

Cryptosporidium and colorectal cancer: a review of epidemiology and possible association

Research Article

Oyetunde T. Oyeyemi^{1*}, Ifeoluwa T. Oyeyemi¹, Nanfizat A. Alamukii¹, Jennifer K. Kone¹, Olawunmi, R. Oyerinde², Iyadunni A. Anuoluwa¹, Oghale O. Ovuakporie-uvu¹, Isaac A. Adesina¹, Yinka D. Oluwafemi¹, Sylvia O. Ogoanah^{1,3}, Adebisi M. Tiamiyu¹

¹Department of Biological Sciences, University of Medical Sciences, Ondo, Ondo State, Nigeria.

²Department of Biological Sciences, Mountain Top University, Km 12, Lagos-Ibadan Expressway, Ogun State, Nigeria

³Department of Animal and Environmental Biology, University of Benin, Benin City, Edo State, Nigeria

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Abstract: Cryptosporidiosis is an important protozoan disease with serious public health implications. The contribution of *Cryptosporidium* to colorectal cancer is still vaguely studied, but little evidence from experimental and epidemiological studies has suggested a possible association. This review discusses the epidemiology of cryptosporidiosis and colorectal cancer and attempts to unravel the possible link between the two diseases using epidemiological, pathological, molecular, and immunological evidence. The review stressed the need to undertake more studies in this relatively neglected field.

Keywords: *Cryptosporidium* spp. • colon cancer • morbidity • association • neglected • public health

1. Introduction

Cryptosporidiosis is a disease caused by *Cryptosporidium* spp., the intracellular obligate protozoan parasites with worldwide distribution^[1, 2]. These parasites invade the gastrointestinal and respiratory epithelium of many vertebrates, including humans^[3]. Cryptosporidiosis has public health implications globally but has shown higher prevalence in the developing world (2.98%–25.9%) than the developed countries (0.1%–9.1%)^[1, 4].

Infection occurs when an oocyst, which is the infective stage of the parasite, is ingested. The excystation of *Cryptosporidium* oocysts in the intestine is triggered by the host's gastrointestinal conditions, such as pH and temperature^[5]. Sporozoites released from the oocyst are attached to the epithelial cells of the hosts. The invasion of the sporozoites results in recruitment of epithelial cells into the site of invasion and the formation of a parasitophorous vacuole^[6]. A feeder organelle develops in sporozoites, and the

morphology of the parasite gradually becomes spherical. Trophozoite is formed and later develops into a Type I meront which can in turn develop into Type II meront. In some instances, the Type I meront may produce 6–8 merozoites. These merozoites may either re-infect the host cells and replicate asexually or develop into Type II meront^[7]. The sexual phase is initiated by Type II meronts, which release a set of four merozoites. The merozoites are differentiated into macrogamonts which produce macrogametes. The invading merozoites also form microgamonts which produce microgametes. Fertilisation occurs when a microgamete penetrates a macrogamete to form a zygote. The zygote divides meiotically to produce sporozoites, which in turn develop into oocysts. The oocysts are differentiated into two types; the thin and the thick-walled oocysts. The latter produce sporozoites, which reinvade the host cells, whilst the former are released into the environment through feces^[7].

* E-mail: ooyeyemi@unimed.edu.ng; zootund@yahoo.com

Cryptosporidiosis is significantly associated with diarrhoea and infections, and the causal parasites could be opportunistic and could cause life-threatening enteric disease, especially in immunocompromised individuals^[8].

Several reports have linked cryptosporidiosis to malignancies^[9, 10] with severe *Cryptosporidium* infection more frequently observed in cancer patients^[11]. A study also showed a 2.8 times greater rate of predisposition to *Cryptosporidium* infection in children with malignancy compared with healthy patients^[12]. Importantly, some studies have related cryptosporidiosis with colorectal adenocarcinoma^[13, 14]. However, the correlation between *Cryptosporidium* infection and human gastrointestinal malignancies is currently unclear. So this review aims to evaluate the possible association between cryptosporidiosis and colorectal cancer using available epidemiological, pathological, and immunological evidence. This may chart a new research direction in this relatively neglected field.

2. Epidemiology of *Cryptosporidium* infection

Cryptosporidiosis is currently endemic in more than 70 countries in the world^[15]. There are more than 30 species of *Cryptosporidium*; however, only two species, *C. parvum* and *C. hominis*, are known to frequently infect humans. Whilst the earlier species is associated with zoonotic transmission, the latter exclusively causes human infection^[16, 17]. The prevalence of *Cryptosporidium* infection is significantly higher in developing countries compared with the developed world since many areas in the former still lack potable water and the level of sanitation is generally poor^[8, 18]. *Cryptosporidium* infection rarely occurs in immunocompetent persons but the infection results in approximately 10%–15% of cases of severe diarrhoea in malnourished children < 5 years old^[8, 18]. A very high prevalence of cryptosporidiosis (77%) was reported among slum-dwelling children in Bangladesh^[19]. In Zambia, *Cryptosporidium* infection was diagnosed in 30.7% of all pre-schoolers in a peri-urban area^[20]. Some other epidemiological studies spanning two decades (2000–2020) documented the prevalence of *Cryptosporidium* in the human population, as presented in Table 1.

In addition to children and those living in areas with poor sanitation facilities, other groups at greater risk of this infection are transplant recipients, AIDS patients, and a host of others whose immune systems have been compromised. The disease could also be

nosocomial. Two or more of these factors often interact to determine the severity of the disease. White^[21] identified *Cryptosporidium* spp. as a common aetiology and a major cause of chronic diarrhoea among immunocompromised patients, and about 60,400 deaths were accrued to the infection. Ninety-three percent (93%) of the 64,818 reported deaths in 2015 in sub-Saharan Africa were mainly among children under the age of five infected with *Cryptosporidium*^[22]. Nevertheless, the 2010 diarrhoeal mortality rate in children under five reported in the Mediterranean African countries was meaningfully lower, varying from 1% to 12% in Liberia and Sudan. Poor surveillance, under diagnosis, or underreporting and poor bookkeeping could be responsible for the lower rate of infection detected in the Mediterranean African countries. Death rate in children below the age of five attributable to cryptosporidiosis in India is about 7.0% based on a multicenter study carried out by Ajampur *et al.*^[23].

3. Colorectal cancer epidemiology and risk factors

Cancer that affects either the colon or rectum is called colorectal cancer. Colorectal cancer is a major public health concern with cases increasing worldwide^[38]. It is the third highest leading cause of cancer death globally^[38]. In Africa, it used to be a rare disease but over the years with changing lifestyle and the adoption of westernized diet, the incidence has increased over time^[39]. According to the data recorded in the last five years, annual age-standardised colorectal cancer incidence was 38.7 per 100,000 persons (2012–2016), and the mortality rate was 13.9 per 100,000 persons (2013–2017)^[40]. Several risk factors can predispose individuals to colorectal cancer including age, gender, unhealthful diet, sedentary lifestyle, obesity, environmental factors, and other underlying diseases such as bowel inflammation^[41–44]. According to Sulżyc-Bielicka *et al.*^[45], the risk of developing colorectal cancer increases with age and has been observed to reach its peak in the eighth decade of life. Though there have been no data showing significant differences in the age range of patients suffering from cryptosporidiosis and colorectal cancer, studies have shown that the median age was between 57.7^[13] and 64.7 years^[45].

Other than the aforementioned factors, there are other genetic predispositions to colorectal cancer: *MHL1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*, *APC*, *MUTYH*, *PTEN*, *POLE*, *POLD1*, *SMAD4*, *GREM1*, *BMPRIA*, and *STK11* are genes associated with hereditary colorectal cancer^[46, 47]. Table 2 shows the prevalence or incidence of colorectal cancer in different regions of the world.

Table 1: Distribution of Cryptosporidium infection in different countries (2000 – 2020).

Country	Location	Population	Sample Size	Prevalence	Year	References
Angola	Cuba	School Children	35.1	2.9%	2015	Dacal et al ^[24] , (2018)
Cameroon	Limbe regional hospital southwest region	Children 0-5 years	112	8.93%	2018	Tombang et al ^[25] (2019)
China	China	HIV patient	89,724	8.69	2018	Wang et al ^[26] , (2018)
Ethiopia	Girar Jarso and Dera Districts of North Shewa Zone, Oromia Region	Children	384	7.8	2013	Wegayehu et al ^[27] , (2013)
Ethiopia	Ethiopia	HIV patient	520	26.9	2014	Adamu et al ^[28] , (2014)
Iran	South west	HIV patient	250	10.8	2018	Ghafari et al ^[29] , (2018)
Jordan	Jordan	Adult hemodialysis patients	133	11	2019	Zueter et al ^[30] , (2019)
Lebanon	North Lebanon	Children	412	10.68	2018	Osman et al ^[31] , (2018)
Malaysia	Tengku Ampuan Afzan (HTAA), Kuantan.	Children	135	25.2	2019	Asady et al ^[32] , (2019)
Nigeria	Calabar	Children	300	19.3%	2007	Inyam-Etoh et al ^[33] , (2007)
Nigeria	Ibadan	Soil & fecal samples of human	512	0.6%	2014	Adekeye et al ^[34] , (2016)
Pakistan	District Buner	Children	425	29.88	2019	Khan et al ^[35] , (2019)
Pakistan	Combined Military Hospital, Lahore Pakistan	Children	200	28	2021	Iqbal et al ^[36] , (2021)
Sri Lanka	Pediatric units of Teaching Hospitals, Kandy and Peradeniya, Sirimawo Bandaranayake Childrens' Hospital, Peradeniya and District General Hospital, Matale	Children	100	5.7	2014	Sirisena et al ^[37] , (2014)

Table 2: Prevalence or incidence of colorectal cancer in different parts of the world.

Country	Population	Sample size	Prevalence/ Incidence	Year	References
China	Adult	75,183	3.8% vs 2.7%	2000-2008, 2009-2018	Wu et al ^[48] , (2019)
China	Adult	50,445	1.9%	2018	Wu et al ^[48] , (2019)
France	Adult	2,097,952	8%	2005	Chauvenet et al ^[49] , (2009)
France	Adult	Over all	12%	2008	Chauvenet et al ^[50] , (2011)
France	Adult	Over all	95000	1994	Colonna et al ^[51] , (2001)
France	Adult	Over all	149.4/100,000	1976 and 1995	Benhamiche-Bouvier et al ^[52] , (2000)
Israel	Adult	1750	0.2%,	1994 - 2014	Leshno et al ^[53] , (2016)
Japan	Gastric cancer patient	332	4.8%	2014	Suzuki et al ^[54] , (2014)
Korea	Gastric cancer patient	543	3.5%	2010	Park et al ^[55] , (2010)
Nigeria	Adult	Over all	5.8%	2018	Eromosele and Irabor ^[56] (2019)
Singapore	Adult	227	1.8%	2018	Soh et al ^[57] , (2018)
Sri Lanka	Adult	Per 100,000	3.2 per 100,000	2008	Perera et al ^[58] , (2008)
USA	Adult	1,573	9.47%	2007-2011	Samadder et al ^[59] , (2014)
USA	Adult	26,742	0.6%	2017	Wong et al ^[60] , (2021)
USA	Elderly African American	Over all	102.6/100,000	2012 and 2017	Chouhan et al ^[61] , (2018)

4. Is there a correlation of risk factors for cryptosporidiosis and colorectal cancer?

It appears the risk factors for cryptosporidiosis and colorectal cancer do not overlap. For example, while *Cryptosporidium* infection is likely to be higher among children, colorectal cancer is more common in the older population. Studies have also revealed that cryptosporidiosis is poverty-related, occurring in many low-middle income countries (Table 1). On the other hand, colorectal cancer is more endemic in the developed countries (Table 2). Each disease, hence, has different drivers, and *Cryptosporidium* appears to be one of the promoters of colorectal cancer.

5. Cryptosporidiosis as a risk factor for colorectal cancer

The contribution of *Cryptosporidium* to benign tumours has been established in vertebrates such as reptiles and birds^[62–64]. The first report of the link between cryptosporidiosis and malignant tumours was established in mice^[65]. Since then, several other mouse models have supported this association^[66, 67]. A recently developed three-dimensional (3D) *in vivo*-like culture model from an adult murine colon also allowed for the maintenance of *Cryptosporidium*. The system allowed for *in vitro* development of low-grade intra-epithelial neoplasia after 27 days post-infection^[68]. Other evidence that suggested the possible association between *Cryptosporidium* and colorectal cancer was extensively discussed in a recent work by Başak *et al.*^[6]. The up-regulation of certain oncomiRNAs and the down-regulation of tumour suppressor miRNAs in the host epithelial cells due to *Cryptosporidium* infection may lead to cancer initiation in humans^[6]. Since *Cryptosporidium* spp. are localised in the colon-rectal region, the most likely cancer-type it may induce is the colorectal cancer.

Although there are limited studies showing a higher prevalence of *Cryptosporidium* spp. in colorectal cancer patients, growing evidence from epidemiological data has shown that *Cryptosporidium* spp. is highly associated with the development of colorectal cancer, thus, indicating that the protozoan parasite could be a potential risk factor for colorectal cancer. For example, a study by Sulżyc-Bielicka *et al.*^[46] has shown that *Cryptosporidium* spp. infections occur more often in patients with colorectal cancer than those without a neoplastic disease. The study corroborated with report

findings from a previous study published in 2012 on the 'Prevalence of *Cryptosporidium* sp. in patients with colorectal cancer' by Sulżyc-Bielicka *et al.*^[45]. According to Sulżyc-Bielicka *et al.*^[45], there's a close relationship between cryptosporidiosis and many cases of human colorectal cancer. Similarly, Osman *et al.*^[13] have shown that DNA and oocysts of *C. parvum* and *C. hominis* are more prevalent in digestive biopsies of patients diagnosed with colon neoplasia/adenocarcinoma compared with patients without digestive neoplasia. Berahmat *et al.*^[10] also reported that the rate of cryptosporidiosis in children with cancer undergoing chemotherapy was higher (3.8%) than those in the control group (0%). Additionally, Shebl *et al.*^[42] reported that cryptosporidiosis was associated with a rare squamous cell carcinoma of the colon in AIDS cases. The findings of Sulżyc-Bielicka *et al.*^[46] have led to the conclusions that (1) *Cryptosporidium* spp. infections appear significantly more frequently in patients with colorectal cancer, before oncological treatment, than in controls, regardless of age and sex; (2) amongst patients with colorectal cancer, the *Cryptosporidium* spp. infection is not associated with the colorectal cancer stage, grade, or location, or with patient age; and (3) males were significantly likely to have an increased frequency of *Cryptosporidium* spp. infections, with the presence of colorectal cancer old or young. It has been observed by Shebl *et al.*^[69] that the risk for developing colorectal cancer is higher in patients who have HIV infection and cryptosporidiosis than in patients who have HIV infections without cryptosporidiosis. In a study conducted by Sulżyc-Bielicka *et al.*^[45], it was observed that *Cryptosporidium* sp. infection was more common in colorectal patients who had tumours on the left side of their colon compared with those who had tumours on the right side. Amongst the patients who had tumours on the left side, it was observed that *Cryptosporidium* sp. infection affected mostly those with tumours located in the sigmoid colon and descending colon. The prevalence of *Cryptosporidium* among colorectal cancer patients has been documented in different populations (Table 3).

6. Pathophysiology of colorectal cancer and a possible contribution from *Cryptosporidium*

Colorectal cancer is a very common malignancy with a high mortality rate, making it one of the leading causes of cancer-related death^[73]. It often arises sporadically but a

Table 3: Prevalence of *Cryptosporidium* spp. among colorectal human cancer patients.

Country	Location	Prevalence	Year	P value	References
Lebanon	Tripoli	Case; 21% (15/72) vs control; 7% (9/146)	2012 - 2013	0.001	Osman et al ^[13] , (2017)
China	Jilin Province	Case; 17.24% (20/116) vs control; 0% (0/141)	2018	<0.001	Zhang et al ^[70] , (2020)
Poland	Szczecin	18% (10/55)	2007		Sulżyc-Bielicka et al ^[45] , (2007)
Poland	Szczecin	12.6% (11/87)	2009 – 2010		Sulżyc-Bielicka et al ^[11] , (2012)
Poland	Szczecin	Case; 13% (14/108) vs control; 4% (5/125)	2009 – 2014		Sulżyc-Bielicka et al ^[46] , (2018)
Poland	Wrocław	1% (1/145)	2017 – 2018		Kopacz et al ^[71] , (2019)
Saudi Arabia	Khalid University Hospital	Case; 70.3% (38/54) vs control; 17.3% (8/42)	-	<0.001	Sanad et al ^[72] , (2014)
United States	Maryland	0.09% (7/7,978)	-		Shebl et al ^[69] , (2012)

significant number of cases are correlated with a specific genetic background^[73]. The development of colorectal cancer is a multi-stage event that involves the stages of initiation, promotion, and progression. The initiation stage involves the development of a stable alteration in the DNA sequence, which is subsequently followed by the uncontrolled expansion of mutated clones which characterises the growth of the tumour^[74]. These events occur over a period of time involving the progression from benign neoplasm to colorectal cancer^[75].

7. Can *Cryptosporidium* contribute to the pathogenesis of colorectal cancer?

The frequent occurrence of *Cryptosporidium* spp. infections in patients with colorectal cancer^[45, 46] suggests its involvement in the pathogenesis of colorectal cancer. *Cryptosporidium* infection mostly affects those with tumours located in the sigmoid colon and descending colon^[45]. Moreover, *Cryptosporidium* infection was observed mostly in patients with grade 2 colorectal cancer^[45]. *Cryptosporidium* infection induces intestinal dysplasia, which is significantly correlated with parasite load, particularly in immunosuppressed mice^[76]. Overexpression of Cyclin D was observed in *Cryptosporidium*-induced intestinal dysplasia^[76], which implied modulation of gene expression in infected patients. The ability of some protozoans to interfere with signaling pathways in host cells has been reported^[77]. The mechanisms of *Cryptosporidium* infection in the pathogenesis of colorectal cancer, however, still remain largely unclear.

8. Immunomodulation in *Cryptosporidium* infection and risk of colorectal cancer

a. Immunomodulation in cryptosporidiosis

The process of immune response begins with the recognition of *Cryptosporidium* by toll-like receptors (TLRs) on epithelial cell surfaces. As reviewed by Ludington and colleagues, several toll-like receptors, including TLRs 2, 4, 5, and 9, have been shown to be involved, and they subsequently aid in parasite clearance^[78]. This brings in other associated proteins that trigger the activation of nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathways^[79], resulting in the release of inflammatory chemokines or cytokines as well as the antimicrobial peptides^[6]. The inflammatory cytokines, such as IL-8 secreted by the intestinal epithelial cells (IECs) and phagocytes, are very crucial to the innate immune response to the parasite^[80]. Likewise, the chemokines CXCL10, CCL2, and CX3CL1 are implicated in the recruitment of inflammatory cells and activation of adaptive immune cells in *Cryptosporidium* infection^[80, 81]. The antimicrobial peptides, such as β-defensins, hinder the growth activity of the parasite and also aid in destruction of the sporozoites *in vitro*^[82, 83].

The adaptive immune responses are triggered by the reactions of innate immunity. Cells involved in the adaptive immune response include CD4+ and CD8+ lymphocytes, natural killer (NK) cells, macrophages, dendritic cells, and innate lymphoid cells^[6].

Despite the activation of both the adaptive and innate immune responses of the host, the parasite is able to evade them with its own defense mechanism. For example, after *Cryptosporidium* infection, there is a depletion of the transcription factor, signaling transducer and activator of transcription1α (STAT1α) in infected

cells, thus, affecting the production of IFN- γ , which is important in the activation of both innate and adaptive immunity^[6]. Likewise, in *C. parvum* infection of neonatal mice, there is a down-regulation of the chemokine CCL20, which is responsible for parasite clearance^[84].

b. Alterations in the immune profile and development of cancer

Cryptosporidium spp. has largely been associated with diarrhoea and particularly the life-threatening form in immune-compromised individuals. The pathophysiological mechanisms of *Cryptosporidium* infection are multifactorial and not completely understood. One of the challenges is the difficulty in translating what obtains in mice models to the human immune system. The invasive process involved in obtaining tissue samples from the intestines of humans is a major limitation in carrying out such studies^[80]. This is due to the variation in clinical symptoms between mice and humans.

The repeated inflammation generated due to the immune responses to *Cryptosporidium* can result in cell proliferation and a destabilised genetic process causing oncogenic mutations^[85]. Quite a number of experimental and epidemiological studies have suggested a link between colorectal cancer and *Cryptosporidium*^[13, 65, 71].

Immunological evidence associating this pathogen with colorectal cancer is based on the established activation of the NF- κ B pathway during *Cryptosporidium* infection^[86]. Increased activation of this pathway potentially affects the initiation, development, and metastasis in human cancer. Thus, the down-regulation of the let-7 family and the modulation of IL-6/STAT3 signaling in response to these inflammatory signals may lead to an uncontrolled proliferation of cells that results in cancer initiation^[87].

9. Other parasites as risk of colorectal cancer

A wide variety of protozoan and helminth parasites that colonise the gastrointestinal tract are known to cause severe pathology in the region, and colorectal cancer is occasionally identified as one of parasite-induced morbidities in endemic areas. Although *Cryptosporidium* spp. appears to be the most frequently encountered protozoa in CRC patients, evidence from Europe, Middle-East, and Central Asia^[88–90] has implicated the opportunistic protozoan *Blastocystis* sp. as a risk factor for CRC. The amoeba *Endolimax nana*, which

is well known not to be pathogenic, has also been reported to be associated with CRC^[89]. These two opportunistic protozoans are known to coexist and cause bowel functional disorder and chronic diarrhoea in immunocompetent individuals^[91, 92]. Although the impact of their concomitant infection on CRC is yet to be explored, the aggravated disease spectrum in the form of severe diarrhoeic condition during concurrent infection is suggestive of a higher risk of colon neoplasia/adenocarcinoma when the two protozoans coexist. Another protozoan linked to adenocarcinoma colon cancer is *Entamoeba histolytica*, but the body of evidence available to support this proposition suggests that the parasite is an unlikely cause of CRC^[93].

It is not certain whether *Blastocystis* sp. is directly involved in the development of colon neoplasia and adenocarcinoma, but studies have shown that the organism alters the gut microbiota community^[94, 95]. The disruption of gut microbiota could induce CRC carcinogenesis through mechanisms such as inflammation, immune regulation, metabolism of dietary components, and genotoxin production^[96]. The interaction of gut microbiota with the host immune system can modulate inflammation in the gastrointestinal tract^[97]. The microbiota produces metabolites that could promote the development of CRC^[98]. Microbiota can also induce the production of DNA-damaging toxins^[99]. The ability of *Blastocystis* sp. to disrupt the gut microbiota (or microbiome) to induce mechanisms that could potentiate the development of CRC may be similar for other earlier discussed protozoan organisms.

Several case-control studies, epidemiological studies, case series, and case reports have shown the helminth *Schistosoma japonicum* as a probable cause of CRC. Much of the pathogenesis of *S. japonicum*-associated CRC still remain unknown, but it shares some similarities to that seen arising in the setting of inflammatory bowel disease^[96]. More importantly, a higher proportion of base-pair substitutions were found at CpG dinucleotides in *S. japonicum*-associated rectal cancers when compared with non-schistosomal rectal cancers^[96]. *S. mansoni* is weakly associated with CRC and the malignant factor Bcl-2 appeared to be the most significantly expressed cancer-related biomarker in *S. mansoni*-associated CRC. Others such as p53 and c-myc were not significantly expressed when compared with non-schistosomal-associated rectal cancer (similar to what was observed in *S. japonicum*)^[96, 100].

10. Conclusion

This review attempts to find a link between *Cryptosporidium* and colorectal cancer. Although the clinical data presented in this review suggest an association between the two, more evidence-based case-control studies will be needed to reach a conclusion. A direct causal link between virulent *Cryptosporidium* factors and the mechanisms of colorectal tumour development is necessary. Recent advances in genomic studies, 3D culture models, gene manipulation using CRISPR/Cas9 system, and clinical trials involving the application of sensitive diagnostic tools can be adopted to study the association between the two. The establishment of a

link between *Cryptosporidium* and colorectal cancer will promote public health measures targeted against cryptosporidiosis which in turn could reduce the risk of colon cancer. In addition, this could stimulate interest in drug and vaccine discovery research, thus, reducing the number of children suffering from cryptosporidiosis-related enteric diseases and mortality. We recommend more research efforts in this field as cryptosporidiosis and colorectal cancer have become persistent public health issues globally.

Disclosure summary

The authors have nothing to disclose.

References

- [1] Kalantari N, Ghaffari S, Bayani M. *Cryptosporidium* spp. infection in Iranian children and immunosuppressive patients: A systematic review and meta-analysis. *Caspian J Intern Med* 2018;9:106–115.
- [2] Kalantari N, Gorgani-Firouzjaee T, Ghaffari S, Bayani M, Ghaffari T, Chehrazhi M. Association between *Cryptosporidium* infection and cancer: a systematic review and meta-analysis. *Parasitol Int.* 2020;74:101,979.
- [3] Ryan U, Zahedi A, Paparini A. *Cryptosporidium* in humans and animals – a one-health approach to prophylaxis. *Parasite Immunol.* 2016;38:535–547.
- [4] Fletcher SM, Stark D, Harkness J, Ellis J. Enteric protozoa in the developed world: a public health perspective. *Clin Microbiol Rev.* 2012;25:420–449.
- [5] Hijjawi NS, Meloni BP, Ryan UM, Olson ME, Thompson RC. Successful in vitro cultivation of *Cryptosporidium andersoni*: evidence for the existence of novel extracellular stages in the life cycle and implications for the classification of *Cryptosporidium*. *Int J Parasitol.* 2002;32:1,719–1,726.
- [6] Başak F, Jainul MA, Yusof AM. *Cryptosporidium*-host interaction alters regulation of oncomiRNAs and tumor suppressor miRNA expression. *J. Biol. Sci* 2012;19:272–279.
- [7] Chen XM, Keithly JS, Paya CV, LaRusso NF. Cryptosporidiosis. *N Engl J Med.* 2002;346: 1,723–1,731.
- [8] Bouzid M, Kintz E, Hunter PR. Risk factors for *Cryptosporidium* infection in low and middle income countries: a systematic review and meta-analysis. *PLoS Negl Trop Dis.* 2018;12:e0006553.
- [9] García-Elorriaga G, Corona-de Los Santos JC, Méndez-Tovar S, del Rey-Pineda G, Pérez-Casillas RX. Opportunistic bacteria and microbial flora in children with leukemia and neutropenic enterocolitis. *Rev Med Inst Mex Seguro Soc.* 2013;51: 424–427.
- [10] Berahmat R, Mahami-Oskouei M, Rezamand A, Spotin A, Aminisani N, Ghoyouchi R, et al. *Cryptosporidium* infection in children with cancer undergoing chemotherapy: how important is the prevention of opportunistic parasitic infections in patients with malignancies? *Parasitol Res.* 2017;116:2,507–2,515.
- [11] Sulżyc-Bielicka V, Kołodziejczyk L, Jaczewska S, Bielicki D, Kładny J, Safranow K, Prevalence of *Cryptosporidium* sp. in patients with colorectal cancer. *Polski Przegląd Chirurgiczny.* 2012;84:348–351.
- [12] Hijjawi N, Zahedi A, Kazaleh M, Ryan U. Prevalence of *Cryptosporidium* species and subtypes in paediatric oncology and non-oncology patients with diarrhoea in Jordan. *Infect Genet Evol.* 2017;55:127–130.
- [13] Osman M, Benamrouz S, Guyot K, Baydoun M, Frealle E, Chabe M, et al. High association of *Cryptosporidium* spp. infection with colon adenocarcinoma in Lebanese patients. *PLoS One.* 2017;12:e0189422.
- [14] Valle L. Genetic predisposition to colorectal cancer: where we stand and future perspectives. *World J Gastroenterol.* 2014;20:9,828–9,849.
- [15] Dong S, Yang Y, Wang Y, Yang D, Shi Y, Li C, et al. Li L, Chen Y, Jiang Q, Zhou Y. Prevalence of *Cryptosporidium* Infection in the Global

- Population: a Systematic Review and meta-analysis. *Acta Parasit.* 2020;65(4):882–889.
- [16] Dixon B, Parrington L, Cook A, Pintar K, Pollari F, Kelton D, et al. The potential for zoonotic transmission of *Giardia duodenalis* and *Cryptosporidium* spp. from beef and dairy cattle in Ontario, Canada. *Vet Parasitol.* 2011;175:20–26.
- [17] Ehsan AM, Geurden T, Casaert S, Parvin SM, Islam TM, Ahmed UM, et al. Assessment of zoonotic transmission of *Giardia* and *Cryptosporidium* between cattle and humans in rural villages in Bangladesh. *PLoS One.* 2015;10:e0118239.
- [18] Shoultz DA, de Hostos EL, Choy RK. Addressing *Cryptosporidium* infection among young children in low-income settings: the crucial role of new and existing drugs for reducing morbidity and mortality. *PLoS Negl Trop Dis.* 2016;10(1):e0004242.
- [19] Korpe PS, Haque R, Gilchrist C, Valencia C, Niu F, Lu M, et al. Natural history of cryptosporidiosis in a longitudinal study of Slum-Dwelling Bangladeshi children: association with severe malnutrition. *PLoS Negl Trop Dis.* 2016;5:e4564.
- [20] Siwila J, Phiri IG, Enemark HL, Nchito M, Olsen A. Seasonal prevalence and incidence of *Cryptosporidium* spp. and *Giardia duodenalis* and associated diarrhoea in children attending preschool in Kafue, Zambia. *Trans R Soc Trop Med Hyg.* 2011;105:102–108.
- [21] White AC Jr. Cryptosporidiosis and the ears of the hippopotamus. *Clin Infect Dis.* 2010;50:1,373–1,374.
- [22] Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet.* 2013;382:209–222.
- [23] Ajampur SS, Liakath FB, Kannan A, Rajendran P, Sarkar R, Moses PD, et al. Multisite study of cryptosporidiosis in children with diarrhea in India. *J Clin Microbiol.* 2010;48:2,075–2,081.
- [24] Dacal E, Saugar JM, de Lucio A, Hernández-de-Mingo M, Robinson E, Köster PC, et al. Prevalence and molecular characterization of *Strongyloides stercoralis*, *Giardia duodenalis*, *Cryptosporidium* spp., and *Blastocystis* spp. isolates in school children in Cubal, Western Angola. *Parasit Vectors.* 2018;11:67.
- [25] Tombang AN, Ambe NF, Bobga TP, Nkfusai CN, Collins NM, Ngwa SB, et al. Prevalence and risk factors associated with cryptosporidiosis among children within the ages 0-5 years attending the Limbe regional hospital, southwest region, Cameroon. *BMC Public Health.* 2019;19:1,144.
- [26] Wang RJ, Li JQ, Chen YC, Zhang LX, Xiao LH. Widespread occurrence of *Cryptosporidium* infections in patients with HIV/AIDS: epidemiology, clinical feature, diagnosis, and therapy. *Acta Trop.* 2018;187:257–263.
- [27] Wegayehu T, Adamu H, Petros B. Prevalence of *Giardia duodenalis* and *Cryptosporidium* species infections among children and cattle in North Shewa Zone, Ethiopia. *BMC Infect Dis.* 2013;13:419.
- [28] Adamu H, Petros B, Zhang G, Kassa H, Amer S, Ye J, et al. Distribution and clinical manifestations of *Cryptosporidium* species and subtypes in HIV/AIDS patients in Ethiopia. *PLoS Negl Trop Dis.* 2014;8:e2831.
- [29] Ghafari R, Rafiei A, Tavalla M, Choghakabodi PM, Nashibi R, Rafiei R. Prevalence of *Cryptosporidium* species isolated from HIV/AIDS patients in southwest of Iran. *Comp Immunol Microbiol Infect Dis.* 2018;56:39–44.
- [30] Zueter AM, Hijawi NS, Hamadeneh KN, Al-Sheyab MM, Hatamleh AM. Cryptosporidiosis among hemodialysis patients in Jordan: first preliminary screening surveillance. *Trop Med Infect Dis.* 2019;4:131.
- [31] Osman M, Benamrouz S, Guyot K, El Safadi D, Mallat H, Dabboussi F, et al. Molecular epidemiology of *Cryptosporidium* spp. in North Lebanon. *J Infect Dev Ctries.* 2018;12:34S.
- [32] Asady A, Ismail S, Marsitah AJ, Pakeer O. Prevalence of *Cryptosporidium* spp. infection among children admitted to Hospital Tengku Ampuan Afzan. *Med J Malaysia.* 2019;74:468–471.
- [33] Inyam-Etoh PC, Etim NG, Useh MF, Udiong CEJ, Essien AW. Cryptosporidiosis and infantile diarrhea in Calabar, Nigeria. *J Med Sci.* 2007;7:1,325–1,329.
- [34] Adekeye TA, Thompson E and Awobode HO. Environmental contamination and public health risk of soil parasites in Ibadan South East Local Government Area, Nigeria. *Zool Ecol.* 2016;26:150–157.
- [35] Khan A, Shams S, Khan S, Khan MI, Khan S, Ali A. Evaluation of prevalence and risk factors associated with *Cryptosporidium* infection in rural population of district Buner, Pakistan. *PLoS One.* 2019;14:e0209188.
- [36] Iqbal MD, Naeem T, Khurshid U, Hameed F. Frequency of cryptosporidiosis in children having persistent diarrhea. *Pak J Med Sci.* 2021;37:121–124.

- [37] Sirisena UM, Iddawela WM, Noordeen F, Wickramasinghe S. Prevalence and identification of *Cryptosporidium* species in paediatric patients with diarrhea. *Ceylon Med J*. 2014;59:75–78.
- [38] Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. *Prz Gastroenterol*. 2019;14:89–103.
- [39] Irabor DO. Emergence of colorectal cancer in West Africa: accepting the inevitable. *Niger Med J*. 2017;58:87–91.
- [40] Siegel RL, Miller KD, Goding Sauer A, Fedewa SA, Butterly LF, Anderson JC, et al. Colorectal cancer statistics, 2020. *CA Cancer J Clin*. 2020;70:145–164.
- [41] Dashti SG, Win AK, Hardikar SS, Glombicki SE, Mallenahalli S, Thirumurthi S, et al. Physical activity and the risk of colorectal cancer in Lynch syndrome. *Int J Cancer*. 2018;143:2,250–2,260.
- [42] Weigl K, Chang-Claude J, Knebel P, Hsu L, Hoffmeister M, Brenner H. Strongly enhanced colorectal cancer risk stratification by combining family history and genetic risk score. *Clin Epidemiol*. 2018;10:143–152.
- [43] Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. *Lancet*. 2019;394:1,467–1,480.
- [44] Zheng Y, Hua X, Win AK, MacInnis RJ, Gallinger S, Marchand LL, et al. A new comprehensive colorectal cancer risk prediction model incorporating family history, personal characteristics, and environmental factors. *Cancer Epidemiol Biomarkers Prev*. 2020;29:549–557.
- [45] Sulzyc-Bielicka V, Kuźna-Grygiel W, Kołodziejczyk L, Bielicki D, Kładny J, Stępień-Korzonek M, et al. Cryptosporidiosis in patients with colorectal cancer. *J. Parasitol*. 2007;93(3):722–724.
- [46] Sulzyc-Bielicka V, Kołodziejczyk L, Jaczewska S, Bielicki D, Safranow K, Bielicki P, et al. Colorectal cancer and *Cryptosporidium* spp. infection. *PLoS One*. 2018;13:e0195834.
- [47] Augustus GJ, Ellis NA. Colorectal cancer disparity in African Americans: risk factors and carcinogenic mechanisms. *Am J Pathol*. 2018;188:291–303.
- [48] Wu JY, Chen DF, Liu TY, Dong WX, Liu X, Wang SN, et al. Reevaluation of a rightward shift in colorectal cancer: A single-center retrospective study in Tianjin. *J Dig Dis*. 2019;20:532–538.
- [49] Chauvenet M, Lepage C, Jooste V, Cottet V, Faivre J, Bouvier AM. Prevalence of patients with colorectal cancer requiring follow-up or active treatment. *Eur J Cancer*. 2009; 45:1,460–1,465.
- [50] Chauvenet M, Cottet V, Lepage C, Jooste V, Faivre J, Bouvier AM. Trends in colorectal cancer incidence: A period and birth-cohort analysis in a well-defined French population. *BMC Cancer*. 2011;11.
- [51] Colonna M, Grosclaude P, Launoy G, Tretarre B, Arveux P, Raverdy N, et al. Estimation of colorectal cancer prevalence in France. *Eur J Cancer*. 2001;37:93–96.
- [52] Benhamiche-Bouvier AM, Clinard F, Phelip JM, Rassiat E, Faivre J. Colorectal cancer prevalence in France. *Eur J Cancer Prev*. 2000;9:303–307.
- [53] Leshno A, Moshkowitz M, David M, Galazan L, Neugut AI, Arber N, et al. Prevalence of colorectal neoplasms in young, average risk individuals: a turning tide between East and West. *World J Gastroenterol*. 2016;22:7,365–7,372.
- [54] Suzuki A, Koide N, Takeuchi D, Okumura M, Ishizone S, Suga T, et al. [Prevalence of synchronous colorectal neoplasms in surgically treated gastric cancer patients and significance of screening colonoscopy. *Dig Endosc*. 2014;26:396–402.
- [55] Park DI, Park SH, Yoo TW, Kim HS, Yang SK, Byeon JS, et al. The prevalence of colorectal neoplasia in patients with gastric cancer: a Korean Association for the Study of Intestinal Disease (KASID) Study. *J Clin Gastroenterol*. 2010;44:102–105.
- [56] Eromosele OB, Irabor DO. Colorectal cancer in Nigeria: changing trends and clinical phenotype. *J Cancer Sci Ther*. 2019;11:287–291.
- [57] Soh NYT, Chia DKA, Teo NZ, Ong CJM, Wijaya R. Prevalence of colorectal cancer in acute uncomplicated diverticulitis and the role of the interval colonoscopy. *Int J Colorectal Dis*. 2018;33:991–994.
- [58] Perera T, Wijesuriya RE, Suraweera PH, Wijewardene K, Kumarage SK, Ariyaratne MH, et al. Prevalence of colorectal cancer and survival in patients from the Gampaha District, North Colombo region. *Ceylon Med J*. 2008;53:17–21.
- [59] Samadder NJ, Curtin K, Wong J, Tuohy TM, Mineau GP, Smith KR, et al. Epidemiology and familial risk of synchronous and metachronous colorectal cancer: a population-based study in Utah. *Clin Gastroenterol Hepatol*. 2014;12:2,078–2,084.e1-2.
- [60] Wong CH, Sundberg T, Chung VC, Voiss P, Cramer H. Complementary medicine use in US adults with a history of colorectal cancer: a nationally representative survey. *Support Care Cancer*. 2021;29:271–278.
- [61] Chouhan V, Mansoor E, Parasa S, Cooper GS. Rates of prevalent colorectal cancer occurrence in persons 75 years of age and older: a population-

- based national study. *Dig Dis Sci*. 2018;63:1,929–1,936.
- [62] Fitzgerald SD, Moisan PG, Bennett R. Aural polyp associated with cryptosporidiosis in an iguana (*Iguana Iguana*). *J Vet Diagn Invest*. 1998;10:179–180.
- [63] Uhl EW, Jacobson E, Bartick TE, Micinilio J, Schimdt R. Aural-pharyngeal polyps associated with *Cryptosporidium* infection in three iguanas (*Iguana iguana*). *Vet Pathol*. 2001;38:239–242.
- [64] Nakagun S, Horiuchi N, Sugimoto M, Tomikawa S, Watanabe K, Kobayashi Y. Proventriculitis associated with *Cryptosporidium baileyi* in a snowy owl (*Bubo scandiacus*) and its epidemiological investigation. *J Parasitol*. 2017;103:451–457.
- [65] Certad G, Ngouanesavanh T, Guyot K, Gantois N, Chassat T, Mouray A, et al. *Cryptosporidium parvum*, a potential cause of colic adenocarcinoma. *Infect Agent Cancer*. 2007;2:22.
- [66] Certad G, Creusy C, Guyot K, Mouray A, Chassat T, Delaire B, et al. Fulminant cryptosporidiosis associated with digestive adenocarcinoma in SCID mice infected with *Cryptosporidium parvum* TUM1 strain. *Int J Parasitol*. 2010;40:1,469–1,475.
- [67] Benamrouz S, Conseil V, Creusy C, Calderon E, Dei-Cas E, Certad G. Parasites and malignancies, a review, with emphasis on digestive cancer induced by *Cryptosporidium parvum* (Alveolata: Apicomplexa). *Parasite*. 2012;19:101–115.
- [68] Baydoun M, Vanneste SB, Creusy C, Guyot K, Gantois N, Chabe M, et al. Three-dimensional (3D) culture of adult murine colon as an in vitro model of cryptosporidiosis: proof of concept. *Sci Rep*. 2017;7:17,288.
- [69] Shebl FM, Engels EA, Goedert JJ. Opportunistic intestinal infections and risk of colorectal cancer among people with AIDS. *AIDS Res Hum Retroviruses*. 2012;28:994–999.
- [70] Zhang N, Yu X, Zhang H, Cui L, Li X, Zhang X, et al. Prevalence and genotyping of *Cryptosporidium parvum* in gastrointestinal cancer patients. *J Cancer*. 2020;11:3,334–3,339.
- [71] Kopacz Ż, Kváč M, Karpiński P, Hendrich AB, Sasiadek MM, Leszczyński P, et al. The first evidence of *Cryptosporidium meleagridis* infection in a colon adenocarcinoma from an immunocompetent patient. *Front Cell Infect Microbiol*. 2019;9:35.
- [72] Sanad MM, Thagfan FA, Al Olayan EM, Almogren A, Al Hammaad A, Al-Mawash A., et al. Opportunistic coccidian parasites among Saudi cancer patients presenting with diarrhea: prevalence and immune status. *Res J Parasitol*. 2014;9:55–63.
- [73] Arnold CN, Goel A, Blum HE, Boland CR. Molecular pathogenesis of colorectal cancer – implications for molecular diagnosis. *Cancer*. 2005;104:2,035–2,047.
- [74] de Leon MP, Percesepe A. 2000. Pathogenesis of colorectal cancer. *Dig Liver Dis*. 2000;32:807–821.
- [75] Grady WM, Markowitz SD. The molecular pathogenesis of colorectal cancer and its potential application to colorectal cancer screening. *Dig Dis Sci*. 2015;60:762–772.
- [76] Abdou AG, Harba NM, Afifi AF, Elnaidany NF. Assessment of *Cryptosporidium parvum* infection in immunocompetent and immunocompromised mice and its role in triggering intestinal dysplasia. *Int J Infect Dis*. 2013;7:e593–e600.
- [77] Sibley LD. Invasion and intracellular survival by protozoan parasites. *Immunol Rev*. 2011;240:72–91.
- [78] Ludington JG, Ward HD. Systemic and mucosal immune responses to *Cryptosporidium*—vaccine development. *Curr Trop Med Rep*. 2015;2:171–180.
- [79] Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on toll-like receptors. *Nat Immunol* 2010, 11: 373–84.
- [80] Pantenburg B, Dann SM, Wang HC, Robinson P, Castellanos-Gonzalez A, Lewis DE, et al. Intestinal immune response to human *Cryptosporidium* sp. infection. *Infect Immun*. 2008;76:23–29.
- [81] Zhou R, Gong AY, Chen D, Miller RE, Eiseheid AN, Chen XM. Histone deacetylases and NF-κB signaling coordinate expression of CX3CL1 in epithelial cells in response to microbial challenge by suppressing miR-424 and miR-503. *PLoS ONE*. 2013;8:e65153.
- [82] Zhou R, Gong AY, Eiseheid AN, Chen XM. miR-27b targets KSRP to coordinate TLR4-mediated epithelial defense against *Cryptosporidium parvum* infection. *PLoS Pathog*. 2012;8:e1002702.
- [83] Carryn S, Schaefer D, Imboden M, Homan E, Bremel R., Riggs, M. Phospholipases and cationic peptides inhibit *Cryptosporidium parvum* sporozoite infectivity by parasitocidal and non-parasitocidal mechanisms. *J Parasitol*. 2012;98:199–204.
- [84] Guesdon W, Auray G, Pezier T, Bussi re FI, Drouet F, Le Vern Y, et al. CCL20 displays antimicrobial activity against *Cryptosporidium parvum*, but its expression is reduced during infection in the intestine of neonatal mice. *J Infect Dis*. 2015;212:1,332–1,340.
- [85] Xia Y, Shen S, Verma IM. NF-κB, an active player in human cancers. *Cancer Immunol Res*. 2014;2:823–830.

- [86] Terzić J, Grivennikov S, Karin E, Karin M. Inflammation and colon cancer. *Gastroenterology*. 2010;138:2,101–2,114.e5.
- [87] Iliopoulos D, Hirsch HA, Struhl K. An epigenetic switch involving NF- κ B, Lin28, Let-7 MicroRNA, and IL6 links inflammation to cell transformation. *Cell*. 2009;139:693–706.
- [88] Sulżyc-Bielicka, V, Kołodziejczyk, L, Adamska, M, Skotarczak B, Jaczevska S, Safranow K, et al. Colorectal cancer and *Blastocystis* sp. infection. *Parasites Vectors*. 2020;14:200.
- [89] Kahyesh RS, Alghasi A, Haddadi S, Sharhani A. Intestinal parasites infection in children with cancer in Ahvaz, Southwest Iran. *Interdiscipl Perspect Infect Dis*. 2020;Article ID 8839740.
- [90] Toychiev A, Abdujapparov S, Imamov A, Navruzov B, Davis N, Badalova N, et al. Intestinal helminths and protozoan infections in patients with colorectal cancer: prevalence and possible association with cancer pathogenesis. *Parasitol Res*. 2018; doi:10.1007/s00436-018-6070-9.
- [91] Shah M, Tan CB, Rajan D, Ahmed S, Subramani K, Rizvon K, et al. *Blastocystis hominis* and *Endolimax nana* co-infection resulting in chronic diarrhea in an immunocompetent male. *Case Rep Gastroenterol*. 2012;6(2):358–364.
- [92] Zara M, Nfn R, Rabab H, Kaleem R. Co-infection of *Blastocystis* and *Endolimax nana* and their association with functional disorders and bowel inflammation. *Amer J Gastroenterol*. 2018;113:S1049.
- [93] Peterson MR, Weidner N. Gastrointestinal neoplasia associated with bowel parasitosis: real or imaginary? *J Trop Med*. 2011;Article ID 234254.
- [94] Kumarasamy V, Anbazhagan D, Subramaniyan V, Vellasamy S. *Blastocystis* sp., parasite associated with gastrointestinal disorders: an overview of its pathogenesis, immune modulation and therapeutic strategies. *Curr Pharm Des*. 2018;24:3,172–3,175.
- [95] Stensvold CR, van der Giezen M. Associations between gut microbiota and common luminal intestinal parasites. *Trends Parasitol*. 2018;34:369–377.
- [96] Tilg H, Adolph TE, Gerner RR, Moschen AR. The intestinal microbiota in colorectal cancer. *Cancer Cell*. 2018;33:954–964.
- [97] Tomkovich S, Yang Y, Winglee K, Gauthier J, Mühlbauer M, Sun X, et al. Locoregional effects of microbiota in a preclinical model of colon carcinogenesis. *Cancer Res*. 2017;77:2,620–2,632.
- [98] Louis P, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. *Nat Rev Microbiol*. 2014;12:661–672.
- [99] Wong SH, Yu J. Gut microbiota in colorectal cancer: mechanisms of action and clinical applications. *Nat Rev Gastroenterol Hepatol*. 2019;16:690–704.
- [100] Zalata KR, Nasif WA, Ming SC, Lotfy M, Nada NA, El-Hak NG, et al. p53, Bcl-2 and C-Myc expressions in colorectal carcinoma associated with schistosomiasis in Egypt. *Cellular Oncol*. 2005;27(4):245–253.