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REVIEW ARTICLE

A meta-analysis of *Cryptosporidium* species in humans from southern Africa (2000–2020)

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Abstract The epidemiology of cryptosporidiosis in southern Africa is largely unknown. The disease is associated with diarrhea and nutritional deficiencies, leading to severe morbidity and mortality among immune-compromised patients. This study aimed to assess the pooled prevalence of *Cryptosporidium* spp. infection among immune-compromised humans in southern Africa over the past 20 years. Reports of *Cryptosporidium* spp. infection in humans published between 2000 and 2020 using Google Scholar, PubMed, Ovid Medline, African Journal Online (AJOL), and Web of Science literature databases were obtained. Inclusion criteria of sorted articles for *Cryptosporidium* spp. infection were standardized using preferred reporting items for systematic reviews and meta-analyses (PRISMA) checklist. A total of 22 eligible studies were sorted for meta-analysis. Overall prevalence of *Cryptosporidium* spp. infection in southern African countries with reports was 16.8% (95% CI 9.7–25.3). Sub-group analysis showed a pooled prevalence of 25.2, 20.5, and 17.9% among HIV/AIDS patients, children, and diarrhoeic individuals, respectively. Pooled prevalence was highest in South Africa and lowest in Zimbabwe across examined individuals. The pooled prevalence of *Cryptosporidium* spp. infections in diarrhoeic patients was highest in individuals from Botswana (17.6%) which is significantly different ($X^2 = 9.337$; $P = 0.002$) from South Africans (12.7%). South African individuals with HIV/AIDS showed the highest pooled prevalence of Cryptosporidium infections than other countries. The high prevalence of *Cryptosporidium* spp. infections among immune-compromised patients in southern Africa showed that the pathogen is of significant importance in this region. Continuous studies on the genetic characterization of *Cryptosporidium* spp. isolates and associated risk factors are needed across southern Africa to identify the predominant subtypes in humans.

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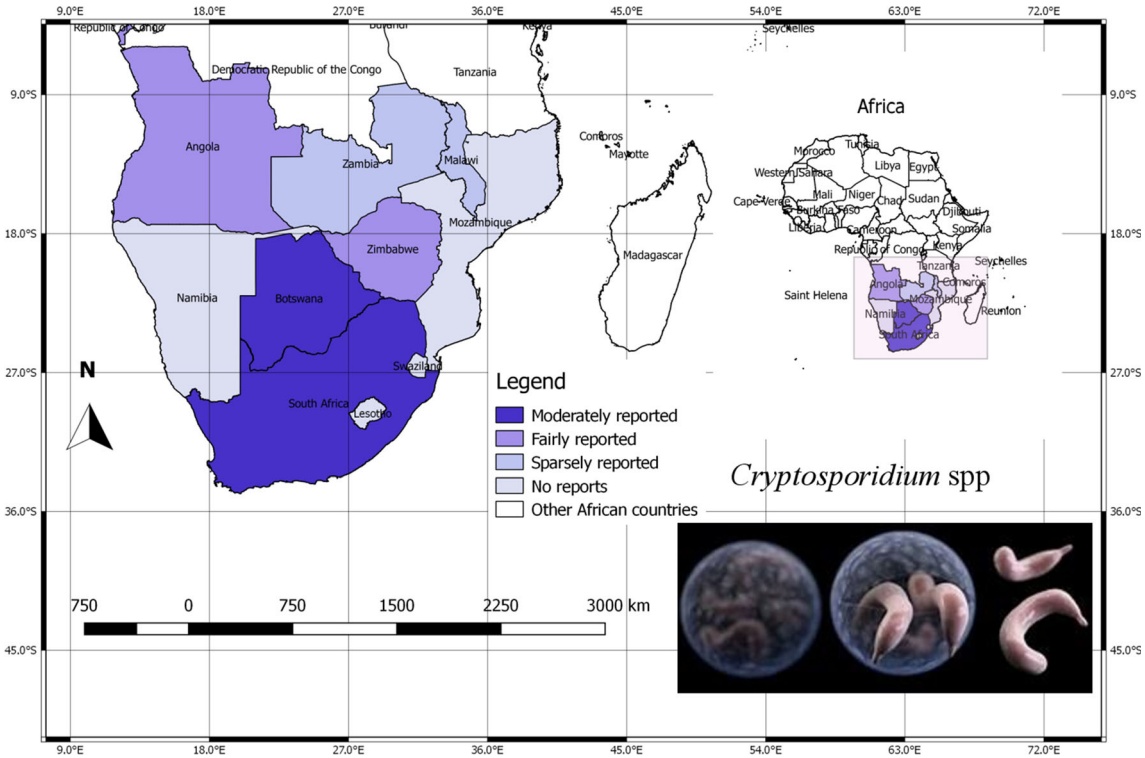
Graphic abstract



Keywords Cryptosporidiosis · Zoonotic disease · Prevalence · HIV · Public health · Protozoa

Introduction

One of the most important neglected tropical diseases is cryptosporidiosis caused by *Cryptosporidium* spp. Cryptosporidiosis is primarily a water-borne disease associated with fatal diarrhea and is often reported in immune-compromised individuals. A joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) expert committee has ranked *Cryptosporidium* spp. enteropathogen fifth among the 24 most significant foodborne parasites in a global ranking (Delahoy et al. 2018; Odeniran and Ademola 2019). Cryptosporidiosis perception among people in southern Africa is low, while the disease is exacerbated by the widespread human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), rural settlements with poor hygiene, and water shortages (Ojuromi and Ashafa 2018).

The disease is common in children, and several identified risk factors include contact with animals, malnutrition, HIV status, and water-related activities (Squire and Ryan 2017; Xiao 2010). Indicators of *Cryptosporidium* spp. infection include presence of watery diarrhoea, nutritional defects, elevated levels of lactoferrin and immune system related defects. Infection can be contracted through direct contact with infected humans, zoonotic (animal), and can likewise be foodborne or waterborne (Xiao 2010; Burnet et al. 2014).

Water left untreated and still used for domestic purposes such as cooking, drinking, swimming, and bathing in many rural African homes mediates the exposure to waterborne *Cryptosporidium* spp. (CDC 2015). *Cryptosporidium* spp. has been identified in animals such as poultry, fish, dogs, horses, sheep, cattle etc. (Amer et al. 2013; Bodager et al. 2015; Odeniran and Ademola, 2019). Only a few studies have been directly conducted in southern Africa on the identification of *Cryptosporidium* spp. species in different vertebrate hosts.

Cryptosporidium spp. enteropathogen genotypes identified in humans from Africa include, *C. andersoni*, *C. bovis*, *C. canis*, *C. cuniculus, C. felis*, *C. hominis*, *C. meleagridis*, *C. muris, C. parvum*, *C. suis, C. ubiquitum*, *C. viatorum*, and *C. xiaoi* (Squire and Ryan 2017). However, most important species identified in southern Africa were *C. hominis, C. meleagridis* and *C. parvum*. Some of the

subtypes of these species are zoonotic and more studies are needed to identify the most prevalent and distributed species in humans from these regions.

To decrease the prevalence of *Cryptosporidium* spp. among HIV patients, the introduction of antiretrovirals have been reported to slightly restore immune function (Missaye et al. 2013; Kiros et al. 2015). Besides, HIV protease inhibitors have been suggested to serve as antiparasitic drugs in cases of cryptosporidiosis. For example, the drugs indinavir, ritonavir, and saquinavir were confirmed to have anti-cryptosporidial effects in experimental studies (Mele et al. 2003). However, their efficacy as an anti-cryptosporidial is limited and cannot be substituted as mainstream antiparasitic drugs.

The diagnosis of cryptosporidiosis in this region is mainly based on the morphological identification of *Cryptosporidium* spp. oocysts in faecal samples by microscopy using acid-fast stains or immunofluorescent antibody staining. More sensitive test such as polymerase chain reaction (PCR) has been limited to research and not diagnostic purposes in Africa due to the cost and expertise required.

Studies conducted on *Cryptosporidium* spp. infection from southern African countries have not been adequately synchronized with its associated risk factors to develop public health implications and the disease's distribution rate. Similarly, meta-analyses conducted have focused on individual studies with a regional explanation of its prevalence with many comparisons rather than improving disease awareness and providing data for policymakers.

Therefore, we investigated the prevalence, risk factors, and sub-group analyses of published data on *Cryptosporidium* spp. infection from humans in southern Africa. Distribution and impact of the pathogen on immune-compromised patients based on reporting was also examined in the study.

Materials and methods

Search strategy

Literature databases (PubMed, Ovid Medline, AJOL, Google Scholar, and Web of Science) were searched for published articles on *Cryptosporidium* spp. infection in southern Africa in the English Language from January 2000 to October 2020. The considered articles were those with full-texts. Keywords for searches include, "Cryptosporid"*, "*parvum*", "*hominis*", "species", "humans", "children", "HIV", "patients", "diarrhoea", "intestinal"*, "prevalence", "epidemiology", "South Africa", "Botswana", "Lesotho", "Malawi", "Angola", "Namibia", "Zambia", "Zimbabwe", "Mozambique", "Swaziland" and "Southern Africa". Missing articles were avoided by carefully examining the references of identified articles, while the authors independently did the extraction process to minimise error.

Inclusion and exclusion criteria

An article's inclusion criterion was primarily identified, provided the conducted study that investigated the prevalence of cryptosporidiosis in humans by a diagnostic tool was a cross-sectional type and such detected positive cases in Southern Africa. Exclusion criteria focused on study review, case reports and letters to the editor, animal studies, duplicated manuscripts, and articles with insufficient information.

Data extraction

All the authors independently examined downloaded articles to avoid bias. Articles with irrelevant objectives were removed after reading through the titles, abstracts, and full texts. Variables were generated for each manuscript and were entered in Microsoft Excel spreadsheet. Some of the variables included record information of the authors, total cases examined, the number of positive cases detected, diagnostic techniques (Microscopy, Polymerase Chain Reaction, and IFAT- indirect fluorescent antibody test), the country where the study was done, study area, age group, and gender. Other attributes were those associated with risk factors such as occupation, contact with animals, water use, frequency of use, disease pathologies etc. Cross evaluation among authors was done to resolve all forms of disagreements in computing the data.

Quality assessment

PRISMA checklist was used to standardize the inclusion criteria of all relevant information in the study analysis (Moher et al. 2010) (Supplementary file). Each article was subjected to a quality assessment scale (1–10) generated in line with the objective of the study (low quality, < 5.0; moderate quality, 5.0–7.5; and high quality, 7.5–10.0). Excluded published articles were those with lesser than acceptable qualities (> 5.0) for the analysis.

Statistical analysis

METAXL® (version 3.1) was used to perform the meta-analysis. The quality effects model was used to obtain the pooled prevalence of the study estimate. The overall prevalence of the quality effects model findings was assessed using the validity of the tests. To evaluate the heterogeneity between test results, Cochran's Q test and the

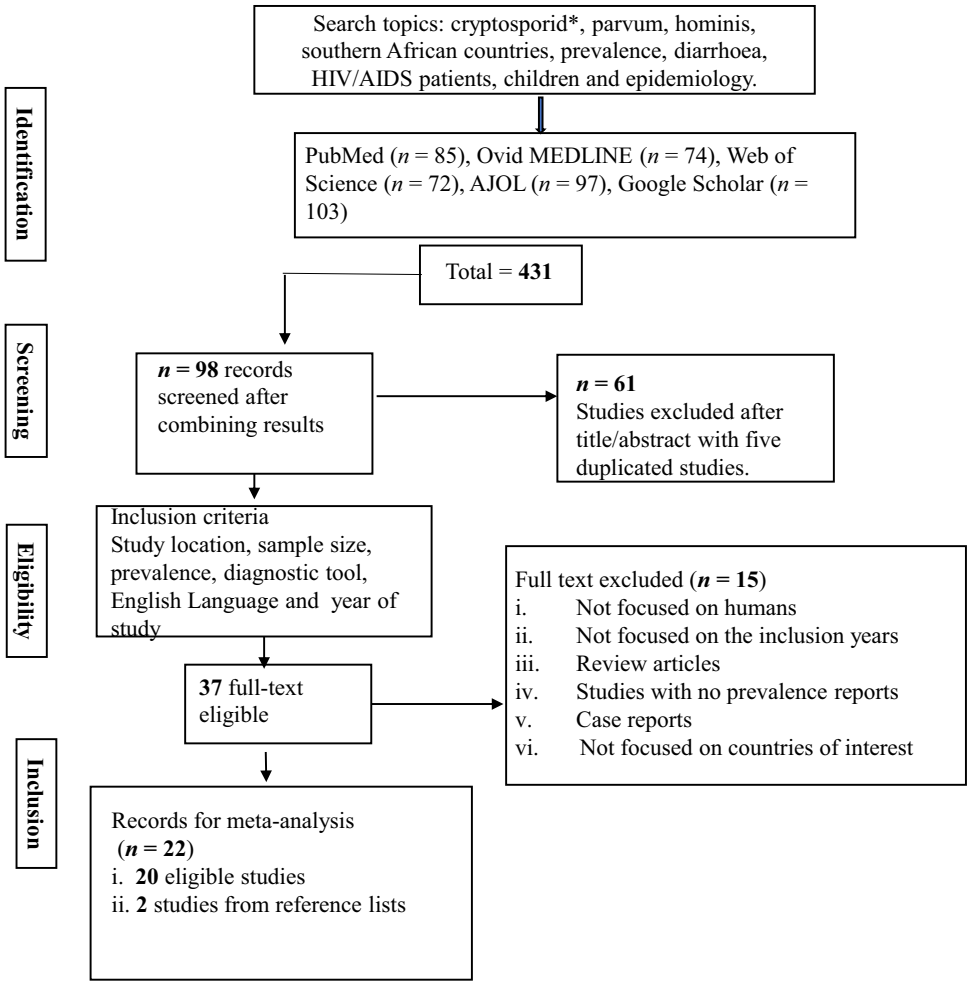


Fig. 1 Literature databases search on *Cryptosporidium* infection of humans in southern Africa

inconsistency (I2) indicator were used. The Luis Frya-Kanamori (LFK) index was obtained by plotting the z-score against the double-arcsin prevalence of the publications examined. This suggests substantial asymmetry when the LFK index reaches ± 2, (publication bias) (Barendregt and Doi 2015). WINPEPI (version11.65) was used for the Pearson chi-square, while Tukey's post-hoc multiple pair-wise one-way ANOVA comparison test was used to compare disease trends among countries using GraphPad Prism (version 5). Confidence intervals was set at ninety-five percent with minimum and maximum values. A map was constructed with qGIS (version 2.8.10) to show *Cryptosporidium* spp. infection distribution in southern African countries.

Results

Study characteristics

A total of 431 published articles were selected on *Cryptosporidium* spp. infection from the screening of five databases and reference lists of downloaded relevant studies (Fig. 1). The initial screening of study titles and abstracts led to the exclusion of 61 studies from the combined searches of 98 records. Further excluded studies due to article duplication during sorting were five studies. The remaining study were read individually by the authors and excluded 15 studies that did not meet the inclusion criteria (these were studies outside the range of selected years). Therefore, 22 studies were included in the meta-analysis (Fig. 1). Examined cases were observed in South Africa (n = 7), Botswana (n = 5), Zambia (n = 4), Malawi (n = 3), Zimbabwe (n = 2) and Angola (n = 1). It was observed that several southern African countries have not published data for human cryptosporidiosis or published in non-visible journals in recent years as observed from the database. In total, 5932 individuals were considered in the study, and the immune-compromised patients include 3959 children, 1722 diarrheic patients, 1073 HIV/AIDS seropositive individuals. A total of 589 individuals were reported as immune-competent. Four studies reported patients who were both children and HIV patients (n = 922), three studies reported patients who were both

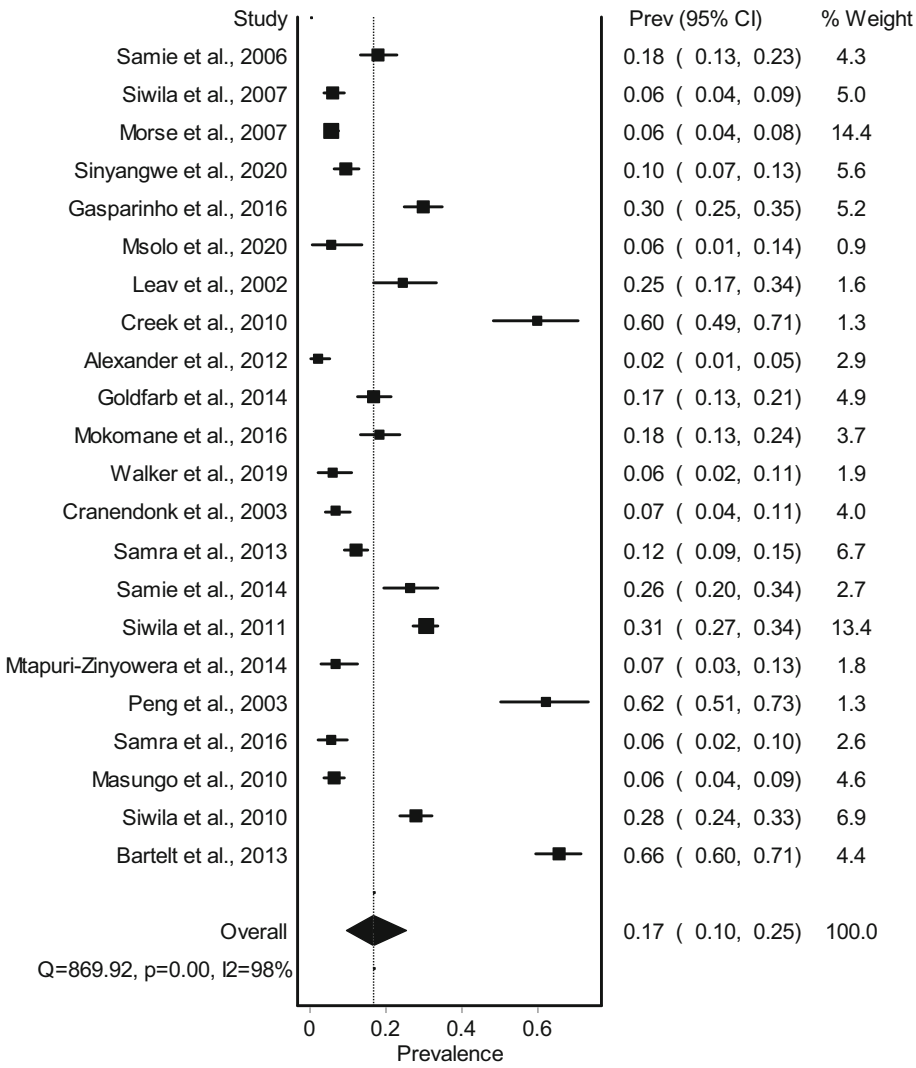


Fig. 2 Forest plot of *Cryptosporidium* infection among human population in southern Africa between 2000 and 2020

children and diarrheic (n = 1059), while a study reported patients who were diarrheic (n = 97) and positive for HIV/AIDS (n = 151).

Cryptosporidium infections in southern Africa

The pooled prevalence estimates of *Cryptosporidium* spp. spp. in humans (n = 22) with individual studies from southern Africa on human *Cryptosporidium* spp. infections are shown in a forest plot (Fig. 2). Studies revealed pooled prevalence of 16.8% (95% CI 9.7–25.3); I^2 = 97.6; Q = 869.9; df = 21; $P < 0.0001$. The prevalence of *Cryptosporidium* spp. of each study varied from 2.4 to 65.7% (median = 14.5), with substantial heterogeneity among studies (Fig. 2). The LFK index of 1.17 showed no asymmetry, indicating that there was no bias. Based on available information from countries with cryptosporidiosis reports, the pooled prevalence was highest in South Africa with 21.8% (95% CI 6.1–42.9), and lowest in Zimbabwe 6.6% (95% CI 4.4–9.3) (Table 1). Only one study met the inclusion criteria in Angola, with prevalence of 29.9%. Statistical analysis showed that there is significant increase (X^2 = 67.9; $P < 0.0001$) in the prevalence of immune-compromised patients (20.3%) compared to immune-competent individuals (6.3%) in southern Africa. The forest plot of the pooled prevalence of immune-compromised patients was reported (Fig. 3). A map showing the recent distribution of *Cryptosporidium* spp. infection studies across southern African countries was illustrated in Fig. 4.

Pooled prevalence and heterogeneity of Cryptosporidium infections in children

Substantial heterogeneity was observed across studies on *Cryptosporidium* spp. infections in children. The pooled prevalence and heterogeneity variables revealed 20.5% (95% CI 9.6–34.1); I^2 = 98.1; Q = 626.7; $P < 0.0001$. The LFK (0.02) indicates that there is no asymmetry.

Table 1 Total report of *Cryptosporidium* infection in humans and associated species in southern Africa between 2000 and 2020

	Sample size	No positive	Pooled Prev	95% CI	Cochran's Q	I^2	*df*	$\alpha_{0.05}$	Chi^2, OR/Anova
Total infection	5932	1121	16.8	9.7–25.3	869.9	97.6	21	< 0.0001	
Countries									
South Africa	1385	339	21.8*	6.1–42.6	289.2	97.9	6	< 0.0001	$P < 0.0001$
Botswana	849	142	13.3	1.8–31.1	119.3	96.6	4	< 0.0001	
Zambia	1804	403	20.3*	7.5–36.8	139.8	97.9	3	< 0.0001	
Malawi	1144	109	8.5	0.0–100.0	112.5	99.1	2	< 0.0001	
Zimbabwe	413	27	6.6	4.4–9.3	0.1	0.0	1	0.729	
Angola	337	101	–	–	–	–	–	–	
Children with Cryptosporidium spp. *infection*									
Children infection	3959	913	20.5	9.6–34.1	626.7	98.1	12	< 0.0001	
Countries									
South Africa	1069	306	24.1*	1.9–56.5	285.9	98.6	4	< 0.0001	$P < 0.0001$
Botswana	327	51	15.3	8.0–24.3	2.1	51.8	1	0.1500	
Zambia	1309	362	26.6*	13.2–42.5	42.7	95.3	2	< 0.0001	
Malawi	917	93	8.5	0.0–100.0	112.5	99.1	1	< 0.0001	
Diarrhoeic patients with Cryptosporidium spp. *infection*									
Diarrhoeic patients	1722	333	17.9	9.8–27.6	126.9	93.7	8	< 0.0001	
Countries									
South Africa	592	81	12.7	3.6–25.6	12.1	83.4	2	0.0020	$X^2 = 9.337$; $P = 0.002$; $OR = 0.63$
Botswana	685	138	17.6#	3.5–37.9	75.8	96.0	3	< 0.0001	
Malawi	108	13	–	–	–	–	–	–	
Angola	337	101	–	–	–	–	–	–	
HIV/AIDS patients with Cryptosporidium spp. *infection*									
HIV/AIDS patients	1073	305	25.2	5.7–51.0	240.5	96.8	4	< 0.0001	
Countries									
South Africa	747	274	34.9	10.9–63.3	143.8	97.9	3	< 0.0001	
Zambia	326	31	–	–	–	–	–		
Diagnostic technique									
ZN	3676	568	13.5	6.1–23.1	332.7	97.3	9	< 0.0001	$F_{2,22}$ $P = 0.5646$ $R^2 = 0.0506$
ELISA	1501	413	24.4#	7.8–45.8	318.9	98.1	6	< 0.0001	
PCR	2486	313	11.0	4.0–20.5	181.1	96.1	7	< 0.0001	
Species in humans									
Cryptosporidium spp. *species characterised*		123	3.8	1.3–7.2	100.1	91.0	9	< 0.0001	
C. hominis	186	105	56.5	–	–	–	5	–	
C. parvum	182	58	31.9	–	–	–	4	–	
C. meleagridis	79	4	5.1	–	–	–	2	–	
C. andersoni	50	1	2.0	–	–	–	–	–	
C. parvum/C. hominis	50	1	2.0	–	–	–	–	–	

I^2, level of inconsistency; *df*, degree of freedom; $\alpha_{0.05}$, level of significance; OR, odd ratio; *F*, variance between and within groups; *R*, coefficient of determination; *CI*, confidence interval; HIV^+, human immunodeficiency virus positive; HIV^-, human immunodeficiency virus negative; *, shows significance at $P < 0.05$; #, no significance at $P > 0.05$

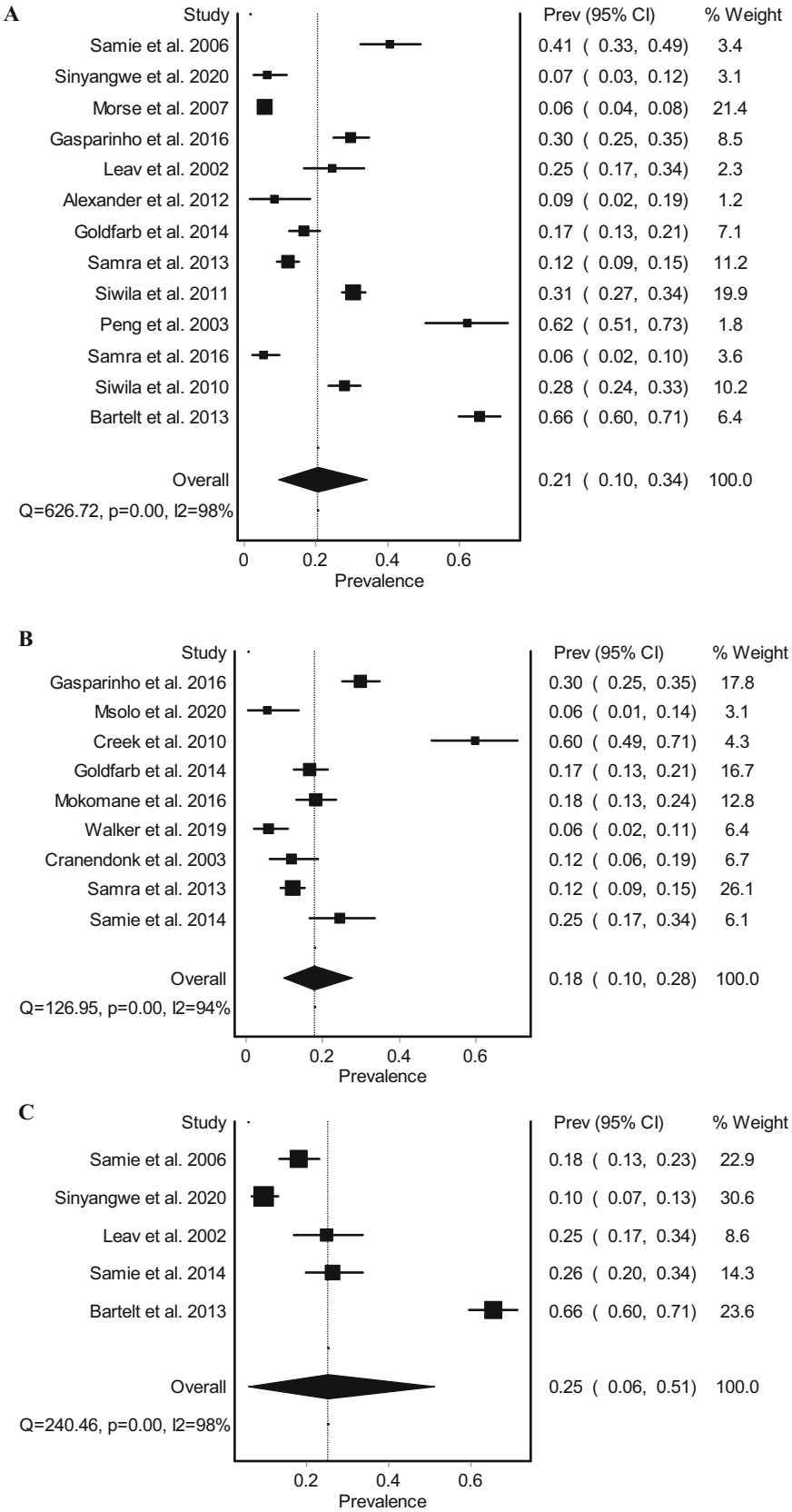


Fig. 3 Forest plot of *Cryptosporidium* infection among immune-compromised patients. A. *Cryptosporidium* infection in children; B. *Cryptosporidium* infection in diarrhoeic patients; C. *Cryptosporidium* infection in HIV/AIDS patients

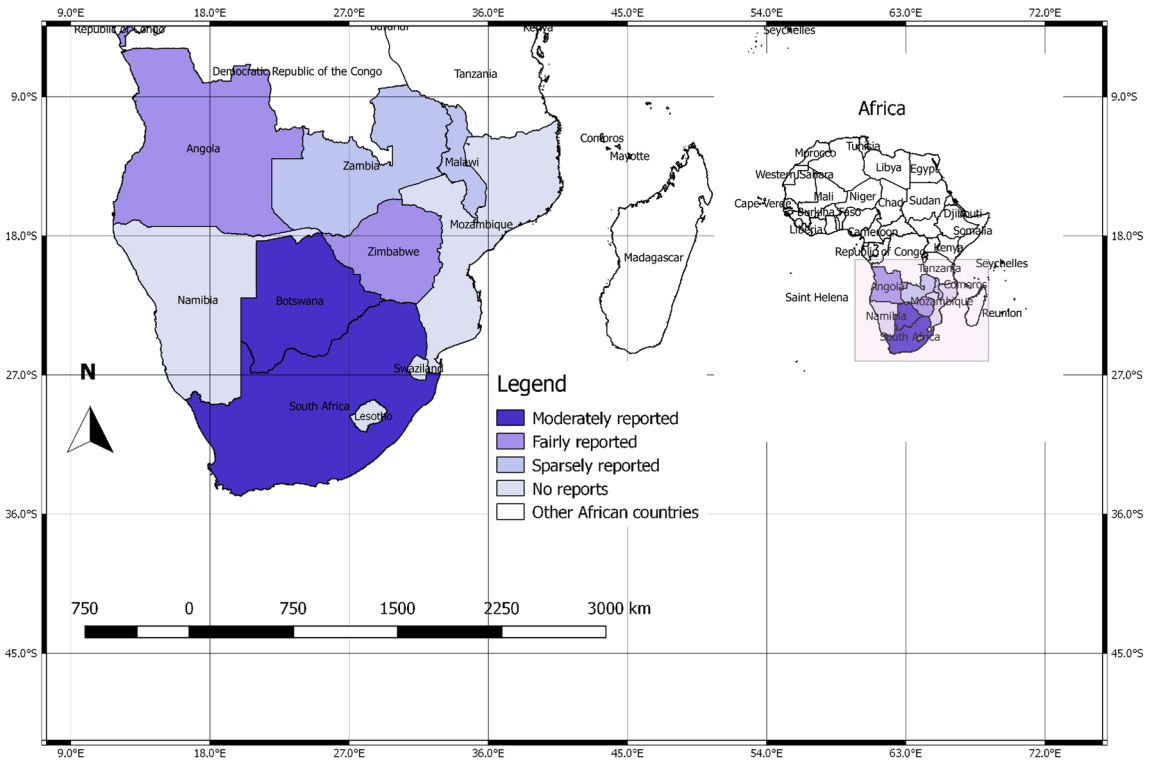


Fig. 4 Map showing southern African countries and the intensity of *Cryptosporidium* infection studies reported in the literature between 2000 and 2020

Cryptosporidiosis pooled prevalence showed that children in Zambia were most vulnerable with 26.6% (95% CI 13.2–42.5), followed by South African children with 24.1% (95% CI 1.9–56.5), and least in Malawian children 8.5% (95% CI 0.0–100) (Table 1). Considering the latter pooled prevalence, the confidence interval showed that the prevalence could rise further depending on the number of studies conducted across the country (Fig. 3a).

Heterogeneity of Cryptosporidium infections in diarrhoeic patients

A total of 1722 individuals with diarrhea were examined for cryptosporidial infections, of which 333 were reported positive. It was observed that most examined studies (98.3%) with diarrhoeic patients were children. Reported studies were from Botswana, South Africa, Malawi, and Angola. The pooled prevalence and heterogeneity variables of diarrhoeic patients with cryptosporidiosis showed 17.9% (9.8–27.6); $I^2 = 93.7$; $Q = 126.9$; $df = 8$; $P < 0.0001$ as observed from the forest plot (Fig. 3b). Most studies were retrieved from Botswana, followed by South Africa. The pooled prevalence was highest in Botswana (17.6%) but not significantly different ($X^2 = 0.357$; $P = 0.550$) from South Africa (12.7%) (Table 1).

Pooled prevalence of Cryptosporidium infections in HIV/AIDS patients in southern Africa

A total of 305 individuals positive with HIV/AIDS were observed to test positive to *Cryptosporidium* spp. infection out of examined 1073 individuals. The LFK index of 0.59 indicates that there is no asymmetry in the analysed studies. There is substantial heterogeneity with pooled prevalence showing 25.2% (5.7–51.0); $I^2 = 98.3$; $Q = 240.5$; $P < 0.0001$ (Fig. 3c). Studies were only reported in South Africa and Zambia (Table 1). The prevalence in South Africa (34.9%) was significantly higher ($X^2 = 81.024$; $P < 0.0001$) than the study from Zambia (9.5%). The presence of lactoferrin was 59.1% in *Cryptosporidium* spp.-positive patients observed in a study.

Diagnostic techniques

Studies were mostly examined with light microscopy using Ziehl–Neelsen staining techniques. The pooled prevalence based on light microscopy, immunoassay (IFAT, ELISA) and PCR methods was 13.5% (6.1–23.1), 24.4% (7.8–45.8) and 11.0% (4.0–20.5), respectively (Table 1). Tukey post-hoc multiple comparison test showed no significant difference ($P = 0.5646$; $R^2 = 0.0506$) between the three diagnostic techniques. Five countries (Zambia, Malawi,

Table 2 Reported genotypes of *C. hominis*, *C. parvum* and *C. meleagridis* in southern Africa from molecular characterisation and sequencing

Species/genotype Total	Host Country	Percentage positives	Subfamily	Subtype	References
C. hominis	Malawi	63.6	Ia, Ib, Id, Ie		Peng et al. (2003)
		95.3			Morse et al. (2007)
		58.1			Gatei et al. (2003)
	South Africa	75.0	Ib, Ie	IbA12G3R2; IbA10G2; IeA11G3T3	Samra et al. (2016)
		76.0	Ia, Ib, Id, Ie, If	IaA20R3; IaA25G1R3; IaA17R3; IbA9G3; IbA10G1; IdA20; IdA25; IdA26; IdA24; IeA11G3T3b; IfA14G1; IfA12G1	Samra et al. (2013)
		75.0	Ia, Ib, Id, Ie		Leav et al. (2002)
		20.0			Siwila et al. (2007)
		81.8			Samie et al. (2006)
	Botswana	41.0			Creek et al. (2010)
C. parvum	Malawi		IIc, IIe		Peng et al. (2003)
	South Africa	20.0	IIb, IIc, IIe	IIbA11; IIcA5G3bb; IIeA12G1	Samra et al. (2013)
		25.0	IIc		Leav et al. (2002)
		80.0			Siwila et al. (2007)
		18.2			Samie et al. (2006)
	Botswana	50.0			Creek et al. (2010)
C. meleagridis	Malawi	4.7			Morse et al. (2007)
	South Africa	4.0	IId	IIIdA4	Samra et al. (2013)

Botswana, South Africa, and Zimbabwe) reported studies using light microscopy and immunoassay, respectively. For the PCR technique, studies were observed in four countries with most reports from South Africa ($n = 3$), while Malawi and Botswana ($n = 2$) followed, and least in Zambia ($n = 1$).

Species variability in southern Africa

A total of eight studies characterized *Cryptosporidium* spp. subtypes with PCR. The characterized *Cryptosporidium* spp. species showed that *C. hominis* was mostly observed with 56.5% distribution, followed by *C. parvum* with 31.9%. *Cryptosporidium* spp. *hominis* sequences analysis with either *HSP70* and *GP60* genes showed the presence of five sub-families (Ia, Ib, Id, Ie and If) across all available studies. The sequence analysis of *GP60* genes of *C. parvum* showed three sub-families (IIb, IIc and IIe) across examined studies. Mixed infection of *C. parvum* and *C. hominis* showed a 2.0% distribution of the examined studies (Table 1). Only one sub-family (IId) was identified for *C. meleagridis* (Table 2). Of the reviewed studies, *C. meleagridis* showed 5.1% distribution, while *C. andersoni* stands at 2.0%.

Sensitivity test and limitations

The stability and reliability of the analysed results were examined from the sensitivity tests of individual data analysed in the METAXL. The analysis from the funnel plot within the 95% confidence interval and doi plot showed LFK index of 1.17 on the overall analysis, which ruled out significant bias risk of the analysed studies on human cryptosporidiosis in southern Africa. The LFK indexes of sub-group analysis have been reported in each sub-section of the result. In cases of publication bias, there is major asymmetry that could result from few cases examined in sub-group assessment.

Discussion

Cryptosporidiosis is a disease of the immune-compromised individuals, and it is often endemic in areas with poor social infrastructures such as lack of safe drinking water and sanitation problems. Studies on *Cryptosporidium* spp. infection in southern Africa within the past 20 years have been scanty, despite the burden of cryptosporidiosis and its associated risk factors. Southern Africa is disproportionately affected by cryptosporidiosis. This may be due to the fact that approximately 70% of its population live with HIV/AIDS, 31% of newly infected individuals with HIV, and 34% of individuals dying from AIDS are in the region (Ojuromi and Ashafa 2018; WHO 2015). South Africa has the largest population living with HIV in Southern Africa (CIA 2016).

The result from this meta-analysis showed that the overall prevalence of 16.8% *Cryptosporidium* spp.

infection among examined humans is high in southern Africa. The published articles showed that perception and awareness of the pathogen are low. The high number of immune-compromised individuals could be correlated with the high prevalence of *Cryptosporidium* spp. infection. For instance, the prevalence rate of HIV individuals was estimated between 1.0 and 26.5% in this region, while adult HIV prevalence exceeded 20% in Lesotho, Swaziland and Botswana. This is higher than pooled prevalence of the disease in HIV/AIDS patients from Nigeria (Karshima and Karshima 2020), and within the global range of prevalence in sub-Saharan Africa (Aldeyarbi et al. 2016). *Cryptosporidium* spp. infection was highest in South Africa compared to other pooled studies from other countries from this study. This could be associated with the high population of immune-compromised individuals within the country.

Notably, *Cryptosporidium* spp. infection was highest in children less than five years old. Meanwhile, children are disproportionately affected by cryptosporidiosis as observed in this study, with highest pooled prevalence in Zambia. The watery diarrhoea accompanied by dehydration and weakness, could lead to death easily. This could contribute significantly to the globally estimated 800,000 deaths in children due to cryptosporidiosis annually (Sow et al. 2016; Odeniran and Ademola 2019).

The high prevalence of *Cryptosporidium* spp. infection observed in diarrhoeic patients, especially studies from Botswana and South Africa, could be linked to the anthroponotic nature of some *Cryptosporidium* spp. subtypes and hygiene. For instance, *Cryptosporidium* spp. oocysts and isolates have been reported to be present in the surface waters of the Vaal Dam, treated effluents, drinking water in South Africa (Dungeni and Momba 2010), and piped-water in Zambia (Nchito et al 1998). The burden of diarrhea is particularly high in children, which could be linked with water-borne cryptosporidiosis. This protozoon is the second cause of severe diarrhea and the leading cause of death in children (Kotloff et al. 2013; Sow et al. 2016). The elevated lactoferrin level observed in *Cryptosporidium* spp. positive individuals in a study (Samie et al. 2016), indicated that inflammation is likely present. However, more studies are needed to correlate elevated lactoferrin and cryptosporidiosis in immune-compromised patients.

Low sensitivity diagnostic techniques are major problems in southern African countries. Even though molecular studies were done, genetic characterization of *Cryptosporidium* spp. species is lacking, which has limited our knowledge of *Cryptosporidium* spp. species and subtypes across the region. The high prevalence observed from studies examined with immunoassay, could be due to circulating IgG antibodies or sensitivity of the rapid diagnostic kits used for diagnosis.

Most characterized species with *GP60 genes* revealed *C. hominis* followed by *C. parvum* across southern Africa in this study, which is similar to an earlier study on Africa (Squire and Ryan 2017). Moreover, *C. hominis* and anthroponotic *C. parvum* subtypes isolated revealed both zoonotic and anthroponotic transmission in southern Africa. Earlier, there have been reports of *C. hominis* subtypes dominance infecting humans in several studies from Africa, regardless of the immune status (Maikai et al. 2012; Helmy et al. 2013; Aldeyarbi et al. 2016). Although only a study from Zambia reported an animal contact rate of 11.4% (Sinyangwe et al. 2020), the association between human and animal with diarrhea, could be responsible for the increasing zoonotic danger of *C. parvum* isolates (Siwila et al. 2007), particularly among the immune-compromised patients in southern Africa. Some *C. parvum* subtypes have been reported to be human-adapted subtypes, that could be transmitted from person to person, with origins from humans (Morse et al. 2007). The reporting of *C. meleagridis* in this study is not surprising, as the African population has a growing immune-compromised population with a previous report of 21% in these populations prone to infection by this pathogen (Morgan et al. 2000; Ben Abda et al. 2011; Aldeyarbi et al. 2016). Cryptosporidial infection was observed to be highest among HIV/AIDS patients, followed by children and then diarrhoeic individuals. The pooled prevalence across these groups was higher than 20%, which indicates that infection is more likely within the immune-compromised patients.

Recent studies showed that the absence of reports for four countries (Lesotho, Namibia, Mozambique, and Swaziland) could be due to low awareness of the importance of cryptosporidiosis or local publication of *Cryptosporidium* spp. infection reports (Fig. 4).

Conclusions

Cryptosporidium spp. infection was highest in HIV/AIDS patients (25.2%), followed by children (20.2%) and diarrheic patients (17.9%). Although, there is no significant difference in the prevalence. These categories of individuals could be at risk of infection if prompt awareness and treatment are neglected. Areas with a high density of livestock and the presence of humans with *Cryptosporidium* spp. infection, especially immune-compromised individuals, could mean that routine screening for opportunistic infections should be prioritized to avoid mortalities and spread of infection. More studies are needed to be conducted in southern Africa, such as correlating the significance of highly active antiretroviral therapy (HAART) positive or negative individuals and evaluating the $CD4^+$ cell counts with *Cryptosporidium* spp. infections.

Awareness on the zoonotic and anthroponotic nature of several *Cryptosporidium* spp. subtypes is needed, while *Cryptosporidium* spp. screening should be included in HIV/AIDS screening procedures in southern Africa.

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Declarations

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