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

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

10 **Results:** 6,796 unique cases were identified. Median age was 1 year (IQR 0-3); 10.4%
11 (435/4,186) of children with available HIV-related records were known HIV-infected. Overall,
12 30.4% (2,067/6,796) had abnormal CSF findings (positive microbiological testing or CSF
13 pleocytosis). Ten-percent (651/6,796) had a confirmed microbiological diagnosis; including
14 26.9% (175/651) *Cryptococcus*, 18.9% (123/651) *Streptococcus pneumoniae*, 20.3% (132/651)
15 *Haemophilus influenzae*, and 1.1% (7/651) *Mycobacterium tuberculosis*. During 2013-2014,
16 national cryptococcal meningitis incidence was 1.3 cases/100,000 person-years (PYO)
17 [95%CI:0.8-2.1] and pneumococcal meningitis incidence 0.7/100,000 PYO (95%CI: 0.3-1.3),
18 with no HiB meningitis diagnosed.

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

24
25

26

27 **Background**

28 Meningitis accounts for a substantial burden of disease in children globally.¹ Of an estimated
29 2.8 million meningitis cases in 2016, 54% occurred in children under the age of five, with 46%
30 of all meningitis deaths in this age group.¹ The burden of disease in sub-Saharan Africa is
31 among the highest globally.¹⁻³ The epidemiology of pediatric meningitis in sub-Saharan Africa
32 has changed over the past two decades with the evolution of the HIV epidemic, including
33 interventions for prevention-of-mother-to-child transmission (PMTCT) and antiretroviral
34 therapy (ART) scale-up, and introduction of vaccines against common bacterial meningitis
35 pathogens.

36
37 Regional meningitis surveillance data among children <5 years in 24 African countries found
38 that only 7% of CSF samples reported were culture-positive from 2002-2008.⁴ The most
39 commonly confirmed bacterial pathogens were *Streptococcus pneumoniae* and *Haemophilus*
40 *influenzae* type B, causing 47% and 34% of cases respectively. Meningitis due to *Streptococcus*
41 *pneumoniae* is associated with a 35% in-hospital case-fatality rate (CFR) and *Haemophilus*
42 *influenzae* type B with a 25% CFR among children in Africa, with a high risk of neurological
43 sequelae a survivors.⁵ Effective vaccines against *Streptococcus pneumoniae* and *Haemophilus*
44 *influenzae* type B have been introduced to national immunization programs in sub-Saharan
45 Africa over the past decades, with a decline in incidence of invasive *Streptococcus pneumoniae*
46 and *Haemophilus influenzae* disease observed in several countries.⁶⁻¹⁰

47
48 The epidemiology of pediatric cryptococcal meningitis and TB meningitis in high HIV-
49 prevalence African settings is poorly characterized. Early laboratory-based surveillance data
50 from 2002-2004 in South Africa found that 0.9% of cryptococcal meningitis cases occurred in
51 children <15 years.¹¹ However, experience from a referral hospital in Malawi 2000-2012 found

52 that cryptococcal meningitis was only observed in children <15 years after 2009, with only a
53 small proportion of cryptococcal compared to bacterial meningitis cases.¹² With maturation of
54 the HIV epidemic and effective PMTCT, cryptococcal meningitis in children should become
55 exceedingly rare. Limited data suggests that TB meningitis, with or without HIV infection,
56 may be under-recognized. For example, 22% (126/557) of cases of pediatric meningitis
57 diagnosed at a hospital in Cape Town 2007-2009 were attributed to tuberculosis, although only
58 10% (13/126) of cases were microbiologically-confirmed.¹³ Another recent study from South
59 Africa found that a confirmed CSF microbiological diagnosis was obtained in only 3%
60 (25/865) of children with suspected TB meningitis from 2010-2014.¹⁴

61

62 Botswana, a country in southern Africa with a population of approximately 2 million, has an
63 adult HIV prevalence of 22.8% in 2017.¹⁵ With an effective PMTCT program, in 2015 the
64 estimated HIV prevalence in children ≤ 14 years was 2%.¹⁵ Botswana has a well-developed
65 antiretroviral therapy (ART) program that started in 2002, as well as a national electronic
66 medical record (EMR) system implemented from 2004 onwards.^{16,17} Childhood HiB
67 vaccination was introduced nationally in 2011, with the United Nations International
68 Children's Emergency Fund (UNICEF) estimating coverage of 96% by 2012.¹⁸ Pneumococcal
69 conjugate vaccine (PCV13) was introduced in July 2012 with estimated 95% coverage by
70 2014.¹⁸ Our primary aim was to describe the epidemiology and temporal trends of meningitis
71 in Botswana children during the evolution of the local HIV epidemic and vaccine rollout.

72

73 **Methods**

74 *Data sources.*

75 We used data from the Botswana National Meningitis Survey (BNMS), a national audit from
76 2000-2015 including all laboratory facilities that analyzed CSF for cases of suspected
77 meningitis, as previously described.¹⁹ Briefly, we scanned paper laboratory records obtained
78 from in-person visits to all facilities and centrally queried CSF and HIV-related records from
79 national electronic medical record (EMR) systems. These included Integrated Patient
80 Management System (IPMS), which started in 2004 at the two national referral hospitals with
81 gradual rollout to additional facilities, and a national electronic TB registry. Paper records were
82 entered into a REDcap database and paper and electronic records merged and de-duplicated to
83 generate a single dataset. Complete CSF data was available for all facilities for 2013-2014, and
84 complete data was available at both national referral hospitals for 2004-2014.

85

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

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

99

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

105

106 *Data Analysis.*

107 A case was defined as a lumbar puncture (LP) performed on a child aged 0-14 years from 2000-
108 2015 with CSF microbiological testing. Although we did not have clinical data on individual
109 patients, nearly all LPs in Botswana are performed for evaluation of central nervous system
110 infection. To exclude repeat LPs, additional LPs from a unique patient within any 14-day
111 period were excluded. For microbiologically-confirmed tuberculous meningitis, additional LPs
112 within 180 days were excluded. Patient demographic and laboratory characteristics were
113 described (using median, interquartile range, and percentages). The cohort was described as a
114 whole, and further sub-categorised within strata; microbiologically-confirmed (including cases
115 with a positive gram stain but negative culture) or in cases with negative microbiological work-
116 up - within meaningful WCC strata²¹; normal (WCC $\leq 5/\mu\text{L}$), mildly abnormal (WCC 6-20/ μL),
117 or markedly abnormal (WCC $>20/\mu\text{L}$). The markedly abnormal group was further sub-divided
118 by neutrophil or lymphocyte predominance (neutrophils $>50\%$ or $\leq 50\%$).

119

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

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

124

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

129

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

137 **Results**

138 *Overall description of cases.*

139 7,238 CSF samples were analysed from children aged 0-14-years between 2000 and 2015.
140 After excluding repeat samples, 6,796 unique episodes were observed in 6,508 children (Figure
141 1). Median age was 1 year (IQR 0-3) and 53.7% (3,647) of episodes occurred in males. HIV-
142 related data was available from the EMR for 61.6% (4,186) of children; of those, 10.4%
143 (435/4,186) were known to be HIV-positive.

144

145 From 2000-2015, 9.6% (651/6,796) of CSF samples yielded a positive microbiological
146 diagnosis (Table 1). *Cryptococcus* was the most common etiology, observed in 26.9%
147 (175/651) of microbiologically-confirmed cases. Median age of children with *Cryptococcus*
148 was 5 years (IQR 1-10, Supplementary Figure 1) and 57.7% (97/175) were male. Minimal CSF
149 inflammatory changes were noted in *Cryptococcus* (median WCC 2/ μ L [IQR 2-33]) and 100%
150 (108/108) of children with data on HIV status were positive.

151

152 Among bacterial pathogens, *Haemophilus influenzae* was cultured in 20.3% (132/651) of
153 microbiologically-confirmed cases. Median age of children with *Haemophilus influenzae* was
154 1 year (IQR 0-2) and 52.9% (65/132) were male. CSF was markedly abnormal, with a median
155 WCC of 1480/ μ L (IQR 246-2000) and 10.1% (7/69) of children with data on HIV status were
156 positive.

157

158 Culture-confirmed *Streptococcus pneumoniae* accounted for 18.9% (123/651) of
159 microbiologically-confirmed cases, with a median age of 3 years (IQR 0-7) and 55.3% (63/123)
160 male. CSF was markedly abnormal for pneumococcal meningitis, with a median WCC of
161 420/ μ L (IQR 120-2000), with neutrophil predominance and 30.2% (16/53) of children with

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

166

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

172

173 Ninety percent (6,145/6,796) of children investigated with LP had no microbiologically-
174 confirmed diagnosis (Table 1). Of these, 17.2% (1,055/6,145) had no WCC recorded. Of cases
175 with a recorded WCC, 11.7% (595/5,090) had mildly abnormal (WCC 6-20/ μ L) and 16.1%
176 (821/5,090) markedly abnormal (WCC >20/ μ L) CSF. Of cases with markedly abnormal CSF,
177 49.5% (406/821) were lymphocyte-predominant, 35.6% (292/821) neutrophil-predominant,
178 and the remainder had no differential cell count.

179

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

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

186 children was 5.4 cases/100,000 PYO (95%CI:4.3-6.7). Highest incidence was observed in the
187 0-4-year age group at 10.5/100,000 PYO (95%CI:6.9-12.4).

188
189 Estimated incidence of culture-confirmed pneumococcal meningitis was 0.7/100,000 PYO
190 (95%CI:0.3-1.3) for all children. Incidence was highest in the 0-4-year and 5-9-year groups at
191 0.6 (95%CI:0.1-1.8) and 1.0 (95%CI:0.3-2.4) per 100,000 PYO, respectively. Estimated
192 incidence of cryptococcal meningitis was 1.3/100,000 PYO (95%CI:0.8-2.1). Incidence was
193 highest in the 0-4-year group at 2.0/100,000 PYO (95%CI:1.0-3.7). Using 2013-2014 UNAIDS
194 estimates of the number of HIV-positive children in Botswana and assuming all cryptococcal
195 meningitis cases occurred in HIV-positive children, HIV-associated incidence was 54/100,000
196 PYO (95%CI:32.5-84.2).

197
198 Cases of *Haemophilus influenzae* declined between 2004-2014 at referral hospitals ($p=0.02$),
199 with no cases observed from year 2013 onward (Figure 2). A decline was not observed for
200 cryptococcal ($p=0.19$) or pneumococcal cases ($p=0.22$). Seasonal trends observed for
201 *Haemophilus influenzae* included a higher number of cases in colder months with lower rainfall
202 (Supplementary Figure 2).

203

204 Discussion

205

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

220

221 Whilst the importance of *Haemophilus influenzae* and *Streptococcus pneumoniae* as causative
222 organisms in pediatric meningitis in the region have been well described,⁴ *Cryptococcus* is not
223 usually considered a major causative organism in pediatric meningitis in sub-Saharan Africa.
224 Population-based laboratory surveillance data from urban Gauteng Province, South Africa
225 showed a similar incidence of cryptococcal meningitis in HIV-infected children to that
226 observed in our study (47 per 100,000 PYO in 0-14-year olds) despite this prior surveillance
227 data being over a decade earlier before ART was available (2002-2004).²⁷ Data from a
228 pediatrics hospital in Cape Town, South Africa from 2007-2009 reported *Cryptococcus* in
229 <0.2% of cases (1/557), with a similar HIV prevalence in this population of 8%.¹³ More recent

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

241
242 The incidence of pneumococcal meningitis observed in 2013-2014 was 0.7 per 100,000 PYO
243 (95% CI 0.3-1.3). This is a minimum estimate as we restricted incidence analysis to culture-
244 confirmed cases. Furthermore, Botswana did not use polymerase chain reaction (PCR) or other
245 advanced diagnostics that might increase diagnostic yield.²⁸ These rates are three-fold higher
246 than the estimated incidence of 0.25 cases per 100,000 PYO in children aged 1-17-years in the
247 United States in 2010.²⁹ No obvious decline was seen in the two years following the roll out
248 of the PCV13 vaccination, although it is unclear what proportion of the susceptible population
249 was vaccinated during this period. Further surveillance and ideally pneumococcal serotyping
250 of isolates is needed to evaluate the impact of childhood pneumococcal vaccination and
251 potential emergence of serotypes not targeted by PCV13.

252
253 We obtained a microbiological diagnosis in only a small proportion (9.6%) of children
254 evaluated for meningitis, with an additional 20.8% (1,416/6,796) having raised CSF white cell

255 counts but no pathogen identified. This is similar to other studies in sub-Saharan Africa
256 investigating meningitis in children; with 3.7% of LPs revealing a microbiological diagnosis
257 in a study from the Ivory Coast²³, 7% in Mozambique,³⁰ and 17% in Angola.²⁴ The low yield
258 can be attributed to a number of factors. Particularly in the neonatal period, LP is frequently
259 performed as part of a sepsis screen, when suspicion for clinical meningitis may be low.
260 However, the large number of cases with markedly abnormal CSF but negative microbiological
261 studies suggests that there were a large number of undiagnosed infections and highlights an
262 urgent need for improved diagnostics to guide therapeutic management including availability
263 of PCR to assess common bacterial and viral pathogens and tailor therapy.³¹ Other factors
264 contributing to the low microbiological testing yield include a proportion of children with
265 bacterial meningitis who were pre-treated with antibiotics before LP, resulting in sterile
266 culture.³²

267

268 Botswana has a high TB incidence,³³ so we would have expected to find more TB meningitis
269 cases, suggesting that TB meningitis was significantly underdiagnosed. A number of factors
270 likely contribute to the low number of confirmed TB meningitis cases; CSF culture and
271 microscopy have low sensitivity, particularly in children.³⁴ However, more importantly, only
272 3% of children had CSF sent for TB culture and only 14% of samples were evaluated by AFB
273 stain. Six percent (406/6,797) of CSF samples had elevated white cell counts with lymphocyte
274 predominance, some of which are likely attributable to TB. A study in Cape Town, South
275 Africa - with similar HIV epidemiology - reported 2.3% (13/557) of pediatric CSF samples
276 were TB culture-positive, significantly higher than observed in our study (0.10% [7/6,796]).¹³
277 Using an expanded definition of TB (CSF with high protein, low glucose, lymphocyte
278 predominance and two clinical characteristics suggestive of TB) 22% of cases were attributed
279 to TB.¹³ As a laboratory-based surveillance study, we are unable to determine the number of

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

283

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

296

297 In conclusion, our study has shown a reduction in *Haemophilus influenzae* meningitis
298 following the national roll out of the vaccine. Conversely, pneumococcal meningitis cases
299 remained common throughout the study period, and further surveillance is needed to assess the
300 impact of childhood pneumococcal vaccination. The high incidence of cryptococcal meningitis
301 reflects gaps in the PMTCT program; effective PMTCT, with detection of exposed infants and
302 appropriate case management and early initiation of treatment of infants who go onto develop
303 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)
<i>Streptococcus pneumoniae</i> (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)
<i>Haemophilus influenzae</i> (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)
<i>Cryptococcus</i> (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 influenzae*, 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

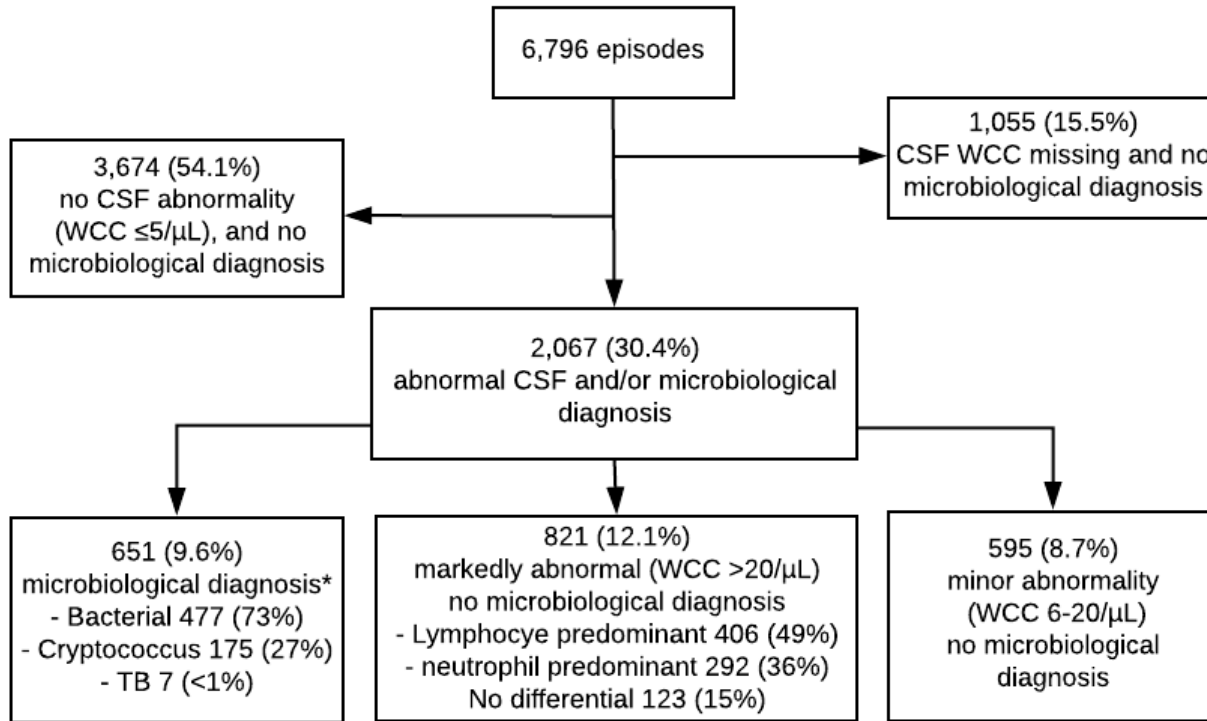
Strata	Age ^a (years)	Number of cases	Person Years	Incidence (95%CI) (per 100,000 person-years)
	0-14	1319	1,449,341	91.0 (86.2-96.1)
Underwent meningitis evaluation by lumbar punctures	0-4	1101	495,308	222.3 (209.4-235.8)
	5-9	118	481,331	24.5 (20.3-29.4)
	10-14	1100	472,702	21.2 (17.2-25.7)
Any abnormal CSF (WCC >20 μ L or non- <i>Cryptococcus</i> 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 <i>Cryptococcus</i>) ^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)
<i>Streptococcus pneumoniae</i>	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)
<i>Cryptococcus</i>	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

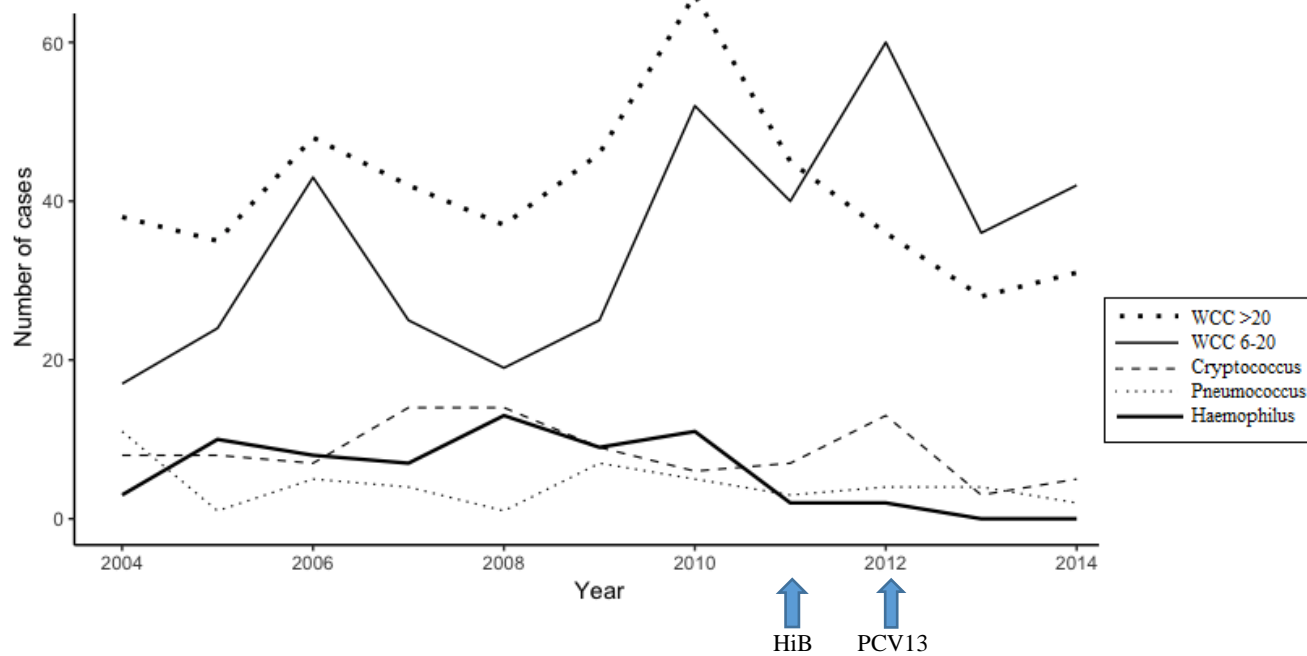
Figure 1. Cerebrospinal fluid findings from 6,796 cases of suspected meningitis in children 0-14 years



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
<i>Streptococcus agalactiae</i> (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

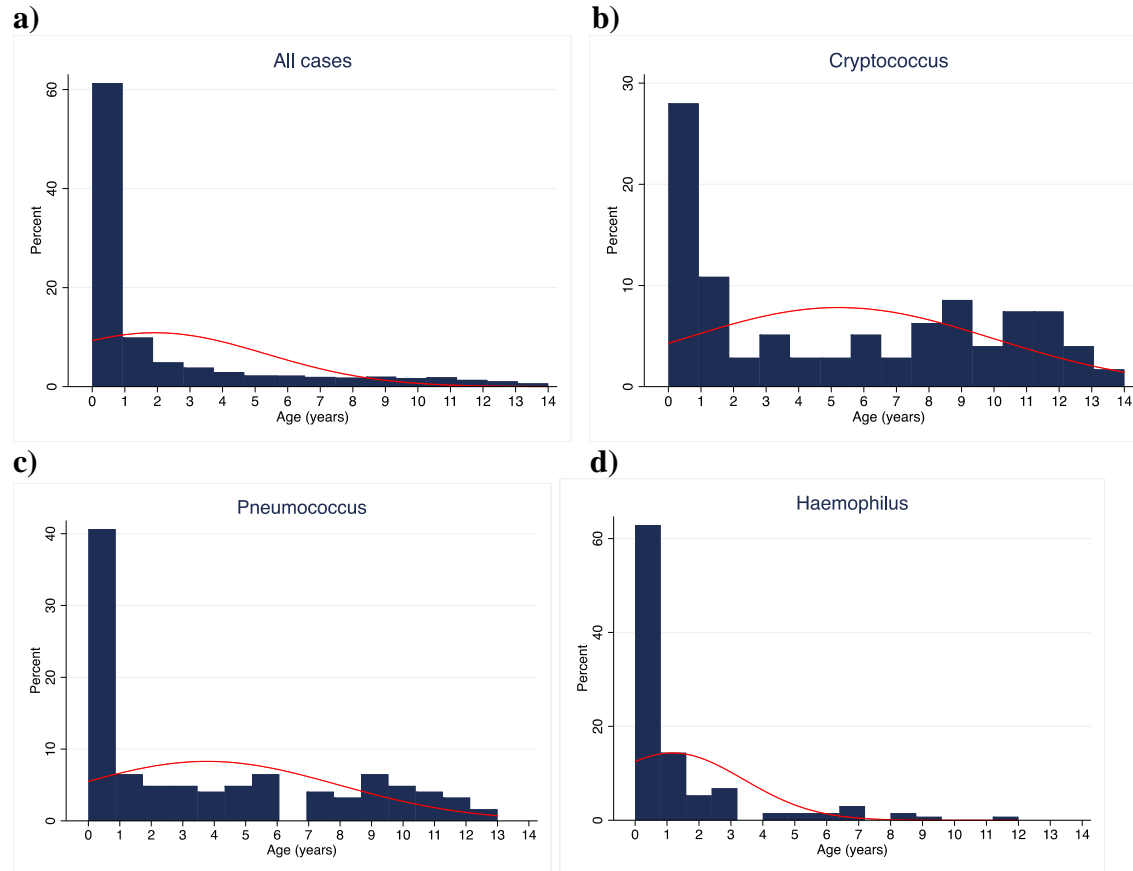
^a missing data on sex for 5.2% (347) of sample

^b Upper limit recorded for WCC 2000/μL

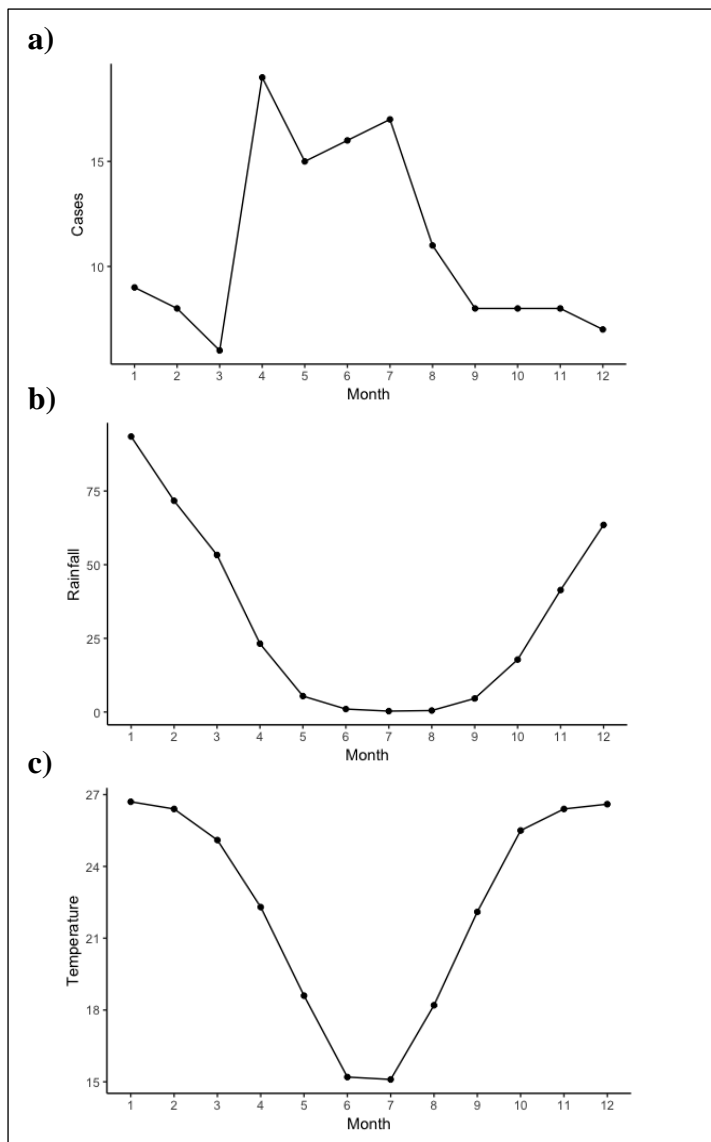
^c missing data on HIV status for 38.4% (2,610) of sample

^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



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>