

## Review Article

# Prevalence of *Cryptosporidium* species among HIV/AIDS patients in Sub Saharan Africa; Systematic Review and Meta-Analysis

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## Abstract

**Background:** *Cryptosporidium* species is increasingly recognized as a leading cause of diarrheal disease with life threatening condition in HIV/AIDS patients. *Cryptosporidium* species is one of the AIDS defining illnesses and associated with an increased risk of death compared to other AIDS-defining illnesses.

**Objective:** To systematically review prevalence of *Cryptosporidium* species among HIV/AIDS patients in Sub Saharan Africa.

**Methods:** A comprehensive search of electronic databases was conducted on PubMed, EMBASE, African Journal Online (AJOL) and advanced Google Scholar. The reference lists of all identified articles were searched for additional studies. Meta-analyses were carried out using review manager 5.3, comprehensive meta-analysis and R software version 3.6.1. Evidence for statistical heterogeneity of results was assessed using Cochrane Q <sup>x2</sup> test and I<sup>2</sup> statistic.

**Results:** A total of 21 studies were included in the systematic review. Meta-analysis by random effect model showed that the estimated pooled prevalence of *Cryptosporidium* infection in people with HIV infection was 11 % (678/6,262; 95% CI: 7–16%). We demonstrated that CD4 level was significantly related to *Cryptosporidium* infection, where the highest risk patients are those with CD4 level < 200 cells/ $\mu$ l (OR: 6.039, 95% CI: 4.441- 8.212, P< 0.0001). The funnel plot demonstrated that there was no publication bias.

**Conclusion:** The results of our meta-analysis show a heavy burden of *Cryptosporidium* infection among HIV/AIDS patients in sub Saharan Africa (11%).

## Abbreviation

AIDS: Acquired Immune Deficiency Syndrome; ART: Anti-Retroviral Therapy; CoCoPop: Condition, Context, Population; HAART: Highly Active Anti-retroviral Therapy; HIV: Human Immune Virus; JBI-MASARI: Joanna Briggs Institute Meta-Analysis of Statistics Assessment for Review Instrument; WHO: World Health Organization

## Background

*Cryptosporidium* species are intestinal protozoan parasites of the phylum Apicomplexa, which cause diarrheal disease in humans worldwide. Although *Cryptosporidium* was discovered in 1907, the first human cases of cryptosporidiosis were reported in 1976. But after the emergence of the HIV/AIDS in the early 1980s, the parasite has become widely recognized as a human pathogen [1,2].



*Cryptosporidium* is primarily transmitted mainly through feco-oral route either by direct contact with an infected human or animal or indirectly via contaminated food or water [3]. The infectious dose of *Cryptosporidium* species is as low as 9–10 oocysts [4,5]. Oocysts of *Cryptosporidium* remain infectious in the environment for at least 6 months if kept moist and resistant to conventional water treatment such as chlorination [6,7].

*Cryptosporidium* species infection is common in many developing countries of sub-Saharan Africa due to poor sanitation, poor hygiene and unavailability of safe drinking water [8,9]. It was previously considered non-pathogenic or with transient pathogenic potential in immune-competent individuals, but nowadays becoming aggressive and cause debilitating illness in HIV/AIDS patients [10].

In patients with HIV/AIDS, prevalence of cryptosporidiosis vary widely, ranging from 0 to 100% with the higher rates reported in patients that have not started ART [11]. According to global disease burden report published in 2010, a prevalence rate of 2.6–21.3% has been documented in Africa. *Cryptosporidiosis* is one of the AIDS-defining illnesses and associated with an increased risk of death compared to other AIDS-defining illnesses [12]. The advent of highly active antiretroviral therapy (HAART) has reduced the prevalence of this disease in AIDS patients [13–15]. The emergence of drug-resistant HIV strain and failure of HAART has been associated with re-emergence of *Cryptosporidium* species infection in HIV/AIDS patients [15].

In 2013, globally an estimated 35.0 million people were living with HIV/AIDS. Sub-Saharan Africa accounts for 71% of the global burden of HIV infection [16]. AIDS-related death declined by about 42% from 2010 to 2017 in eastern and southern Africa, reflecting that the rapid growth of treatment scale-up in the region [17]. About 80% of AIDS patients died from AIDS-related disease including intestinal parasites rather than HIV infection itself. *Cryptosporidium* species on the other hand is an emerging cause of chronic diarrhea with life-threatening conditions in HIV/AIDS patients [18].

Diarrhea occurs in about 90% HIV/AIDS patients in developing countries, including sub-Saharan Africa [19]. *Cryptosporidium* species is increasingly recognized as a leading cause of diarrheal disease, and its largest burden occurs in HIV/AIDS patients [20]. HIV/AIDS patients with CD4 count falls below 100 cells/mm<sup>3</sup> the risk increases for severe disease accompanied by malabsorption, weight loss, and high case fatality [21].

In 2004, cryptosporidiosis was added to the WHO's 'Neglected Diseases Initiative' which includes diseases that occur mainly in developing countries [22]. *Cryptosporidium* co-infections and associated morbidities are common among people living with HIV/AIDS and have implications for their treatment and care. So, it is found mandatory to know the magnitude of the neglected *Cryptosporidium* infection among HIV/AIDS patients so as to develop strategies to prevent and control the disease. The main objective of this review was to summarize data on the prevalence of *Cryptosporidium* species infection among HIV/AIDS patients in sub-Saharan Africa.

## Methods

### Search strategy

In this review all studies published in the English language from 1990 to October 2019 were searched. The search strategy was conducted in three steps; initially, the search was conducted on PubMed followed by the analysis of text words contained in the title and abstract, and index terms used to describe the article. Secondly using all identified keywords, and mesh terms where each factor was combined using the "OR" operator and "AND" operator was used to search studies across PubMed, Embase, African Journal Online (AJOL) and Google scholar. Database search terms included were those that used to describe *Cryptosporidium* species and Human Immuno Virus (HIV) infection as well as terms that describe the context of this study. The search terms were: coccidian parasite, Cryptosporidiosis, *Cryptosporidium parvum*, opportunistic parasite, prevalence, and human immunodeficiency virus, HIV, AIDS, HIV/AIDS, Africa, and Sub Saharan Africa). Full-text articles were retrieved after review of the title and abstract. Thirdly, the reference lists of all identified studies were searched for additional studies that are relevant to this study. An Endnote software version 5 was used to manage references in this review.

### Inclusion and exclusion of studies

**Inclusion criteria:** To review the prevalence of *Cryptosporidium* species infections in HIV/AIDS patients, we considered the following criteria: i) Studies on the prevalence of *Cryptosporidium* among HIV/AIDS patients were included in the study. ii) Only a study conducted in sub-Saharan African countries were considered. iii) Studies published in English were selected. iv) No restriction on methods of diagnosis.

**Exclusion criteria:** Observational studies including case reports and case series were excluded.

### Assessment of study quality

Studies selected for inclusion were assessed for methodological quality by two independent reviewers using standard critical appraisal instruments for prevalence study Condition, context and population (CoCoPop) from the Joanna Briggs Institute Meta-Analysis of Statistics Assessment for Review Instrument (JBI-MAStARI). A 10-point scoring system was used to rate the quality of the articles retrieved. Scoring was conducted by two independent investigators using a modification of the Downs and Black checklist [23]. For inclusion in the review, both reviewers (E.A and K.D) agreed that a cut-off a score of five out of 10 was used to determine acceptable quality for inclusion. Disagreements were resolved by consensus.

The study quality assessments criteria were: objective of the study clearly described, study design clearly stated, the sample size representativeness of the population from which they were recruited, method of identification of the parasite identification clearly identified, outcome assessed with the objective criteria, were confounders reported, were potential



biases reported, was outcome clearly described, appropriate statistical analysis method used, and if whether the context of the study is sub-Saharan Africa (Table 1).

### Data extraction

The two authors (E.A and K.D) extracted the data from included studies using a standardized form independently and checked the data together. A critical appraisal checklist for observational studies (prevalence study) adopted from JBI was used to assess the overall methodological quality of the included studies [24]. From each included studies, detail description of study subjects, report on the study area, year of publication, study design, sample size, method of *Cryptosporidium* species screening was extracted. Secondary outcomes for this study included clinical data including the presence or absence of diarrhea, whether the patient started ART treatment or not, a CD4 count of the patients, and distribution of *Cryptosporidium* species by age, sex of the patients (Table 2).

### Data analysis and data synthesis

Meta-analyses were carried out using review manager 5.3, comprehensive meta-analysis and R software version 3.6.1 with user contributed commands for meta-analyses: metan, metainf, metabias, and metareg [25]. The effect sizes and SEs of the studies were pooled using a random-effects model to calculate the pooled prevalence of *Cryptosporidium* species

among HIV/AIDS patients. The association of prevalence of cryptosporidium species with CD4 count and with the presence and absence of diarrhea will be evaluated and odds ratio was used to present association.

### Risk of bias and sensitivity analysis

The random-effects meta-analysis models were chosen because heterogeneity was demonstrated [26]. Evidence for statistical heterogeneity of results was assessed using Cochrane  $Qx^2$  test and  $I^2$  statistic. A significance level of  $P < 0.10$  and  $I^2 > 50\%$  was interpreted as evidence of heterogeneity [27]. A potential source of heterogeneity was investigated by subgroup analysis and meta-regression analysis [28]. Where statistical pooling was not possible the findings were presented in a narrative form including tables and figures to aid in data presentation where appropriate.

Sensitivity analyses were conducted to weigh up the relative influence of each individual study on the pooled effect size using user-written function, metainf [25]. The presence of publication bias was assessed informally by visual inspections of funnel plots [29].

## Results

### Study selection

As shown in the flowchart above (Figure 1) the literature

**Table 1:** Methodological quality of included studies.

	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Total points 100%
G. Hunter	6	5	7	7	6	5	5	7	7	10	65
Fisseha B	6	7	8	7	6	8	7	7	6	10	72
MarianneLebbad	9	8	7	8	9	6	9	8	7	9	79
Maiga MY	5	8	7	7	9	8	5	8	9	9	71
Adjei A	7	5	6	6	7	7	7	5	5	7	62
ERIC R. HOUP	6	7	8	7	6	8	7	7	6	10	71
Frederick O. A	9	8	7	8	9	6	9	8	7	9	79
Ce' line Nguefeu	5	8	7	7	9	8	5	8	9	9	75
Dibua	7	5	6	6	7	7	7	5	5	7	62
Leopold G. Lehman	6	5	7	7	6	5	5	7	7	10	65
Zelalem Teklemariam	6	7	8	7	6	8	7	7	6	10	72
Haileyesus Adamu	6	5	7	7	6	5	5	7	7	10	65
Mekonnen Girma	6	7	8	7	6	8	7	7	6	10	72
Jane W. Wanyiri	9	8	8	7	9	6	9	8	7	9	79
Sekesai Mtapuri-Zinyowera1	5	8	7	7	9	8	5	8	9	9	75
Marius Zambou Vouking	7	5	6	6	7	7	7	5	5	7	62
Habtom Kiros	6	7	8	7	6	8	7	7	6	10	72
Dickson Shey Nsagha	9	7	8	8	9	6	9	8	7	9	79
Dereje Gedle	5	8	7	7	9	8	5	8	9	9	75
Veronica Casmo	7	5	6	6	7	7	7	5	5	7	62
Juliet Nakibirango	6	7	5	7	6	5	5	7	7	10	65

**Notes:** Quality appraisal questions

Q1: Were objective of the study clearly described? Q2: Were study design clearly stated? Q3. Was sample size representativeness of the population from which they were recruited? Q4: Were methods of identification of the parasite identification clearly identified? Q5: Were outcome assessed with the objective criteria? Q6: Were confounders reported? Q7: Were potential biases reported? Q8: Was outcome clearly described? Q9: Were appropriate statistical analysis method used? Q10. Were the context of the study being sub Saharan Africa



**Table 2:** Data extraction form used in this systematic review and meta-analysis.

Data extraction form field	Information to consider in data extraction
Reviewer identification	Review author ID; date
Study identification	Study ID; report ID; citation; author contact details; publication yr; country; source of data
Methods	Study design; setting
Study setting	Saharan Africa country (country of the study)
Participant characteristics	Total number of participant, age, sex; HIV sero-status
Disease characteristics	Naive HIV (not on HARRT) and ART patients (on HAART), CD4 level, diarrhea
Diagnostic test characteristics	Methods of stool examination: Wet mount microscopic examination, stool concentration techniques, modified AFB staining. ELISA, PCR
Outcome	Outcome definition (unit of measurement: prevalence in number of events (n/N) or in percent (%))
Results to include in a meta-analysis	Dichotomous outcomes: no. of events/no. of participant
Risk of bias	Cochrane RoB tool for prevalence study COCOPOP

infection from the included study. Due to the variability of data quality and reporting system, we only extracted and analyzed the data on diarrhea, ART, and CD4 count. According to the

search resulted in 37 studies. Eight duplicates were removed. After a review of titles and abstracts of 29 studies, 6 studies were excluded from the systematic review as they no longer met the inclusion criteria. From these four papers were unavailable for full text. One study excluded after full-text screen due to unclear study site and unclear study population and another one study excluded after quality appraisal. After the end of quality appraisal, 21 studies met the inclusion criteria and were retrieved for full text. A total of 21 studies were included in this systematic review and meta-analysis, and the extracted data are summarized in (Table 1). In the included studies a total of 6, 262 HIV-infected patients were assessed for *Cryptosporidium* species infection and the total events of *Cryptosporidium* infections were 678. These studies were done in 10 different countries. All papers were written in English.

### Study characteristics

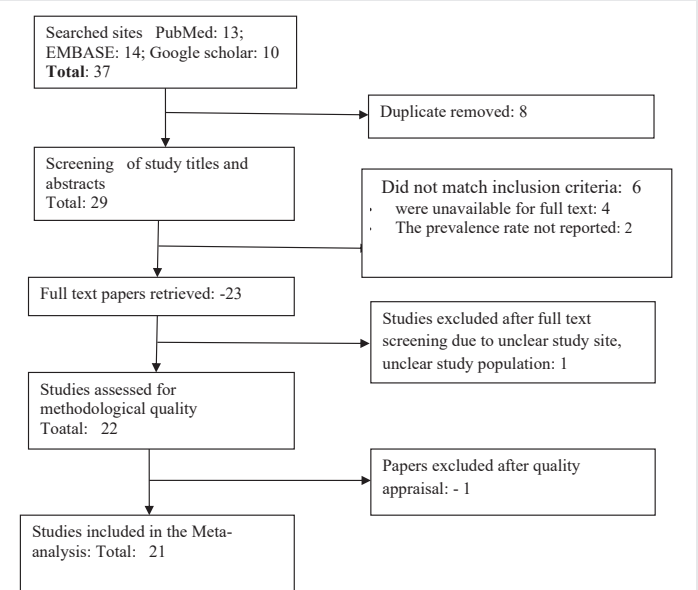
Of the 21 studies included in the Meta-analysis, one was a prospective study [30], one retrospective study [15], Two was case-control studies [31,32], and 17 were cross sectional studies [33-50]. Most of the studies conducted a parasitological investigation by microscopic examination of wet mount preparation and concentration techniques while 4 studies employed molecular techniques [43]. Characteristics of the included studies have been shown in Table 3.

### Synthesis of result

The prevalence of *Cryptosporidium* infection among HIV/AIDS patients in sub-Saharan Africa ranged between 2.17 and 44% (Table 1). A Meta-analysis by random effect model showed that the estimated pooled prevalence of *Cryptosporidium* infection in people with HIV infection was 11% (678/6,262; 95% CI: 7-16%). Test of heterogeneity showed that it heterogeneous (Quantifying heterogeneity: tau<sup>2</sup> = 0.9755; H= 4.94; I<sup>2</sup>= 95.9%, P< 0.0001) (Figure 2).

### Additional analysis

This review also extracted additional data on *Cryptosporidium*



**Figure 1:** Flow chart of the search and study inclusion.

**Table 3:** Included studies of *Cryptosporidium* infection in people with HIV listed in order of year publication.

S. No.	Author	Country	Year of publication	Total sample size	no events	Prevalence
1	G. Hunter	Zambia	1992	90	2	2.20%
2	Fisseha B	Ethiopia	1998	246	38	25.90%
3	Marianne Lebbad	Guinea-Bissau	2001	37	9	25%
4	Maiga MY	Mali	2002	434	71	16.30%
5	Adjei A	Ghana	2003	21	6	28.60%
6	ERIC R. HOUP	TANZANIA	2005	127	22	17%
7	Frederick O. A	Nigeria	2010	2,000	80	4%
8	Ce' line Nguefeu	Cameroon	2013	396	42	11
9	Dibua	Nigeria	2013	50	4	8%
10	Leopold G. Lehman	Cameroon	2013	201	13	6.46%
11	Zelalem Teklemariam	Ethiopia	2013	371	8	2.20%
12	Haileeyesus Adamu	Ethiopia	2014	378	32	8.50%
13	Mekonnen Girma	Ethiopia	2014	268	92	34.30%
14	Jane W. Wanyiri	Kenya	2014	164	56	34%
15	Sekesai Mtapuri-Zinyowera	Zimbabwe	2014	29	5	17.20%
16	Marius Zambou Vouking	Cameroon	2014	207	15	12.60%
17	Habtom Kiros	Ethiopia	2015	399	23	5.80%
18	Dickson Shey Nsagha	Cameroon	2016	300	132	44%
19	Dereje Gedle	Ethiopia	2017	323	19	5.90%
20	Veronica Casmo	Mozambique	2018	83	6	7.20%
21	Juliet Nakibirango	Uganda	2019	138	3	2.17%



pooled data of five studies in sub-Saharan African countries, we demonstrated that CD4 level was significantly related to *Cryptosporidium* infection, where the highest risk patients are those with CD4 level < 200 cells/μl (OR: 6.039, 95% CI: 4.441-8.212, P< 0.0001) (Figure 3).

### Heterogeneity and risk of bias

Subgroup analysis of five studies showed the pooled prevalence of *Cryptosporidium* infection in HIV-infected patients was significantly higher in patients with diarrhea (OR=1.779 95% CI: 1.057-2.994, p= 0.030) and the pooled prevalence of six studies in sub-Saharan Africa showed the highest prevalence *Cryptosporidium* infection in HIV/AIDS patients was significantly higher in Naïve HIV/AIDS Patients (OR= 1.559; 95% CI= 1.100-2.209; P= 0.013).

The funnel plot helped us distinguish between publication bias and other causes of the asymmetry. It showed that small studies were found not only in the areas of statistical significance. We demonstrated no publication bias (t= -1.3505, df= 19, p-value= 0.1927).

## Discussion

### Summary of evidence

In sub-Saharan Africa high burden of *Cryptosporidium* infection in this review indicate the importance of routine testing for *Cryptosporidium* species in all HIV-infected people.

To our knowledge, this is the first systematic review on prevalence of *Cryptosporidium* species among HIV infected

people in sub-Saharan Africa. Our findings demonstrated evidence for a high prevalence of *Cryptosporidium* species among HIV-infected patients in sub-Saharan Africa.

In HIV infected patients, a high prevalence has been reported in Cameroon (44%) [35], Ethiopia (34.30%) [45], Kenya (34%) [40], Ghana (28.60%) [51] and Guinea-Bissau (25%) [43] for *Cryptosporidium* infection. In contrast, a low prevalence has been shown in Uganda (2.17%) [41], Zambia (2.20%) [37], and Nigeria (4%) [32].

The prevalence of *Cryptosporidium* infection varies depending on the presence or absence of diarrhea, level of CD4 count, ART status [46]. On the other hand, the prevalence of *Cryptosporidium* infection among HIV/AIDS may vary even within a country or among different populations of the country. For example, in Ethiopia, the prevalence of *Cryptosporidium* infection in Butajira was 5.90% [34] while it was 34.03% in Yirgalem Hospital South of Ethiopia [45]. This might be due to the diagnostic method used to detect *Cryptosporidium* species.

In the present study; we demonstrated that diarrhea, ART, CD4 count was significantly related to *Cryptosporidium* infection in HIV/AIDS patients in comparison with their control. HIV/AIDS patients with CD4 level less than 200 cells/μl are three times likely to be infected by *Cryptosporidium* species (RR=2.95; 95% CI: 2.53-3.43) and Naïve HIV patient 1.75 times likely to be infected by *Cryptosporidium* species than ART started patients (RR= 1.75 95% CI: 1.42-2.17). So, policy makers need to plan and give emphasis to allocate resource for improving health care of HIV infected patients with CD4 count <200 cells.

To explain the possible causes of heterogeneity, we conducted meta-regression and subgroup analyses on various sources including country, patients with diarrhea, and found different main causes of heterogeneity for *Cryptosporidium* infection. These may come from publication year (P = 0.0001), selection of participant with or without diarrhea (P= 0.001) and ART status (P< 0.0001). Other potential causes of heterogeneity may include CD4 level, sample size, and detection methods. Due to missing data we did not analyze them.

### Limitation

However, this systematic review came up with the prevalence of *Cryptosporidium* species among HIV/AIDS patients in sub-Saharan Africa, we acknowledge few limitations of the present meta-analysis, which may affect the results. First of all four relevant studies which were identified through our literature search were excluded due to unavailability for full-text review. Secondly, the majority of the studies were of moderate or low quality, as most of the data was from the conventional microscopic examination techniques; that have lower sensitivity than ELISA and polymerase chain reaction. So, use of different diagnostic tests with varying diagnostic sensitivity is the other limitation of this study.

## Conclusions

The results of our meta-analysis show a heavy burden of *Cryptosporidium* infection among HIV/AIDS patients in sub-

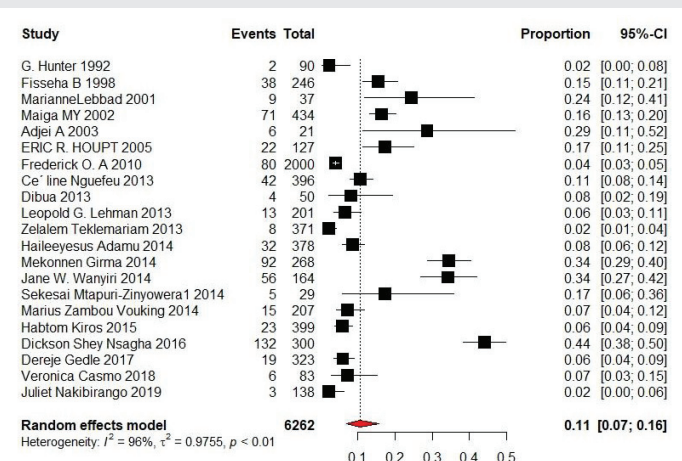


Figure 2: Prevalence of *Cryptosporidium* species among HIV/AIDS patients in sub-Saharan Africa

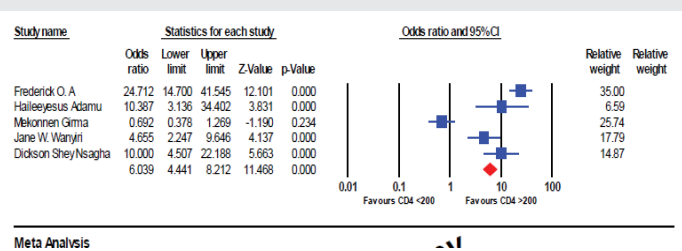


Figure 3: Comparative prevalence of *Cryptosporidium* species in HIV/AIDS patients according to CD4 level.



Saharan Africa (11%). Thus, routine screening of *Cryptosporidium* species should be done, particularly for those who have a CD4 count less than 200 cells/ $\mu$ l and early treatment should be administered. Patient with a CD4 count > 350 and those who had started ART have the lowest prevalence.

### Availability of data and materials

All the datasets generated and analyzed during the review are included in this article.

### Author's contribution

E. A, K. D, A.G and F. W designed the study, extracted, critically reviewed and analyzed data and wrote the first draft of the manuscript, and approved the manuscript.

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