Background: Central nervous system (CNS) infections are an important cause of childhood morbidity and mortality in high HIV prevalence settings of Africa. We evaluated the epidemiology of pediatric meningitis in Botswana during the rollout of antiretroviral therapy, pneumococcal conjugate vaccine (PCV13), and *Haemophilus influenzae* type B (HiB) vaccine.

Methods: We performed a cross-sectional study of children (<15 years) evaluated for meningitis by cerebrospinal fluid (CSF) examination from 2000-2015, with complete national records for 2013-2014. Clinical and laboratory characteristics of microbiologically-confirmed and culture-negative meningitis were described and incidence of *Streptococcus pneumoniae*, *Haemophilus influenzae* and cryptococcal meningitis estimated for 2013-2014.

Results: 6,796 unique cases were identified. Median age was 1 year (IQR 0-3); 10.4% (435/4,186) of children with available HIV-related records were known HIV-infected. Overall, 30.4% (2,067/6,796) had abnormal CSF findings (positive microbiological testing or CSF pleocytosis). Ten-percent (651/6,796) had a confirmed microbiological diagnosis; including 26.9% (175/651) *Cryptococcus*, 18.9% (123/651) *Streptococcus pneumoniae*, 20.3% (132/651) *Haemophilus influenzae*, and 1.1% (7/651) *Mycobacterium tuberculosis*. During 2013-2014, national cryptococcal meningitis incidence was 1.3 cases/100,000 person-years (PYO) [95%CI:0.8-2.1] and pneumococcal meningitis incidence 0.7/100,000 PYO (95%CI: 0.3-1.3), with no HiB meningitis diagnosed.

Conclusions: Following HiB vaccination, a marked decline in microbiologically confirmed cases of *Haemophilus influenzae* meningitis occurred. Cryptococcal meningitis remains the most common confirmed etiology, demonstrating gaps in prevention-of-mother-to-child transmission and early HIV diagnosis. The high proportion of abnormal CSF samples with no microbiological diagnosis highlights limitation in available diagnostics.

Background

Meningitis accounts for a substantial burden of disease in children globally.[1] Of an estimated 2.8 million meningitis cases in 2016, 54% occurred in children under the age of five, with 46% of all meningitis deaths in this age group.[1] The burden of disease in sub-Saharan Africa is among the highest globally.[1–3] The epidemiology of pediatric meningitis in sub-Saharan Africa has changed over the past two decades with the evolution of the HIV epidemic, including interventions for prevention-of-mother-to-child transmission (PMTCT) and antiretroviral therapy (ART) scale-up, and introduction of vaccines against common bacterial meningitis pathogens.

Regional meningitis surveillance data among children <5 years in 24 African countries found that only 7% of CSF samples reported were culture-positive from 2002-2008.[4] The most commonly confirmed bacterial pathogens were *Streptococcus pneumoniae* and *Haemophilus influenzae* type B, causing 47% and 34% of cases respectively. Meningitis due to *Streptococcus pneumoniae* is associated with a 35% in-hospital case-fatality rate (CFR) and *Haemophilus influenzae* type B with a 25% CFR among children in Africa, with a high risk of neurological sequelae a survivors.[5] Effective vaccines against *Streptococcus pneumoniae* and *Haemophilus influenzae* type B have been introduced to national immunization programs in sub-Saharan Africa over the past decades, with a decline in incidence of invasive *Streptococcus pneumoniae* and *Haemophilus influenzae* disease observed in several countries.[6–10]

The epidemiology of pediatric cryptococcal meningitis and TB meningitis in high HIV-prevalence African settings is poorly characterized. Early laboratory-based surveillance data from 2002-2004 in South Africa found that 0.9% of cryptococcal meningitis cases occurred in children <15 years.[11] However, experience from a referral hospital in Malawi 2000-2012 found

that cryptococcal meningitis was only observed in children <15 years after 2009, with only a small proportion of cryptococcal compared to bacterial meningitis cases.[12] With maturation of the HIV epidemic and effective PMTCT, cryptococcal meningitis in children should become exceedingly rare. Limited data suggests that TB meningitis, with or without HIV infection, may be under-recognized. For example, 22% (126/557) of cases of pediatric meningitis diagnosed at a hospital in Cape Town 2007-2009 were attributed to tuberculosis, although only 10% (13/126) of cases were microbiologically-confirmed.[13] Another recent study from South Africa found that a confirmed CSF microbiological diagnosis was obtained in only 3% (25/865) of children with suspected TB meningitis from 2010-2014.[14]

Botswana, a country in southern Africa with a population of approximately 2 million, has an adult HIV prevalence of 22.8% in 2017.[15] With an effective PMTCT program, in 2015 the estimated HIV prevalence in children ≤14 years was 2%.[15] Botswana has a well-developed antiretroviral therapy (ART) program that started in 2002, as well as a national electronic medical record (EMR) system implemented from 2004 onwards.[16,17] Childhood HiB vaccination was introduced nationally in 2011, with the United Nations International Children's Emergency Fund (UNICEF) estimating coverage of 96% by 2012.[18] Pneumococcal conjugate vaccine (PCV13) was introduced in July 2012 with estimated 95% coverage by 2014.[18] Our primary aim was to describe the epidemiology and temporal trends of meningitis in Batswana children during the evolution of the local HIV epidemic and vaccine rollout.

Methods

Data sources.

We used data from the Botswana National Meningitis Survey (BNMS), a national audit from 2000-2015 including all laboratory facilities that analyzed CSF for cases of suspected meningitis, as previously described.[19] Briefly, we scanned paper laboratory records obtained from in-person visits to all facilities and centrally queried CSF and HIV-related records from national electronic medical record (EMR) systems. These included Integrated Patient Management System (IPMS), which started in 2004 at the two national referral hospitals with gradual rollout to additional facilities, and a national electronic TB registry. Paper records were entered into a REDcap database and paper and electronic records merged and de-duplicated to generate a single dataset. Complete CSF data was available for all facilities for 2013-2014, and compete data was available at both national referral hospitals for 2004-2014.

Standard CSF evaluation in Botswana throughout the study period included white cell count (WCC) and differential (if WCC >10 cells/μL), protein and glucose, microscopy (India ink, Gram stain), bacterial culture with blood and chocolate agar, and fungal culture with Sabouraud agar. Tuberculous meningitis is evaluated, per ordering clinician request, from Acid-fast bacillus (AFB) stain (Ziehl-Neelsen), rather than being part of a standard evaluation on all samples. TB culture is available on request as a send out to a single central TB laboratory. Gene Xpert MTB/RIF was not performed on CSF. Cryptococcal antigen testing was performed infrequently using latex agglutination testing (performed on 4% of children during the study period). Information on HIV status (HIV tests, CD4 count, viral load) was available on a subset of patients with data in the national EMR. To derive HIV prevalence estimates, any child with a positive HIV test, documentation of CD4 T-cell count monitoring, and/or HIV viral load

testing with a detectable viral load was considered to be HIV-infected. National HIV guidelines recommend HIV-exposed infant screening through PCR evaluation.[20]

The study was approved by institutional review boards at the University of Pennsylvania, University of Washington, University of Botswana, the Ministry of Health's Health Research and Development Committee (HDRC), and all hospitals with independent research ethics committees in Botswana. A waiver of informed patient consent was obtained for retrospective collection of routine data.

Data Analysis.

A case was defined as a lumbar puncture (LP) performed on a child aged 0-14 years from 2000-2015 with CSF microbiological testing. Although we did not have clinical data on individual patients, nearly all LPs in Botswana are performed for evaluation of central nervous system infection. To exclude repeat LPs, additional LPs from a unique patient within any 14-day period were excluded. For microbiologically-confirmed tuberculous meningitis, additional LPs within 180 days were excluded. Patient demographic and laboratory characteristics were described (using median, interquartile range, and percentages). The cohort was described as a whole, and further sub-categorised within strata; microbiologically-confirmed (including cases with a positive gram stain but negative culture) or in cases with negative microbiological work-up - within meaningful WCC strata[21]; normal (WCC $\leq$5/µL), mildly abnormal (WCC 6-20/µL), or markedly abnormal (WCC >20/µL). The markedly abnormal group was further sub-divided by neutrophil or lymphocyte predominance (neutrophils >50% or $\leq$50%).

Cryptococcal meningitis was defined as positive CSF microscopy (gram stain or India ink), culture, and/or CrAg. Tuberculous meningitis was defined as positive CSF culture for

Mycobacterium tuberculosis and/or AFB smear. Other organisms were defined through positive CSF culture.

Complete CSF records were obtained from all laboratories 2013-2014. Overall population incidence rates and incidence by category (cryptococcal, pneumococcal and markedly abnormal CSF) were calculated using UNAIDs population denominators, stratified by age. Poisson distribution was used to calculate 95% confidence intervals.

For the time period from 2004-2014, when full CSF laboratory records were available at the two national referral hospitals, the number of cases of pneumococcal, cryptococcal, and *Haemophilus influenzae* meningitis in children were plotted by year to evaluate temporal trends and crude overall trends over time were assessed using unadjusted Poisson regression. Microbiologically confirmed cases of *Haemophilus influenzae* were plotted by month of diagnosis to look for seasonal trends (Supplementary material). Analyses were performed in Stata (Version 14, College Station, TX) and the ggplot2 package in R.[22]

Results

Overall description of cases.

7,238 CSF samples were analysed from children aged 0-14-years between 2000 and 2015. After excluding repeat samples, 6,796 unique episodes were observed in 6,508 children (Figure 1). Median age was 1 year (IQR 0-3) and 53.7% (3,647) of episodes occurred in males. HIV-related data was available from the EMR for 61.6% (4,186) of children; of those, 10.4% (435/4,186) were known to be HIV-positive.

From 2000-2015, 9.6% (651/6,796) of CSF samples yielded a positive microbiological diagnosis (Table 1). *Cryptococcus* was the most common etiology, observed in 26.9% (175/651) of microbiologically-confirmed cases. Median age of children with *Cryptococcus* was 5 years (IQR 1-10, Supplementary Figure 1) and 57.7% (97/175) were male. Minimal CSF inflammatory changes were noted in *Cryptococcus* (median WCC 2/µL [IQR 2-33]) and 100% (108/108) of children with data on HIV status were positive.

Among bacterial pathogens, *Haemophilus influenzae* was cultured in 20.3% (132/651) of microbiologically-confirmed cases. Median age of children with *Haemophilus influenzae* was 1 year (IQR 0-2) and 52.9% (65/132) were male. CSF was markedly abnormal, with a median WCC of 1480/µL (IQR 246-2000) and 10.1% (7/69) of children with data on HIV status were positive.

Culture-confirmed *Streptococcus pneumoniae* accounted for 18.9% (123/651) of microbiologically-confirmed cases, with a median age of 3 years (IQR 0-7) and 55.3% (63/123) male. CSF was markedly abnormal for pneumococcal meningitis, with a median WCC of 420/µL (IQR 120-2000), with neutrophil predominance and 30.2% (16/53) of children with

data on HIV status were known positive. A further 63 cases had gram stain showing gram positive cocci but negative cultures (Supplementary Table 1). Fifteen cases of *Streptococcus agalactiae* and 45 cases of meningitis caused by gram negative rods were observed (Supplementary Table 2).

Tuberculous meningitis was diagnosed in 1.1% (7/651) of microbiologically-confirmed cases, with a median age of 3 years (IQR 1-7) and a majority diagnosed by CSF *Mycobacterium tuberculosis* culture (5/7) and 2 by AFB stain. CSF TB evaluation was uncommon: culture was performed in 2.9% (198/6,796) of cases and AFB smear in 14.4% (975/6,796). Other uncommon culture-positive pathogens are included the footnote of Table 2.

Ninety percent (6,145/6,796) of children investigated with LP had no microbiologically-confirmed diagnosis (Table 1). Of these, 17.2% (1,055/6,145) had no WCC recorded. Of cases with a recorded WCC, 11.7% (595/5,090) had mildly abnormal (WCC 6-20/μL) and 16.1% (821/5,090) markedly abnormal (WCC >20/μL) CSF. Of cases with markedly abnormal CSF, 49.5% (406/821) were lymphocyte-predominant, 35.6% (292/821) neutrophil-predominant, and the remainder had no differential cell count.

National incidence from 2013-2014 and temporal trends at referral hospitals.

National incidence estimates were calculated during 2013-2014 (Table 2). Among children aged 0-14-years an estimated 91.0 LPs were performed per 100,000 person-years of observation (PYO) [95%CI:86.2-96.1]. Highest incidence was observed in the 0-4 age group with 222.3 LPs/100,000 PYO (95%CI:209.4-235.8). Restricted to cases with positive microbiology findings (smear, culture, and/or CrAg), the overall meningitis incidence for

children was 5.4 cases/100,000 PYO (95%CI:4.3-6.7). Highest incidence was observed in the 0-4-year age group at 10.5/100,000 PYO (95%CI:6.9-12.4).

Estimated incidence of culture-confirmed pneumococcal meningitis was 0.7/100,000 PYO (95%CI:0.3-1.3) for all children. Incidence was highest in the 0-4-year and 5-9-year groups at 0.6 (95%CI:0.1-1.8) and 1.0 (95%CI:0.3-2.4) per 100,000 PYO, respectively. Estimated incidence of cryptococcal meningitis was 1.3/100,000 PYO (95%CI:0.8-2.1). Incidence was highest in the 0-4-year group at 2.0/100,000 PYO (95%CI:1.0-3.7). Using 2013-2014 UNAIDS estimates of the number of HIV-positive children in Botswana and assuming all cryptococcal meningitis cases occurred in HIV-positive children, HIV-associated incidence was 54/100,000 PYO (95%CI:32.5-84.2).

Cases of *Haemophilus influenza*e declined between 2004-2014 at referral hospitals (p=0.02), with no cases observed from year 2013 onward (Figure 2). A decline was not observed for cryptococcal (p=0.19) or pneumococcal cases (p=0.22). Seasonal trends observed for *Haemophilus influenza*e included a higher number of cases in colder months with lower rainfall (Supplementary Figure 2).

Discussion

Using a large, nationally representative sample of almost 7,000 children (aged 0-14-years) being investigated for suspected meningitis over a 16-year period in Botswana, we have described the epidemiology and evolution of pediatric meningitis in a high HIV-prevalence region in sub-Saharan Africa. Although *Haemophilus influenzae* accounted for over 20% of microbiologically-confirmed cases of meningitis over the 16-year study period, a marked decline was observed following introduction of HiB vaccination, with only two microbiologically-confirmed cases after vaccine introduction in 2011 and none from 2013 onward. Declines in incidence of *H. influenzae* meningitis have also been observed in other African settings. [23–26] Strikingly, despite over a decade of ART scale-up in Botswana and a successful PMTCT program, *Cryptococcus* remained the most common confirmed etiology of paediatric meningitis through 2013-2014. During this period, we observed an incidence of 1.3 cases per 100,000 PYO in children aged 0-14-years and an HIV-associated incidence of 54 cases per 100,000 PYO. This is just over half the incidence rate observed in HIV-positive adults in Botswana.[19]

Whilst the importance of *Haemophilus influenzae* and *Streptococcus pneumoniae* as causative organisms in pediatric meningitis in the region have been well described,[4] *Cryptococcus* is not usually considered a major causative organism in pediatric meningitis in sub-Saharan Africa. Population-based laboratory surveillance data from urban Gauteng Province, South Africa showed a similar incidence of cryptococcal meningitis in HIV-infected children to that observed in our study (47 per 100,000 PYO in 0-14-year olds) despite this prior surveillance data being over a decade earlier before ART was available (2002-2004).[27] Data from a pediatrics hospital in Cape Town, South Africa from 2007-2009 reported *Cryptococcus* in <0.2% of cases (1/557), with a similar HIV prevalence in this population of 8%.[13] More recent

pediatric data from a single center in Ivory Coast from 2012-2013 found cryptococcal meningitis accounted for 6.5% (2/31) of microbiologically-confirmed meningitis cases, but the small sample limits inference.[23] The high relative incidence observed in Botswana likely has to do, in part, with excellent case ascertainment with standard India ink stain and fungal culture testing on all CSF samples. We found that there was a peak in cryptococcal meningitis in the youngest age group (Supplementary Figure 1). This early-onset cryptococcal meningitis almost certainly represents disease in immunosuppressed infants following mother-to-child transmission of HIV, with a lower ongoing incidence of cryptococcal meningitis occurring in children either not diagnosed in infancy or failing ART. Botswana has a successful prevention of mother-to-child transmission (PMTCT) program;[15] however, the continued presence of cryptococcal meningitis highlights the challenges of achieving full coverage.

The incidence of pneumococcal meningitis observed in 2013-2014 was 0.7 per 100,000 PYO (95% CI 0.3-1.3). This is a minimum estimate as we restricted incidence analysis to culture-confirmed cases. Furthermore, Botswana did not use polymerase chain reaction (PCR) or other advanced diagnostics that might increase diagnostic yield.[28] These rates are three-fold higher than the estimated incidence of 0.25 cases per 100,000 PYO in children aged 1-17-years in the United States in 2010.[29] No obvious decline was seen in the two years following the role out of the PCV13 vaccination, although it is unclear what proportion of the susceptible population was vaccinated during this period. Further surveillance and ideally pneumococcal serotyping of isolates is needed to evaluate the impact of childhood pneumococcal vaccination and potential emergence of serotypes not targeted by PCV13.

We obtained a microbiological diagnosis in only a small proportion (9.6%) of children evaluated for meningitis, with an additional 20.8% (1,416/6,796) having raised CSF white cell

counts but no pathogen identified. This is similar to other studies in sub-Saharan Africa investigating meningitis in children; with 3.7% of LPs revealing a microbiological diagnosis in a study from the Ivory Coast[23], 7% in Mozambique,[30] and 17% in Angola.[24] The low yield can be attributed to a number of factors. Particularly in the neonatal period, LP is frequently performed as part of a sepsis screen, when suspicion for clinical meningitis may be low. However, the large number of cases with markedly abnormal CSF but negative microbiological studies suggests that there were a large number of undiagnosed infections and highlights an urgent need for improved diagnostics to guide therapeutic management including availability of PCR to assess common bacterial and viral pathogens and tailor therapy.[31] Other factors contributing to the low microbiological testing yield include a proportion of children with bacterial meningitis who were pre-treated with antibiotics before LP, resulting in sterile culture.[32]

Botswana has a high TB incidence, [33] so we would have expected to find more TB meningitis cases, suggesting that TB meningitis was significantly underdiagnosed. A number of factors likely contribute to the low number of confirmed TB meningitis cases; CSF culture and microscopy have low sensitivity, particularly in children.[34] However, more importantly, only 3% of children had CSF sent for TB culture and only 14% of samples were evaluated by AFB stain. Six percent (406/6,797) of CSF samples had elevated white cell counts with lymphocyte predominance, some of which are likely attributable to TB. A study in Cape Town, South Africa - with similar HIV epidemiology - reported 2.3% (13/557) of pediatric CSF samples were TB culture-positive, significantly higher than observed in our study (0.10% [7/6,796)]).[13] Using an expanded definition of TB (CSF with high protein, low glucose, lymphocyte predominance and two clinical characteristics suggestive of TB) 22% of cases were attributed to TB.[13] As a laboratory-based surveillance study, we are unable to determine the number of

children who are treated empirically for TB, but improved TB diagnostic testing to guide therapy should be prioritized including expanded use of PCR-based testing (e.g. XPert MTB/RIF) and culture.[35]

Our study had some important limitations. Firstly, we had incomplete HIV-related details, limited to data available in the EMR. However, for 61% of children in the study with electronic records, 10% were known HIV-infected which is substantially higher than general population prevalence estimates for children ≤14-years in Botswana (2%).[15] It is unlikely that many HIV-exposed but uninfected infants would have been misclassified as HIV-infected due to serologic testing detecting residual maternal antibody as national guidelines recommend PCR testing rather than serology for HIV diagnosis in exposed infants.[20] Secondly, our data was limited to routinely-collected laboratory data, meaning detailed clinical information was unavailable. Information on treatment given, outcomes, ART status, and immunization and nutritional status were not available. Thirdly, age in children was generally recorded as a whole number (e.g. 0 years, 1 year); therefore, neonatal meningitis could not be clearly delineated from meningitis later in the first year of life.

In conclusion, our study has shown a reduction in *Haemophilus influenzae* meningitis following the national roll out of the vaccine. Conversely, pneumococcal meningitis cases remained common throughout the study period, and further surveillance is needed to assess the impact of childhood pneumococcal vaccination. The high incidence of cryptococcal meningitis reflects gaps in the PMTCT program; effective PMTCT, with detection of exposed infants and appropriate case management and early initiation of treatment of infants who go onto develop HIV is central to reducing this high incidence. Importantly, the low proportion of cases with

304 microbiological confirmation highlight the urgent need for enhanced diagnostics to guide
305 appropriate clinical management and prevention strategies.
306

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Table 1. Clinical and laboratory characteristics of children 0-14 years evaluated for meningitis from 2000-2015

Pathogen	Age (years), median (IQR)	Sex (male), % (n)	CSF WCC (/µL), median (IQR)[b]	Lymphocytes, % (IQR)	Neutrophil % (IQR)	Protein (g/dL), median (IQR)	Glucose (mmol/L), median (IQR)	HIV positive, % (n)[c]
All non-TB bacterial[d] (n=468)	1 (0-4)	56.2 (243)	443 (70-2000)	10 (5-24)	90 (76-95)	2.9 (1.5-5.2)	0.4 (0.1-1.7)	14.5 (36)
Streptococcus pneumoniae (n=123)	3 (0-7)	55.3 (63)	420 (120-2000)	10 (5-20)	90 (80-95)	3.5 (2.0-5.8)	0.1 (0.0-1.0)	30.2 (16)
Haemophilus influenzae (n=132)	1 (0-2)	52.9 (65)	1480 (246-2000)	10 (4-20)	90 (80-96)	2.4 (1.1-3.9)	0.4 (0.1-1.2)	10.1 (7)
TB (n=7)	3 (1-7)	57.1 (4)	140 (10-270)	60 (30-90	40 (10-70)	---	---	50.0 (1)
Cryptococcus (n=175)	5 (1-10)	57.7 (97)	2 (2-33)	90 (55-96)	10 (4-45)	0.6 (0.3-1.7)	2.5 (1.8-3.4)	100.0 (108)
CSF WCC 0-5 (n=3,674)	1 (0-2)	56.8 (2,002)	2 (0-2)	75 (35-96)	25 (4-65)	0.4 (0.2-0.8)	3.7 (3.0-4.6)	7.3 (179)
CSF WCC 6-20 (n=595)	0 (0-2)	61.4 (337)	10 (8-15)	90 (65-98)	10 (2-35)	0.7 (0.3-1.1)	3.3 (2.5-4.6)	5.6 (20)
CSF WCC >20 lymphocyte predominant (n=406)	1 (0-5)	55.0 (210)	125 (45-400)	90 (78-95)	10 (5-22)	1.5 (0.7-2.6)	2.2 (1.1-3.1)	12.0 (32)
CSF WCC >20 neutrophil predominant (n=292)	1 (0-7)	48.9 (134)	322 (90-1843)	15 (5-30)	85 (70-95)	1.8 (1.0-3.0)	1.8 (0.8-2.8)	9.9 (16)

IQR = interquartile range; WCC = white cell count

[a] missing data on sex for 5.2% (347) of sample

[b] Upper limit recorded for WCC of 2000/µL

[c] missing data on HIV status for 38.4% (2,610) of sample, HIV prevalence stated represents prevalence among those with available HIV status data

[d] positive bacterial culture (346) or gram stain (122) [excluding *Mycobacterium tuberculosis*]. Culture positive: 132 *Haemophilus influenza*, 123 *Streptococcus pneumoniae*, 15 *streptococcus agalactiae*, 12 *Salmonella Spp*, 12 *Staphylococcus aureus*, 9 *Escherichia coli,* 9 *Klebsiella pneumoniae,* 7 *coagulase negative Staphylococcus*, 7 *Pseudomonas Spp.* 2 *Enterobacter Spp*, 1 *Listeria monocytogenes,* 1 *Neisseria meningitides,* 1 *Proteus mirabilis,* 15 other or unidentified and *62 gram positive cocci, 43 gram negative rods, 12 gram negative cocci, 5 gram positive rods.*

Table 2. 2013-2014 National incidence of meningitis in children 0-14 years in Botswana

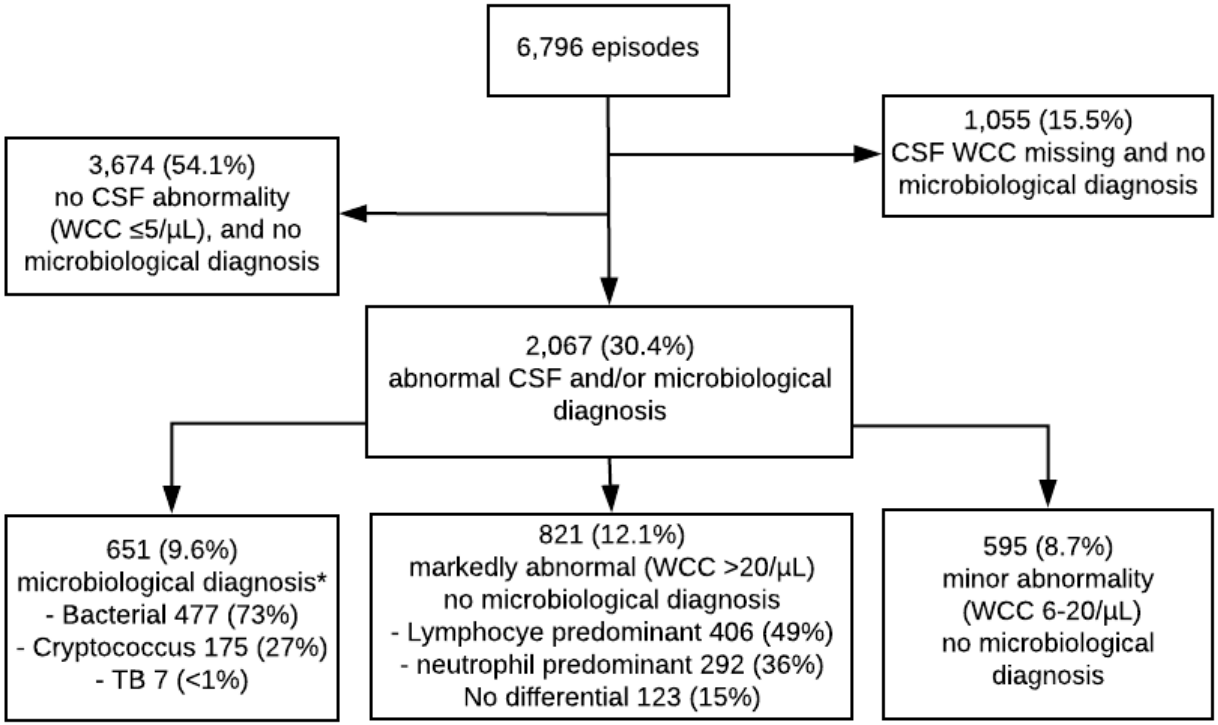
Strata	Age[a] (years)	Number of cases	Person Years	Incidence (95%CI) (per 100,000 person-years)
Underwent meningitis evaluation by lumbar punctures	0-14	1319	1,449,341	91.0 (86.2-96.1)
	0-4	1101	495,308	222.3 (209.4-235.8)
	5-9	118	481,331	24.5 (20.3-29.4)
	10-14	1100	472,702	21.2 (17.2-25.7)
Any abnormal CSF (WCC >20μL or non-*Cryptococcus* pathogen identified)	0-14	171	1,449,341	11.8 (10.1-13.7)
	0-4	117	495,308	23.6 (19.5-28.3)
	5-9	28	481,331	5.8 (3.9-8.4)
	10-14	26	472,702	5.5 (3.6-8.1)
Any microbiological diagnosis (excluding *Cryptococcus*)[b]	0-14	78	1,449,341	5.4 (4.3-6.7)
	0-4	52	495,308	10.5 (7.8-13.8)
	5-9	16	481,331	3.3 (1.9-5.4)
	10-14	10	472,702	2.1 (1.0-3.9)
Streptococcus pneumoniae	0-14	10	1,449,341	0.7 (0.3-1.3)
	0-4	3	495,308	0.6 (0.1-1.8)
	5-9	5	481,331	1.0 (0.3-2.4)
	10-14	2	472,702	0.4 (0.1-1.5)
Cryptococcus	0-14	19	1,449,341	1.3 (0.8-2.1)
	0-4	10	495,308	2.0 (1.0-3.7)
	5-9	4	481,331	0.8 (0.2-2.1)
	10-14	5	472,702	1.1 (0.3-2.5)

CI = confidence interval; WCC = white cell count

[a] Three percent of cases from 2013 and 2014 did not have documented age. It was assumed the distribution of ages in those with no documented age was the same as those who had age documented. Samples missing age were allocated to different age categories in these proportions. For example, if 10% of cryptococcus cases occurred in children aged 0-15 then 10% of samples missing age were allocated to the 0-15 age group.

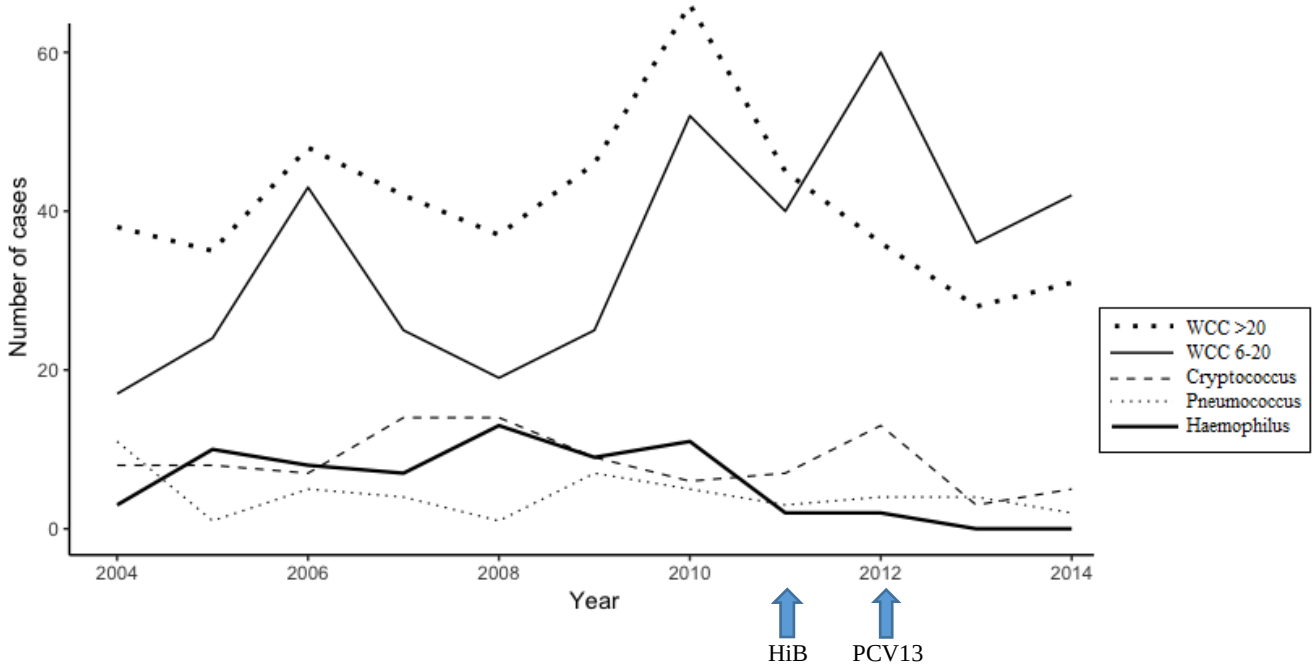
[b] Microbiological diagnosis= positive CSF microscopy (gram stain or AFB) or culture excluding any confirmed cryptococcal cases

Figure 1. Cerebrospinal fluid findings from 6,796 cases of suspected meningitis in children 0-14 years



CSF = cerebrospinal fluid; TB = tuberculosis; WCC = white cell count
* Microbiological diagnosis = positive CSF microscopy (gram stain, India ink or AFB), culture or CrAg

Figure 2. Trends in meningitis cases in children aged 0-14 years at two national referral hospitals from 2004-2014 (arrows showing roll out of vaccination against haemophilus and pneumococcus)



Supplementary table 1. Clinical and laboratory characteristics of children 0-14 years with *Streptococcus pneumoniae* meningitis diagnosed by culture only and using composite definition of culture or gram positive cocci on gram stain without positive culture

Pathogen	Age (years), median (IQR)	Sex (male), % (n)	CSF WCC (/µL), median (IQR)[b]	Lymphocyte, % (IQR)	Neutrophil % (IQR)	Protein (g/dL), median (IQR)	Glucose (mmol/L), median (IQR)	HIV positive, % (n)[c]
Culture-positive only (n=123)	3 (0-7)	51.2 (63)	420 (120-2000)	10 (5-20)	90 (80-95)	3.5 (2-5.8)	0.1 (0.0-1.0)	30.2 (16)
Composite definition[d] (n=186)	2 (0-7)	48.4 (90)	410 (90-2000)	10 (5-20)	90 (80-95)	3 (2-5.8)	0.3 (0.1-1.2)	24.3 (18)

IQR = interquartile range; WCC = white cell count
[a] missing data on sex for 5.2% (347) of sample
[b] Upper limit recorded for WCC 2000/µL
[c] missing data on HIV status for 38.4% (2,610) of sample
[d] Composite definition = positive culture or gram positive cocci

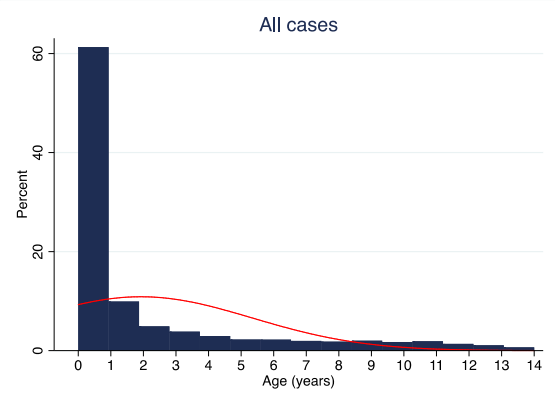
Supplementary table 2. Clinical and laboratory characteristics of children 0-14 years with *Streptococcus agalactiae* and gram negative rod meningitis

Pathogen	Age (years), median (IQR)	Sex (male), % (n)	CSF WCC (/µL), median (IQR)[b]	Lymphocytes, % (IQR)	Neutrophil % (IQR)	Protein (g/dL), median (IQR)	Glucose (mmol/L), median (IQR)	HIV positive, % (n)[c]
Streptococcus agalactiae (n=15)	0 (0-0)	73 (11)	1180 (50-2000)	10 (5-20)	90 (80-95)	3.9 (2.0-4.4)	1.3 (0.8-2.4)	0.0 (0)
Gram negative rods[d] (n=88)	0 (0-1)	56.6 (47)	200 (20-2000)	10 (5-29)	90 (71-95)	2.4 (0.9-6.3)	0.3 (0.1-2.3)	12.1 (7)

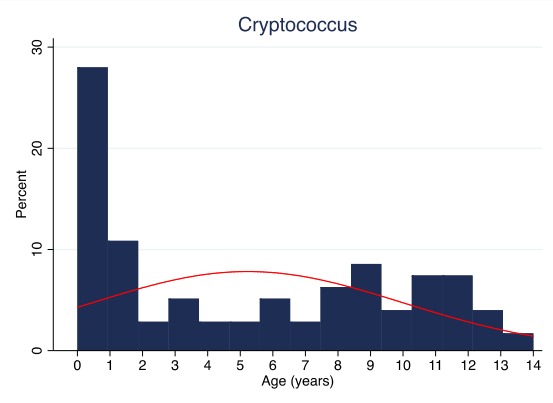
IQR = interquartile range; +ve = positive; WCC = white cell count
[a] missing data on sex for 5.2% (347) of sample
[b] Upper limit recorded for WCC 2000/µL
[c] missing data on HIV status for 38.4% (2,610) of sample
[d] Gram negative rod=gram negative rod on gram stain or culture of gram negative rod organism

Supplementary figure 1: Histogram of distribution of ages for a) all cases, b) *Cryptococcus*, c) Pneumococcus and d) Haemophilus

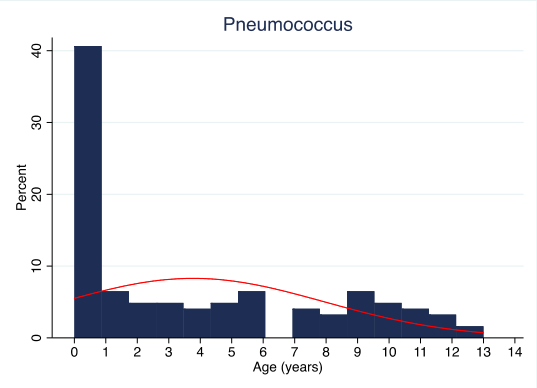
a)



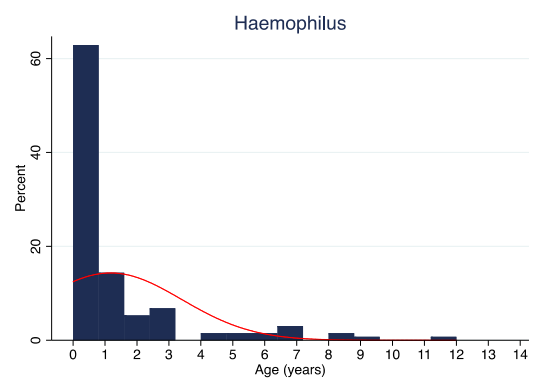
b)



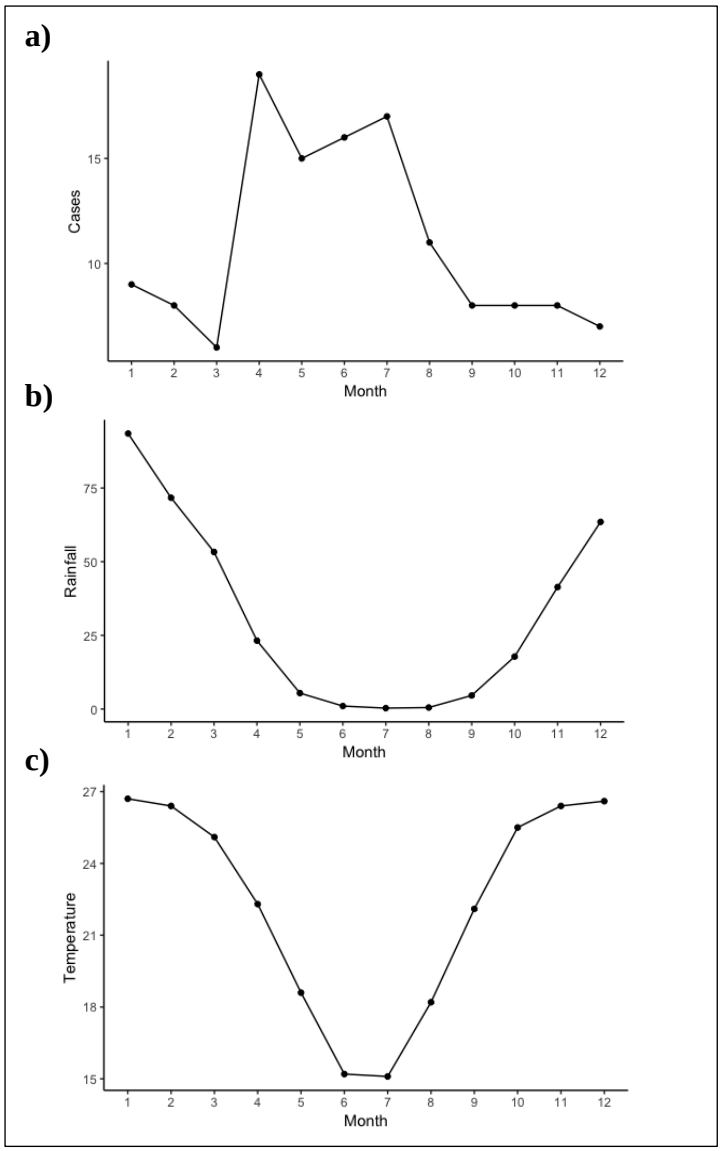
c)



d)



Supplementary Figure 2: (A) Culture-confirmed *Haemophilus influenzae* meningitis cases, (B) average rainfall (centimeters), and (C) average temperature (Celsius) by month



Average monthly temperature and rainfall in Botswana calculated 2000-2015. Source: World Bank Group – Climate Change Knowledge Portal. Available at: https://climateknowledgeportal.worldbank.org/country/botswana