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Association of *Cryptosporidium* spp. Infection with Colorectal Cancer in the Patients Attended Al-Amal Center for Tumors Treatment in Taiz City, Yemen

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تلازم الإصابة بطفيل الأبواغ الخفية مع سرطان القولون والمستقيم في المرضى المترددين على مركز الأمل لمعالجة الأورام في مدينة تعز، اليمن

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الملخص

داء الكريبتوسبوريديم هو مرض يسببه أنواع من طفيل الأبواغ الخفية المسماه بالكريبتوسبوريديم، وهي طفيليات أولية إجبارية داخل خلوية ذات توزيع عالمي. هدفت الدراسة الحالية إلى تحديد مدى انتشار أنواع الكريبتوسبوريديم وبعض عوامل الخطر المرتبطة بالعدوى لدى مرضى سرطان القولون والمستقيم والأفراد الأصحاء. شملت هذه الدراسة 200 مشارك (130 مريضًا بسرطان القولون والمستقيم و70 فردًا صحيحًا) يرتادون مستشفى الأمل لمرضى السرطان في مدينة تعز–اليمن خلال الفترة من مايو 2022 إلى أغسطس 2023. تم جمع عينات براز طازجة وفحصها بواسطة الصبغة الدائمة لمسحات البراز باستخدام تقنية صبغة زيل–نيلسن المعدلة للكشف عن أكياس الكريبتوسبوريديم. تم التحقيق من البيانات الديموغرافية وعوامل الخطر المحتملة مثل العمر والجنس ومكان الإقامة ومصدر مياه الشرب لدى جميع المشاركين في الدراسة باستخدام استبان خاصة. كان معدل انتشار أنواع الكريبتوسبوريديم بين مرضى سرطان القولون والمستقيم أعلى بكثير مقارنة بمعدل الانتشار بين الأفراد الأصحاء (54,62%، 10%) على التوالي ($P < 0.001$). أيضًا، لوحظ أعلى معدل للعدوى في الفئة العمرية 18–38(66.67%) بين مرضى سرطان القولون والمستقيم و13.64% بين أفراد العينة الضابطة. وفقًا لذلك، فإن الفرق في معدلات عدوى أنواع الكريبتوسبوريديم بين مرضى سرطان القولون والمستقيم وأفراد المجموعة الضابطة ذو دلالة إحصائية عالية ($P = 0.005$). كان معدل عدوى أنواع الكريبتوسبوريديم أعلى بين المرضى الذكور بسرطان القولون والمستقيم (58.21%) وبين الإناث في المجموعة الضابطة (14.71%). كلا مرضى سرطان القولون والمستقيم والأصحاء القادمين من المناطق الريفية كان لديهم معدلات أعلى للعدوى بأنواع الكريبتوسبوريديم (65.15%، 18.52%) على التوالي. بالإضافة إلى ذلك، لوحظ أعلى معدل إيجابي لأنواع الكريبتوسبوريديم في مرضى سرطان القولون والمستقيم الذين يشربون مياه الآبار (73.33%)، بينما لوحظ في أفراد المجموعة الضابطة الذين يشربون مياه الجداول ليكون 42.86%. أخيرًا، كان معدل الانتشار في مرضى سرطان القولون بأنواع الكريبتوسبوريديم أعلى قليلاً (56.34%) مقارنة بمرضى سرطان المستقيم (52.54%). استنتجت هذه الدراسة أن العدوى بأنواع الكريبتوسبوريديم تحدث بشكل أعلى بكثير في مرضى سرطان القولون والمستقيم مقارنة بالمجموعة الضابطة.

الكلمات المفتاحية: داء الكريبتوسبوريديم، مرضى سرطان القولون والمستقيم، زيل–نيلسن المعدلة، تعز، اليمن.

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Abstract

Background: Cryptosporidiosis is a disease caused by *Cryptosporidium* species, the intracellular obligate protozoan parasites with worldwide distribution.

Aim: The present study aimed to determine the prevalence of *Cryptosporidium* spp. and some associated risk factors with infection in colorectal cancer patients and healthy controls.

Method: This study included 200 participants (130 colorectal cancer patients and 70 healthy individuals) attending Al-Amal Hospital for cancer patients in Taiz city-Yemen during the period from May 2022 to August 2023. Fresh stool specimens were collected and examined by permanent staining of faecal smears using Modified Ziehl-Neelsen staining technique for detection of *Cryptosporidium* oocysts. Demographic and possible risk factors such as age, gender, residence and source of drinking water were investigated in all the studied participants using a structured questionnaire.

Results: The prevalence of *Cryptosporidium* spp. among colorectal cancer patients was 54.62%. This prevalence was significantly higher compared to 10% of that prevalence among healthy individuals ($P < 0.001$). Also, the highest rate of infection was found in the age group of 18-38 years at 66.67% among colorectal cancer patients and 13.64% among healthy controls. Accordingly, the difference in the rates of *Cryptosporidium* spp. infection between the patients with colorectal cancer and control group is statistically high significant ($P = 0.005$). The rate of *Cryptosporidium* spp. infection was higher among male patients with colorectal cancer (58.21%) and among females in control group (14.71%). Both colorectal cancer and healthy participants coming from rural areas had a higher rates of infection with *Cryptosporidium* spp. (65.15%, 18.52%) respectively. In addition, the highest positive rate of *Cryptosporidium* spp. was observed in colorectal cancer patients drinking wells water (73.33%), while it was showed in healthy controls drinking stream water to be 42.86%. Finally, the colon cancer patients had slightly higher rate of *Cryptosporidium* spp. infection (56.34%) than patients with rectal cancer (52.54%).

Conclusion: The results of this study can be concluded that *Cryptosporidium* spp. infections occurred significantly higher in patients with colorectal cancer compared to controls.

Keywords: Cryptosporidiosis, Colorectal cancer patients, Modified Ziehl-Neelsen, Taiz, Yemen.

Introduction

Cryptosporidiosis is an important protozoan disease caused by intestinal protozoan parasites called *Cryptosporidium* species that is an obligate intracellular protozoan microorganism with worldwide distribution. It is a significant diarrheal disease for both people and animals worldwide. Several species of the protozoan parasite *Cryptosporidium* can cause this disease (Innes *et al*., 2020).

Currently, Cryptosporidium has 41 reported species with more than 60 valid genotypes. Amongst them, 21 species and genotypes have been identified in humans, out of which *C. parvum* and *C. hominis* are the most common pathogenic species, causing more than 90% of infections in humans (Holubová *et al.*, 2019).

Globally, diarrhea diseases have killed 1.6 million people in 2017. One third of these deaths were children under 5 years, and their highest mortality are from sub-Saharan Africa and South Asia. This was because of unsafe drinking water and poor sanitation (Dadonaite *et al.*, 2020).

Cryptosporidium oocysts can transmit through water, making it one of the most important causes of human and livestock infectious diarrhoea (Karanis *et al*., 2007; Baldursson and Karanis, 2011; Efstratiou *et al*., 2017). Contamination with *Cryptosporidium* oocysts has been identified as the leading cause of 905 worldwide waterborne outbreaks (Karanis *et al*., 2007; Rosado-García *et al*., 2017). In most cases, *Cryptosporidium* infection results in gastrointestinal problems such as severe diarrhoea in both immunocompromised and immunocompetent people (Current *et al*., 1983; Fayer and Ungar, 1986). In industrialized countries, *Cryptosporidium* causes diarrhea in 10.0% to 20.0% of people with acquired immunodeficiency syndrome (AIDS), while in developing countries, this rate is up to 50.0% (Gerace *et al.*, 2019).

Furthermore, *Cryptosporidium* has been reported to be responsible for more than 8 million cases/year of foodborne illness and 25 documented foodborne outbreaks (Ahmed and Karanis, 2018; Ryan *et al*., 2018). Weak institutional infrastructure, political conflict, inadequate water supplies, unclean water, underdevelopment, poverty and illiteracy, population density, high levels of malnutrition, social unrest, poor hygiene and sanitation, climate

change, and water crises have all been cited as negative factors that are promoting cryptosporidiosis and other diarrhea diseases (Aldeyarbi *et al*., 2016; Ahmed *et al*., 2018; GBD, 2018).

Cryptosporidium oocysts transmission can occur following direct or indirect contact with an infected host usually via the faecal–oral route. Person-to-person contact, zoonosis, and the consumption of contaminated food or water are well known mechanisms for faecal–oral transmission (Plutzer and Karanis, 2009; Efstratiou *et al*., 2017) with a significant risk of infection from the ingestion of a single oocyst (Messner and Berger, 2016). When the oocysts enter the gastrointestinal tract, the invasive stage of *Cryptosporidium* causes damage to the small intestinal epithelium. It disrupts the barrier function and absorption capability that leads to mild-to-severe diarrhoea and other abdominal symptoms. In immunocompetent adults, *Cryptosporidium* infection is usually asymptomatic or mild, which is generally self-limiting (Ahmed and Karanis, 2020). *Cryptosporidium* can survive in temperatures of -20˚C and in salt water (Carey *et al*., 2004).

In addition, children infected with *C. hominis* shed higher levels of oocysts because they have underdeveloped immune system and oocysts can proliferate easier, possibly contributing to the increased prevalence and spread of *C. hominis* within these communities (McLauchlin *et al.*, 2000; Xiao *et al*., 2001).

Cryptosporidium spp. infections have been suggested to be associated with several cancers, such as hematological malignancies, colorectal cancer, and liver cancer. It was postulated that *Cryptosporidium* spp. infections might be related to the development and progression of colon cancer via triggering colonic mucosal dysplasia (Taghipour *et al*., 2022).

Colorectal cancer is one of the most common malignant neoplasms in humans. Its incidence is the third highest among malignant neoplasms worldwide, and it accounts for 9.7% of all tumors (Ferlay *et al.*, 2010). Colorectal cancer arises from an accumulation of genetic and epigenetic changes that transform healthy epithelial cells into cancer cells. In addition to genetic factors, environmental factors contribute to the genesis of colorectal cancer, like red meat-rich diets, low physical activity, and chronic nicotine use (Kerr *et al*., 2017). There is also evidence that bacteria in intestinal flora

play a significant role in colorectal cancer development (Keku *et al.*, 2015). *Cryptosporidium* spp. have been suggested as important intestinal parasites in colorectal cancer patients and the severe form of these diseases occurs most frequently in such patients (Sulżyc-Bielicka *et al*., 2018).

Additionally, the team in Lebanon has discovered that *Cryptosporidium parvum*, a species frequently isolated from humans and animals, is able to induce digestive adenocarcinoma in a rodent model, being the first time that an eukaryotic microorganism has been associated with neoplastic changes in the digestive epithelium of a mammalian host (Certad *et al*., 2007; Certad *et al*., 2010; Benamrouz *et al*., 2012; Certad *et al*., 2012).

Consistently, some epidemiological studies have reported an association with cryptosporidiosis in patients with colorectal adenocarcinoma (Sulżyc-Bielicka *et al*., 2007; Shebl *et al*., 2012; Sulżyc-Bielicka *et al.*, 2012).

Osman *et al*. (2017), reported a high rate (21%) of *Cryptosporidium* infection was found in biopsies from Lebanese patients with recently diagnosed colonic neoplasia/adenocarcinoma before any treatment. These results provide new data about a potential role of this parasite in the development of colon adenocarcinoma.

Considering the prevalence of *Cryptosporidium* spp. infection, a few studies on this protozoan parasite were conducted in different regions of Yemen. The occurrences of *Cryptosporidium* spp. in Yemen have been evaluated among orphans (Al-Shibani *et al*., 2009), children (Al-Shamir *et al*., 2010), immunocompromised patients (Qasem *et al*., 2022), and among cancer and hemodialysis patients (Al-Shehari *et al*., 2023). However, to our knowledge there were no published studies on the *Cryptosporidium* spp. infections among the patients who suffer from colorectal cancer in Yemen. Accordingly, the present study is the first work performed in Yemen comparing the prevalence of *Cryptosporidium* spp. infections between colorectal cancer patients and healthy people. Therefore, the main aim of this study was to determine the prevalence of *Cryptosporidium* spp. and some associated risk factors with infection in the colorectal cancer patients compared to the healthy controls attending Al-Amal hospital cancer patients in Taiz city, Yemen.

Materials & Methods

Study design and study population

This is a cross-sectional descriptive study conducted at Al-Amal Hospital for cancer patients in Taiz city-Yemen from May, 2022 until August, 2023 in order to investigate the presence of *Cryptosporidium* infection in colorectal cancer patients, and to compare them with healthy individuals. In all, 200 participants were included in this study, consisted of 130 colorectal cancer patients (65%) and 70 apparently healthy individuals (35%) were selected as a control group. Oral consent was obtained from all the participants in the study, including adult participants and legal guardians of children who participated in the study before the investigation. All the participants filled out a questionnaire containing information about age, gender, residence and drinking water source.

Sample collection and examination

A fresh stool sample was collected from each participant in a clean, dry labeled plastic container. The collected stool samples were then transferred to the laboratory in the same Hospital for microscopic examination. For every sample, a direct microscopic examination of prepared smears applying normal saline and formalin ether concentration techniques was carried out. In addition, all slides were stained with a modified Ziehl-Neelsen (Modified Acid-Fast Stain) method for detecting *Cryptosporidium* oocysts. To investigate the presence of *Cryptosporidium* oocysts, the stained slides were examined at a 100x oil immersion lens under a light microscope.

Statistical analysis

The collected data were statistically analyzed by commercially available software package SPSS version 22 and Graph pad prism. The one way ANOVA and Pearson *Chi*-square (χ^2) or Fisher's exact test were used to assessing the differences between different groups. A *P*-value less than 0.05 was considered statistically significant.

Results

In this study, a total of 200 participants were tested in order to investigate the prevalence of *Cryptosporidium* spp. infection, the majority of them 130 (65%) were patients with colorectal cancer and 70 (35%) were healthy participants (control group). On the other hand, the colorectal cancer patients

group was consisted of 67 males and 63 females with the average age of 58.3±12.51 years. Also, the control group was included 36 males and 34 females, and has an average age of 45.8±14.5 years (Table 1). The detailed information of cancer patients and control individuals were shown in Table (1). A stool specimen was collected from each of them and microscopically examined by using modified Ziehl-Neelsen method to detection *Cryptosporidium* spp. oocysts. In thin stool smear, a *Cryptosporidium* spp. oocysts are shown at the front of the arrow with bright red color, under oil immersion lens (Figure 1). Out of the total (200) participants examined, 78 (39.0%) were found to be positive for *Cryptosporidium* spp. infection. On the other hand, the highest prevalence of *Cryptosporidium* spp. infection was 54.62% (71/130) among colorectal cancer patients compared with 10.0% (7/70) among the healthy individuals in the control group. This revealed a high significant difference (χ^2= 38.070; P < 0.001) in the prevalence of *Cryptosporidium* spp. infections among the participants in the colorectal cancer patients compared with the control group. Moreover, the patients with colorectal cancer also had a 10.8 times higher risk of *Cryptosporidium* infection than those in control group (OR = 10.831 with the confidence interval between 4.612 – 25.436) (Table 2). Furthermore, the prevalence rate of *Cryptosporidium* spp. infection was higher among the different age groups of colorectal cancer patients compared to controls, with highest prevalence was observed in the age group of 18-38 years in both colorectal cancer patients and controls (66.67% and 13.64%) respectively. There was high statistically significant in the prevalence of *Cryptosporidium* spp. infection among the different age groups in the colorectal cancer patients compared with healthy controls (χ^2= 17.028; P = 0.005); as shown in Table (3).

Table 1:

Socio-demographic characteristics of study participants (130 colorectal cancer patients and 70 healthy controls) attending Al-Amal hospital in Taiz city, Yemen from May 2022 to August 2023.

Characteristics		Colorectal cancer patients	Healthy controls
		No (%)	No (%)
Age		58.3±12.51*	45.8 ± 14.5*
Gender	Male	67 (51.5%)	36 (51.4%)
	Female	63 (48.5%)	34 (48.6%)
	Total	130 (100%)	70 (100%)
Water source	Treated water	21 (16.2%)	16 (22.9%)
	Well water	30 (23.1%)	18 (25.7%)
	Tank water	23 (17.7%)	18 (25.7%)
	Bottled water	12 (9.2%)	11 (15.7%)
	Streams water	44 (33.8%)	7 (10.0%)
	Total	130 (100%)	70 (100%)
Residence	Urban	64 (49.2%)	43 (61.4%)
	Rural	66 (50.8%)	27 (38.6%)
	Total	130 (100%)	70 (100%)
Site of cancer	Colon cancer	71 (54.6%)	-
	Rectum cancer	59 (45.4%)	-
	Total	130 (100%)	-

** Mean ± SD.*

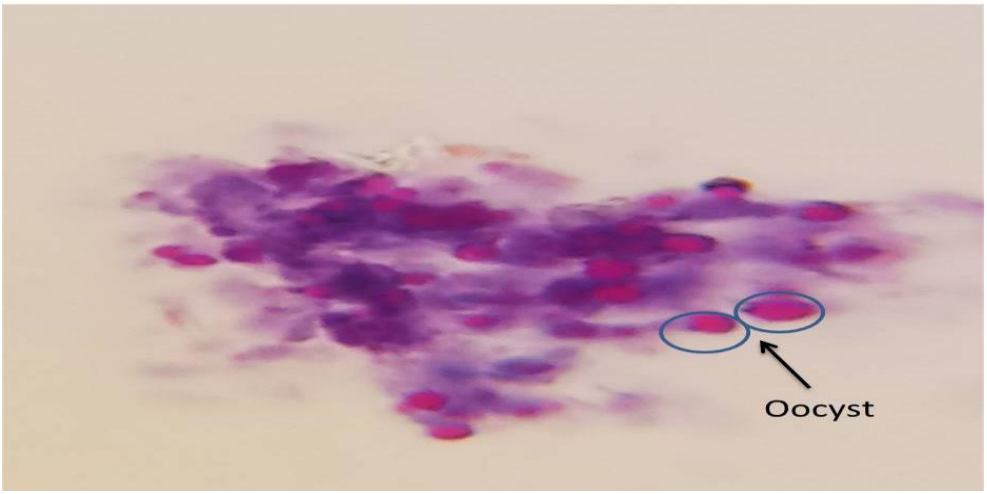

Figure 1: Photomicrograph of *Cryptosporidium* spp. showing Oocysts (Arrowhead), in thin smear of human feces stained with modified Ziehl Neelsen (100x).

Table 2:

Prevalence of Cryptosporidium spp. Among colorectal cancer patients and healthy controls.

Cryptosporidium spp.	Colorectal cancer patients	Healthy controls	Total	OR (95%CI)	χ^2	*P*-value
	No. (%)	No (%)	No (%)			
Positive	71 (54.62%)	7 (10.0%)	78 (39.0%)	10.831 (4.612 – 25.436)	38.070	0.000*
Negative	59 (45.4%)	63 (90.0%)	122 (61.0%)			
Total	130 (100%)	70 (100%)	200 (100%)			

****High significant difference compared with the healthy control people (χ^2= 38.070; P<0.001). OR= Odds ratio (10.831); CI= Confidence interval (4.612-25.436).***

Table 3:

Comparison of Cryptosporidium spp. infections between colorectal cancer patients and healthy controls regarding age.

Age group (years)	Positive *Cryptosporidium* spp.			χ^2	*P*- value
	Colorectal cancer patients	Healthy controls	Total		
	No +ve (%)	No +ve (%)	No +ve (%)		
18-38	2/3 (66.67%)	3/22 (13.64%)	5/25 (20%)	17.028	0.005*
39-58	34/63 (53.97%)	2/31 (6.45%)	36/94 (38.30%)		
>58	35/64 (54.69%)	2/17 (11.76%)	37/81 (45.68%)		
Total	71/130 (54.62%)	7/70 (10%)	78/200 (39%)		

**** High significant difference compared with the healthy control people (χ^2= 17.028; P = 0. 005).***

According to the gender, the positivity rate of *Cryptosporidium* spp. infection in males and females were 58.21% and 50.79% in colorectal cancer patients respectively, compared with 5.56% in males and 14.71% in females among the participants in the control group. Although the statistical results showed that the chance of exposure to *Cryptosporidium* spp. in the patients with colorectal cancer was 3 times higher than that in the control group (OR = 3.047 with the confidence interval between 0.554 and 16.765), but there was no significant difference in the prevalence of *Cryptosporidium* spp. infections among the participants in the colorectal cancer compared with control groups (χ^2= 1.775; *P*= 0.247); (Table 4).

As shown in Table (5), the infection rate of *Cryptosporidium* spp. was higher in the colorectal cancer patients were coming from rural areas (65.15%) than those inhabit urban area (43.75%). In comparison, the prevalence of *Cryptosporidium* spp. was 65.15% and 18.52% among colorectal cancer patients and healthy controls living in the rural areas, respectively; while in participants living in urban area, it was 43.75% and 4.65% among colorectal cancer patients and healthy controls, respectively. However, this difference was statistically not significant between the groups (χ^2= 0.318; *P*= 0.701).

Table (6) shows the relationship between water sources and the prevalence rate of *Cryptosporidium* spp. infection among colorectal cancer patients and healthy controls. The highest rate of *Cryptosporidium* spp. infection was observed in the colorectal cancer patients drinking from well water (73.33%) and 42.86% in healthy controls drinking from streams water. Accordingly, there was no significant difference (χ^2= 4.991; *P*= 0.230) in relation to the source of water and *Cryptosporidium* spp. infections between the participants in the colorectal cancer and control groups.

In relation to positivity of *Cryptosporidium* spp. infection and the site of cancer (in colon or rectum), the rate of *Cryptosporidium* spp. infection was slightly higher in colon cancer patients (56.34%) compared to 52.54% in rectum cancer patients. However, there was no significant difference between site of cancer and a positivity of *Cryptosporidium* spp. (OR (95%CI) = 1.16 (0.58 -2.33); χ^2 = 0.18; *P* =0.665), as shown in table (7).

Table 4:

Comparison of Cryptosporidium spp. infections between colorectal cancer patients and healthy control group according to the gender

2 Gender	Positive *Cryptosporidium* spp.			χ^2	OR (95%CI)	1 *P*-value
	Colorectal cancer patients	Healthy controls	Total			
	No +ve (%)	4 No +ve (%)	3 No +ve (%)			
8 Male	39/67 (58.21%)	2/36 (5.56%)	7 41/103 (39.81%)	6 1.775	3.047 (0.554-16.765)	5 0.247
9 Female	32/63 (50.79%)	5/34 (14.71%)	37/97 (38.14%)			
11 Total	71/130 (54.62%)	7/70 (10%)	10 78/200 (39%)			

Not significant difference (χ^2 = 1.775; P >0.05); OR= Odds ratio (3.047); CI= Confidence interval (0.554-16.756).

Table 5:

Comparison of Cryptosporidium spp. infections between colorectal cancer patients and healthy control group according to residency.

Residence	Positive *Cryptosporidium* spp.			χ^2	OR (95%CI)	*P*- value
	Colorectal cancer patients	Healthy controls	Total			
	No +ve (%)	No +ve (%)	No +ve (%)			
Rural	43/66 (65.15%)	5/27 (18.52%)	48/93 (51.61%)	0.318	0.614 (0.111-3.388)	0.701
Urban	28/64 (43.75%)	2/43 (4.65%)	30/107 (28.03%)			
Total	71/130 (54.62%)	7/70 (10%)	78/200 (39%)			

Not significant difference ($\chi 2 = 0.318$; P >0.05); OR= Odds ratio (0.614); CI= Confidence interval (0.111-3.388).

Table 6:

The association between drinking water sources and the positivity of Cryptosporidium spp. among colorectal cancer patients and healthy controls.

Source of drinking water	Positive *Cryptosporidium* spp.			χ^{2*}	*P*- value
	Colorectal cancer patients	Healthy controls	Total		
	No +ve (%)	No +ve (%)	No +ve (%)		
Treated water	14/21 (66.67%)	0/16 (0%)	14/37 (37.84%)	4.991	0.230
Well water	22/30 (73.33%)	1/18 (5.56%)	23/48 (47.92%)		
Tank water	6/23 (26.09%)	2/18 (11.11%)	8/41 (19.51%)		
Bottled water	5/12 (41.67%)	1/11 (9.09%)	6/23 (26.09%)		
Streams water	24/44 (54.55%)	3/7 (42.86%)	27/51 (52.94%)		
Total	71/130 (54.62%)	7/70 (10%)	78/200 (39%)		

Not significant difference ($\chi^2 = 4.991$; P>0.05); *: Fisher's exact test.

Table 7:

The association between the positivity of Cryptosporidium spp. and the site of cancer.

Cryptosporidium spp.	Site of cancer		Total	χ^2	OR (95%CI)	*P*- value
	Colon cancer	Rectum cancer				
	No (%)	No (%)	No (%)			
Positive	40 (56.34%)	31 (52.54%)	71 (54.62%)	0.187	1.165 (0.582 – 2.332)	0.665
Negative	31(43.66%)	28 (47.46%)	59 (45.38%)			
Total	71 (54.62%)	59 (45.38%)	130 (100%)			

Not significant difference (χ^2= 0.187; P>0.05; OR= 1.165; CI= 2.582-3.32).

Discussion

The present study revealed that the overall prevalence of *Cryptosporidium* spp. infection among all participants (colorectal cancer patients and healthy controls, together) was 39.0%. Whereas, the prevalence rate in colorectal cancer patients was 54.62%, while in healthy controls was 10.0%. From these results we can notice that colorectal cancer patients have higher infection with *Cryptosporidium* spp. Compared with the healthy controls. This difference is in agreement with results of other studies which revealed that high prevalence rate of *Cryptosporidium* infections among colorectal cancer patients were reported in each of Egypt (47.5% versus 5.0% of control group) by Abd El-Latif *et al*. (2023), in Saudi Arabia (70.3% vs. 17.30%) reported by Sanad *et al*. (2014) and in Iran (42.5% vs. 12.6%) reported by Ghanadi *et al*. (2022). Also, Sulżyc-Bielicka *et al*. (2007) reported that the frequency of *Cryptosporidium* spp. infections in colorectal cancer patients with or without diarrhea was 43.5% and 18%, respectively. High prevalence of Cryptosporidiosis in the present study may be due to lifestyle, health status, immunological condition, outdoor and indoor pollution of environment, personal hygiene habits, individual and community health education about communicable diseases, management of sewage water, drinking water, and economic status of poor families (Nasir *et al*., 2020). In addition to these, the geographical distribution and the parasitological techniques applied in each study could influence the outcome of results. These reasons rise the hypothesize that people with colorectal cancer are more exposed to *Cryptosporidium* spp. infection.

Other studies revealed disagreement in the prevalence rate between *Cryptosporidium* spp. infections in colorectal cancer and healthy controls. Sulżyc-Bielicka *et al*. (2018) in Poland, Osman *et al*. (2017) in Lebanon, and Zhang *et al*. (2020) in China reported low prevalence rate (13% vs. 4%), (21% vs. 7%) and (17.24% vs. 0.0%) respectively among colorectal cancer and non-colorectal cancer. These low rates of infection because of undergoing chemotherapy also may be due to environmental factors, sample size, adequate sanitation, clean water, suitable sewage treatment, high economic status and high level of health education.

In the current study, there is a statistically significant association between *Cryptosporidium* spp. infection and colorectal cancer, pathogenesis of *Cryptosporidium* spp. infections in colorectal cancer patients has not been defined clearly. However, this study may contributed to the belief that *Cryptosporidium* spp. infections are more prevalent among people with colorectal cancer, but it is not clear whether colorectal cancer have more susceptibility to this infection or *Cryptosporidium* spp. infections increases the susceptibility to colorectal cancer.

In the present findings of this study, the colorectal cancer patients were associated with a higher prevalence rate of infection with *Cryptosporidium* spp. among all the age groups compared with healthy controls, with highest prevalence was observed in the age group 18-38 and >58 years in both colorectal cancer patients and healthy controls (66.67% vs. 13.64% and 54.69% vs. 11.76) respectively, the exact reason for this finding is unclear. Statistical analysis showed high significant differences in the positivity of *Cryptosporidium* spp. between the age groups in colorectal cancer patients compared with healthy controls. This study is consistent with other studies conducted on cancer patients in Sulaimani Province, Iraq by Nasir *et al*. (2020) and in Ibb city, Yemen by Al-Shehari *et al*. (2023). In Isfahan province, central Iran, Mohaghegh *et al*. (2017) showed high prevalence rate of *Cryptosporidium* spp. infection among young ages of hemodialysis patients.

Regarding gender, this study reveals no statistically significant association between gender and infection with cryptosporidiosis for both colorectal cancer patients and control, the risk of *Cryptosporidium* spp. infection increased 3 times in the patients with colorectal cancer than that in the control group (OR: 3.047; CI: 0.554 – 16.765; $P = 0.247$). Also among the colorectal

cancer patients, the rate of infection with *Cryptosporidium* spp. in males (58.21%) was higher than 50.79% in females. This is in agreement with that was observed among the colorectal cancer patients in Poland, the prevalence rate was 61.1% in males and 38.9% in females (Sulżyc-Bielicka *et al*., 2018). Also, among cancer patients, in Isfahan province, central Iran, the prevalence of *Cryptosporidium* spp. infection was higher in males (9.6%) than in females (2.2%), which was statistically significant (Pestechian *et al*., 2022). In Egypt, it also was 67.5% in males and 32.5% in females (Abd El-Latif *et al.*, 2023). The males obtained higher prevalence of *Cryptosporidium* infection because they most prone to parasite exposure compare to females due to the nature of men's lifestyle such as type of occupation and work environment (Mohaghegh *et al*., 2017 & Sulżyc-Bielicki *et al*., 2018). In contrast, the results of Sherchand and Shrestha (1996) in Nepal were strongly disagreed with the current study, they reported a high prevalence rate in females than in males for all age groups, they explained this high rate of cryptosporidiosis among females due to close contact of the females with children than males, and females often spend more time with child care (Sherchand and Shrestha, 1996).

In this study, there is a statistically no significant association between *Cryptosporidium* spp. infection and residence among colorectal cancer patients compared with healthy individuals. The higher prevalence rates were found in colorectal cancer patients and healthy individuals coming from the rural areas (65.15% vs. 18.52% respectively), while lower prevalence rates were recorded in an urban area (43.75% vs. 4.65%) among patients with colorectal cancer and control individuals, respectively. These results were in agreement with the previous studies that have been recorded the high rate of *Cryptosporidium* infection among cases coming from the rural areas (Al-Shamiri *et al*., 2010; Mohaghegh *et al*., 2017; Qasem *et al*., 2022 and Abd El-Latif *et al*., 2023 and Al-Shehari *et al*., 2023). The high prevalence of *Cryptosporidium* parasite in rural areas could be due to the social habits of the rural people in which keep the animals in their houses (Al-Shamiri *et al*., 2010) and many factors such as lack of hygienic practices, agriculture background, lack of clean drinking water, and contact directly with domestic animals (Qasem *et al*., 2022).

The present study showed no significant association between water sources and infections with *Cryptosporidium* spp. among colorectal cancer patients and control individuals. However, the highest prevalence rate of

Cryptosporidium spp. (73.33%) was in the patients with colorectal cancer whose source of water for drinking was wells, while the lowest prevalence rate (26.09%) was in those who relied on tanks water. This could be attributed to high level of contamination of wells water by the infective oocysts of this parasite resulting from human and domestic animals feces during the rainy season. These results are in agreement with studies conducted by Izadi *et al*. (2012) in Isfahan city, Isfahan province, central Iran, Alyousefi *et al*. (2013) in Yemen, Kipchirchir (2016) in Kenya and Al-Shehari *et al*. (2023) in Ibb city, Yemen. On the other hand, The current study is disagreement with study conducted in Taiz, Yemen by Al-Shamiri *et al*. (2010) and also another study conducted in Ibb city, Yemen by Qasem *et al*. (2022). The reason for this difference may be attributed to the variance of diagnostic methods and studied community.

In the current study, the prevalence of *Cryptosporidium* spp. infection was slightly higher among the patients with colon (56.34%) compared with 52.54% among patients with rectal cancer, this difference was not statistically significant. Similar conducted by Sulżyc-Bielicka *et al*. (2012), they reported the prevalence of *Cryptosporidium* spp. infection was twice higher in patients with rectal cancer (16.7%) as compared with those with colon cancer (8.9%), although this difference was not statistically significant. However, they attributed these results to the small size of the studied group.

Conclusion

In conclusion, the results in the present study showed that the colorectal cancer patients are at a higher risk for *Cryptosporidium* spp. infection in comparison with the healthy individuals in Taiz, Yemen. This study also found higher rates of *Cryptosporidium* spp. infections and highly significant difference in the age groups among colorectal cancer patients compared with healthy controls. Among patients with colorectal cancer, the *Cryptosporidium* spp. infection was not associated with gender, although the prevalence of this parasitic infection was higher in males than in females. On the other hand, residency in rural areas and using wells water for drinking might be considered as a risk factor for cryptosporidiosis among colorectal cancer patients. A further molecular investigation is required for species and molecular characterization of *Cryptosporidium*.

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