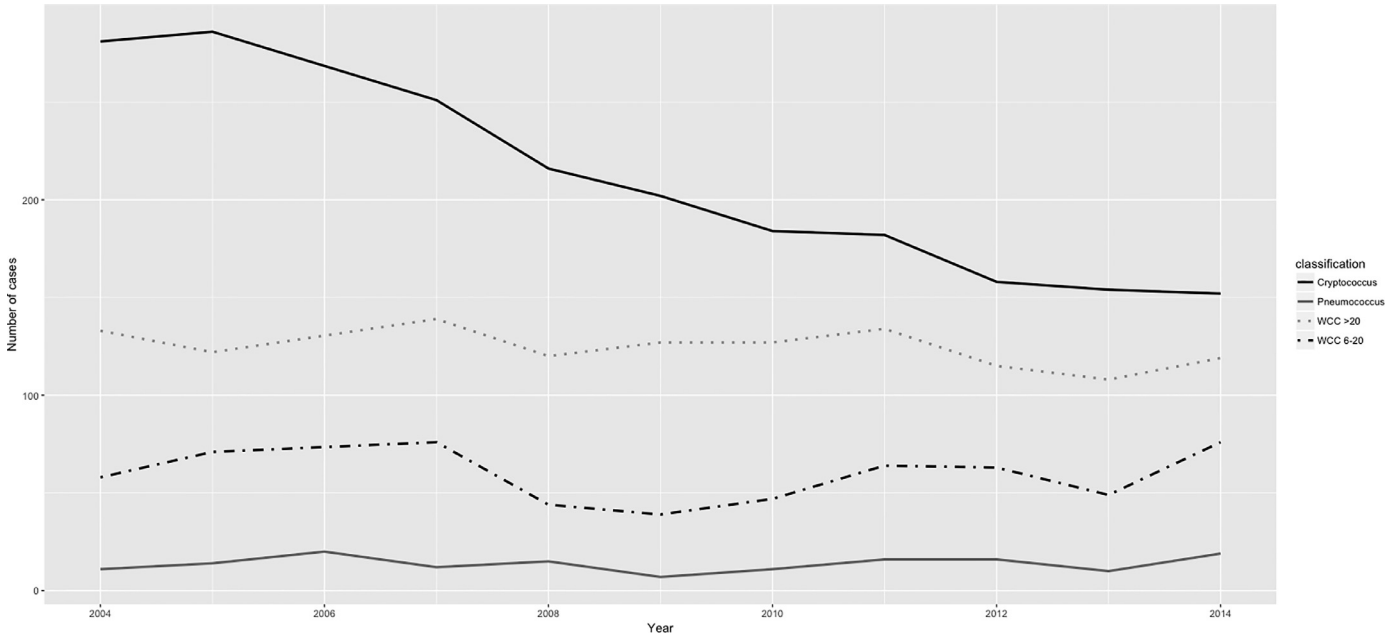

Fig. 2. Trends in meningitis cases at Botswana national referral hospitals, 2004–2014.
WCC 6–20 cells/μL and >20 cells/μL were used to reflect minor and marked inflammation of the CSF suggesting infection or other central nervous system pathology
CSF - cerebrospinal fluid; WCC - cerebrospinal fluid white cell count
Classifications for pneumococcal meningitis restricted to culture-positive cases. WCC >20 and WCC 6–20 groups excluded cases of diagnosed cryptococcal meningitis.
* Excluded 2006 data for Cryptococcus, WCC>20 and WCC 6–20; a large drop in cryptococcal meningitis cases and concurrent rise in cases with WCC 6–20 and WCC>20 likely represented missed diagnoses of cryptococcal meningitis in this year.

However, similar trends were not observed for pneumococcal cases ($p = 0.68$), cases with minor WCC abnormalities (6–20 cells/μL) [$p = 0.94$], or cases with markedly abnormal CSF (WCC >20 cells/μL) [$p = 0.16$]. No decrease in adult pneumococcal meningitis cases was observed 2013–2014 following childhood PCV13 introduction, compared to the earlier period, although based on a small number of cases from referral centres. In seasonal analysis of culture-confirmed pneumococcal meningitis, cases peaked in the winter and early spring (Fig. 3).

Discussion

Using a nationally representative sample of over 20,000 adult episodes of suspected meningitis over 16 years, this study provides novel insights into the epidemiology of meningitis in high HIV-prevalence regions of Africa. About three-quarters of adult patients evaluated for CNS infection were known to be HIV-infected. Evaluation for CNS infections was common in the HIV-infected adults, with over 400 LPs/100,000 person-years. Most HIV-infected individuals undergoing LP were profoundly immunocompromised (median CD4 <100 cells/μL) despite almost half having a history of ART use. This mirrors findings from recent studies showing approximately half of patients with cryptococcal meningitis are now ART-experienced,[19–21] reflecting a combination of late presentation to care with severe immune-suppression,[9] default from care, and ART failure from resistance. Interpreted alongside recent data showing that approximately half of patients with culture-negative suspected meningitis and two-thirds diagnosed with cryptococcal meningitis die within one year of presentation in Botswana,[13,22] these findings demonstrate that CNS infections remain an important cause of mortality in HIV-infected individuals over a decade into the ART era.

Our findings highlight severe limitations in routine strategies for the diagnosis of meningitis common throughout much of sub-Saharan Africa. Consistent with previous studies from South Africa,[3,4] Zambia,[23] and Uganda,[24] cryptococcal meningitis was the most common aetiology of meningitis accounting for about 90% of cases. This high case ascertainment reflects both the still-high burden of advanced HIV/AIDS and excellent diagnostic yield combining rapid cryptococcal testing (usually India ink) with more sensitive fungal culture in all cases. Without use of the highly-sensitive CrAg lateral flow assay (LFA) [IMMY, Norman, OK] in Botswana,[25]

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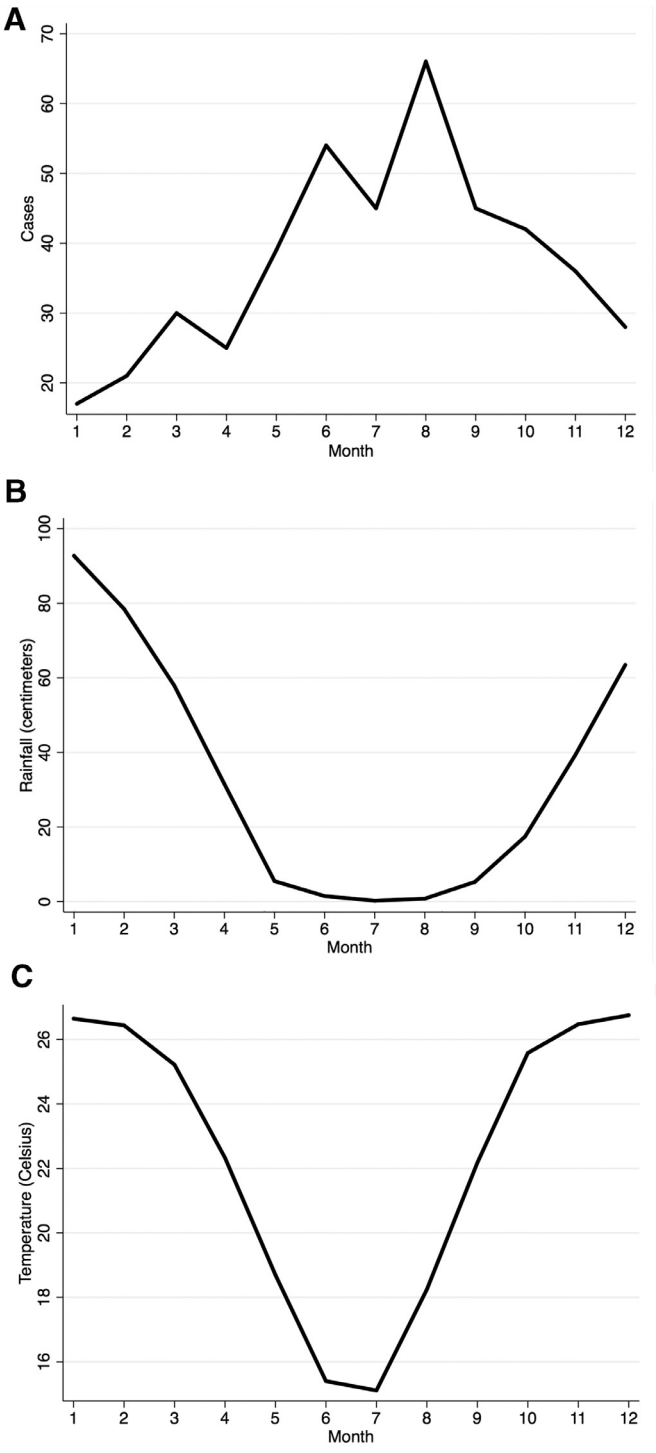

Fig. 3. (A) Culture-confirmed pneumococcal meningitis cases, (B) average rainfall (centimetres), and (C) average temperature (Celsius) by month.
* Month: 1=January, 2=February, etc.; the number of cases each month represent the total number of cases of pneumococcal meningitis diagnosed during the calendar month over the full observation period; graphed monthly temperature and rainfall also represent mean values observed during a calendar month over the full observation period.

a small number of additional cases of cryptococcal meningitis may have been misclassified as having a negative diagnostic work-up. Given the poor sensitivity of India ink stain, LFA CrAg testing should be used as a preferred rapid diagnostic test for cryptococcal meningitis.

Microbiological diagnoses other than cryptococcal meningitis were infrequent despite a significant proportion of cases with abnormal CSF WCC. TB accounted for only 1% of cases with positive microbiological findings. This contrasts with surveillance data from South Africa 2009–2012, where almost 25% of microbiologically-confirmed adult meningitis cases had TB confirmed by smear, culture and/or Xpert. Although the incidence of TB is lower in Botswana than in South Africa (estimated at 300/100,000 versus 567/100,000, respectively, in 2017[26]), a 25-times lower proportion of TB meningitis among patients being investigated for meningitis in Botswana compared to South Africa is unlikely. Of cases in Botswana with pleocytosis and/or a positive microbiological finding, 15% (1348/8759) had a WCC >20/μL with lymphocytic predominance and negative standard work-up, much of which likely reflects undiagnosed TB meningitis. The small number of confirmed TB diagnoses in Botswana almost certainly reflects infrequent TB testing despite culture availability. Reasons for infrequent TB diagnostic testing are probably multifactorial, including lack of decentralized testing and intermittent TB laboratory stoppages; however a major factor driving clinicians' low testing rates is likely the poor sensitivity of AFB smear and delays in culture results with lack of actionable data on which to base clinical decisions.[27] Patients with TB meningitis and advanced immune suppression due to HIV also often lack significant CSF pleocytosis or other "classic" CSF findings,[28] possibly reducing clinical suspicion. Rapid next-generation Xpert (Ultra) has recently shown improved yield over CSF culture in HIV-infected Ugandan patients,[29] and gives actionable results within hours, providing an attractive diagnostic option for TB meningitis which may overcome many of the existing diagnostic challenges and should be more widely implemented for the diagnostic work-up of meningitis.[30]

Bacterial meningitis accounted for 11% of cases with positive microbiological findings with a predominance of *Streptococcus pneumoniae*. Even using a conservative definition restricted to culture-confirmed cases the adult national incidence of 1.8 cases / 100,000 person-years is similar to a reported 2012 incidence of culture-confirmed cases in Gauteng Province, South Africa of 2.5 cases / 100,000 person-years but approximately 6 times higher than the rate estimated in the United States in 2010[4,31] (or 9 times higher with an expanded case definition combining culture and gram stain results). HIV-specific pneumococcal meningitis incidence was approximately 15–20-fold higher than in the general U.S. population. Our pneumococcal meningitis incidence estimates in Botswana are certainly an underestimate of true population rates. First, we found a large number of culture-negative cases with markedly elevated CSF white cell count (>20/μL) and neutrophil predominance (632 cases versus 278 culture-confirmed pneumococcal meningitis cases), probably in-part representing rapid CSF sterilization and decreased culture sensitivity with pre-LP antibiotic administration.[32] Pneumococcal meningitis occurred year-round but peaked during winter and early spring, as previously described.[33] Cases of pneumococcal meningitis over *a* >10-year period at the two referral hospitals were largely unchanged over time and did not show any appreciable decline following introduction of PCV13 vaccination in 2012, although our analysis was restricted to just two full years following introduction. Pneumococcal vaccination trials in HIV-infected adults in Africa have shown mixed results,[34,35] but paediatric vaccination may provide some protection through herd immunity,[36] and further surveillance, preferably with serotype data, is warranted.

Our study has several limitations. We relied on routinely collected laboratory data, did not have detailed clinical information, and lacked HIV-related clinical data for some patients (those with only paper records). A lack of detailed clinical information limits our ability to apply published diagnostic algorithms to better differentiate between potential meningitis etiologies.[37] As most

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records with national identification used for HIV database linkage were from referral hospitals, HIV prevalence data may not be fully representative of the general population. HIV prevalence was likely under-ascertained in our study; although 73% of adults evaluated for meningitis were documented as HIV-infected, this is lower than observed in other high HIV prevalence African settings.[3,38] This results in conservative HIV-associated meningitis incidence estimates. Additionally, other than years 2013–2014, we had incomplete CSF laboratory records and were unable to evaluate national incidence trends over the full study period. However, given stable referral pathways and health utilization reflected in the number of lumbar punctures per year, we evaluated cases over 2004–2014 at national referral hospitals as a crude measure of long-term meningitis trends. Finally, we lacked data from 2016 including after adoption of the HIV "Treat All" strategy (with ART recommended regardless of CD4 count or HIV clinical stage) in Botswana.[14] However, the proportion of patients presenting with advanced HIV disease (CD4 count <200 cells/μL) in Botswana has remained steady from 2015–2017 and diagnostic methods for suspected cases of meningitis are unchanged,[39] suggesting a similar epidemiology in more recent years.

In conclusion, using a nationally representative sample of suspected meningitis cases, we provide insights into the epidemiology of meningitis in high HIV prevalence sub-Saharan African settings. Cryptococcal meningitis remains the predominant aetiology identified. However, our findings highlight a large burden of culture-negative meningitis, some of which likely represents partially-treated pyogenic bacterial meningitis or TB meningitis, but may also include a large number of other potential pathogens. Our findings support the need for scale-up of molecular diagnostic platforms, particularly for TB evaluation, and to better characterize meningitis aetiology to inform treatment approaches.

Declarations of interest

None

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2019.06.013.

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