

COCCIDIAN INTESTINAL PARASITES AMONG CHILDREN IN AL-TORBAH CITY IN YEMEN: IN COUNTRY WITH HIGH INCIDENCE OF MALNUTRITION**ABSTRACT**

Diarrhoea is an important cause of malnutrition, morbidity and mortality among children in Yemen. *Coccidian* parasitic infections are an important cause of diarrhea in children particularly malnutrition and immune-compromised patients, but their investigations are rarely required by the treating physicians in apparently immunocompetent children. This study was aimed to find the prevalence of intestinal *coccidian* parasites in country with high incidence rate of malnutrition. Between May 2016 and October 2016, 228 fecal samples from 228 selected school children in Al Turbah city, Taiz governorate, Yemen, aged between 6 and 15 years were examined using wet-mount preparations and formal concentration method then films stained by modified acid-fast staining. Also data of children were collected including demographic data, and sources of water. Findings of positive intestinal *coccidian* parasites were analyzed in relation with demographic data, and sources of water. The prevalence of *Cryptosporidium* species, *Cyclospora* species and *Isospora belli* were 75.9%, 45.6% and 1.75% respectively. There was significant association between positive of *Cryptosporidium* species and females ($OR= 2.1$ times, $P=0.01$), and spring water source ($OR=4$ times, $P=0.04$), while there was no significant association between positive of *Cryptosporidium* species and others factors studied. Also there was no significant association between positive of *Cyclospora* species and *Isospora belli* and children sex, age groups, or different sources of water. In conclusion the study highlights the high prevalence of *coccidian* parasites among immunocompetent school children in Yemen. The clinicians in Yemen need to be aware that *coccidian* parasites are a potential cause of childhood diarrhea even in immunocompetent children.

KEYWORDS: *Coccidian*, *Cryptosporidium*, *Cyclospora*, *Isospora*, children, Yemen

INTRODUCTION

Coccidian is a primary microscopic parasite that infects the intestinal system of most human and animal organisms. These organisms are one of the main concerns of doctors, especially with increasing the rate of HIV. *Coccidian* parasites (*Cryptosporidium* spp., *Isospora belli*, and *Cyclospora* spp.) are the most common intestinal parasites in immunocompromised patients that can usually lead to fatal acute diarrhea while causing moderate and limited gastrointestinal disorders in individuals with a natural immune system.^{1,2,3} *Cryptosporidium* and *Isospora* have been identified as important and widespread causes of diarrheal disease in both immunocompromised individuals and those with immunodeficiency causes such as acute malnutrition as well as people who move from a clean environment to a contaminated environment such as tourists so it has been described as traveler disease.^{4,5} Acute or chronic diarrhea syndromes caused by these parasites are usually accompanied by weight loss, dehydration, abdominal pain and malabsorption syndrome in immunocompromised patients.⁶ Chronic diarrhea in malnourished children can also increase the incidence of these parasites and mortality in these patients.⁶ *Coccidiosis* diarrhea has been observed with fluid loss of 25 l / day in infected patients, which can last for weeks in immunocompromised patients.⁷

Isospora belli is one of the opportunistic *coccidian* parasites that affects HIV⁺/AIDS patients, especially in developing countries of Africa, Asia, and Latin America with low levels of hygiene. It is always considered as a neglected parasite and there is lack of enough investigation, particularly in immune-compromised patients, so the previous factors lead to underestimate this infection.⁸ Various risk factors for *coccidian* parasites such as use of contaminated drinking water, exposure to animals, lack of sewage, poverty, etc., have been reported to be associated with *coccidian* gastrointestinal infections.⁹ In Yemen, the prevalence of *coccidian* parasites has not been studied, not among immunocompromised patients as well as children with diarrhea or malnutrition in which the malnutrition rate among children in Yemen raised due

to the Saudi-Emirati aggression against Yemen.¹⁰ Therefore, it may not be out of context to suggest that identification of these so-called opportunistic pathogens should be an essential part of investigations for childhood diarrhea, particularly with the rise of malnutrition rate among children in Yemen which affect the immune status of the children. With this aim, this study was conducted to find the prevalence of intestinal *coccidian* parasites among children in country with high incidence of malnutrition and determine some risk factors associated with this infection in Al Turbah city, Taiz governorate, Yemen.

SUBJECTS AND METHODS

The study conducted in the Faculty of Sciences, Taiz University, Taiz city, Yemen. Between May 2016 and October 2016, 228 fecal samples from 228 selected school children in Al Turbah city, Taiz governorate, aged between 6 and 15 years were examined. As a standard protocol, after receiving the samples in the laboratory, stool samples were processed and wet-mount preparations with both saline and iodine were prepared and screened within 2 h of sample collection to look for motile Trophozoites, larvae, ova, and cyst. Stool samples were also processed by the formalin-ether concentration technique and reexamined with saline and iodine wet-mount preparations as well as stained with Modified acid-fast stain³ to look for *Cryptosporidium*, *Cyclospora*, and *Isospora* oocysts. Each wet-mount preparation and stained fecal smears were examined by a clinical microbiologist and the researcher (ENAS) independently and findings were cross-checked. After those children demographic data and sources of water were collected in standard questionnaire. Next, findings of positive intestinal *coccidian* parasites were analyzed in relation with demographic data, and sources of water.

RESULTS

The study results illustrated in tables 1 to 7. The prevalence of *Cryptosporidium* spp, *Cyclospora* spp and *Isospora belli* were 75.9%, 45.6% and 1.75% respectively. There was significant association between positive of *Cryptosporidium* species and females ($OR= 2.1$ times, $CI=1.1-3.9$, $P=0.01$), and spring water source ($OR=4$ times, $CI=1.0-17$ $P=0.04$), while there was no significant association between positive of *Cryptosporidium* spp and others factors studied. Also there was no significant association between positive of *Cyclospora* spp, *Isospora belli* and children sex, age groups, or different sources of water.

DISCUSSION

Cryptosporidium, *Isospora* and *Cyclospora* have become increasingly prevalent in patients with immune deficiency and normal immunity people. Humans can infect *Coccidian* infections through fecal-oral route, through direct person-to-person or animal-to-person contact in addition to consuming contaminated water or food¹¹ while no animal reservoir for human *Isospora* has been identified.¹² In the current study the prevalence of *Cryptosporidium* spp was 75.9%, while *Cyclospora* spp was next commonest *coccidian* pathogen (45.6%). The results of this study are higher than that reported in general population of developing and developed countries in which the rate of *Cryptosporidium* oocysts was recorded from 6.1 and 2.1%, respectively.¹³ Also the current study rate of *Cryptosporidium* spp (75.9%) was even higher than the prevalence rates of *Cryptosporidiosis* among HIV⁺/AIDS diarrheic patients which ranged from 10% to 33.4%^{13,14} or among diarrheic children with normal immunity (7%).¹⁵ In the current study, there was significant association between positive of *Cryptosporidium* species and females ($OR= 2.1$ times, $CI=1.1-3.9$, $P=0.01$) (table 2). The current result is different from other studies carried out in developed and developing countries in which the rate of *Cryptosporidium* species infections are roughly equal in both sexes.^{13,15,16}

Although animals are known to be the potential source of *Cryptosporidiosis*, aquatic sources are also known as one of the major sources of *Cryptosporidium*.¹³ In the current study, there was a significant correlation between positive *Cryptosporidium* species and the source of spring water ($OR = 4$ times, $CI = 1.0-17$, $P = 0.04$) (Table 3). This association can be explained by that *Cryptosporidium* spp. which can be found in surface water and groundwater resources through fecal contamination, which can affect drinking water resources.¹⁷ Interestingly, *Cryptosporidium* oocysts are able to pass through the water treatment process because of their resistance to routine disinfectants and their small size.¹⁸

The prevalence of *Isospora* spp in the current study was very low (1.75%) comparing with high rates of *Cryptosporidium* spp (75.9%) and *Cyclospora* spp (45.6%). Although, *Isosporiasis* has worldwide distribution especially in tropical and subtropical regions, but there are rare reports of this infection.¹⁹ *Isospora belli* is considered as an opportunistic infection in immunocompromised individuals, mainly AIDS patients, all over the world. Moreover, *Isosporiasis* has been reported as the most prevalent intestinal parasitic disease among AIDS patients.^{20,21} Assis and colleagues reported the frequency rate 10.1% and 6.7% in HIV-positive patients for *Cryptosporidium* spp and *Isospora belli*, respectively.²² In another study, the prevalence rate of *Isosporiasis* in Nigeria was reported 3.1% in HIV-positive patients while no *Isospora* infection was observed in the healthy controls.¹⁹ As well, the current study finding is in agreement with other studies that have suggested low prevalence rate of *Isosporiasis* in immunocompetent or immunocompromised patients. However, treatment of *Isosporiasis* usually is successful in all types of cases but recurrence cases are common.²⁰ It is known that detection of *Isospora* in direct examination of stool samples in most of laboratories is unusual. Alternatively, cases of *Isosporiasis* has being raised up together with increase of HIV-infected subjects that can increase gastrointestinal complications in immunocompromised patients. *Isosporiasis* is generally transmitted through ingestion of sporulated oocysts from contaminated food and water.¹² Although some cases of homosexuality have been reported to have *Isosporiasis* more than other individuals²³, but because of the fact that *Isospora* oocysts require to mature and become infectious in the environment, direct contact with faeces is unlikely to be the usual course of transmission.²⁴ Therefore, sanitation for water and food is very important in prevention programs. However, as explained, although reports of *Isosporiasis* cases are low, but this infection should be considered a neglected disease in Yemen, especially in people with immune disorders.

The results of this study highlight the fact that *coccidian* parasites should not be overlooked by the clinical microbiologists, while investigating cases of diarrhea, even if these are not requested by the physician. The fact that 75.9% of *Cryptosporidium* cases were seen among immunocompetent cases shows the existence of this pathogen even in non-immunocompromised pediatric population. *Cryptosporidium* is an important etiological agent and its diagnosis is of utmost importance as this is a useful guide for the prompt treatment of such cases. More importantly, it can be diagnosed by relatively simple and inexpensive techniques such as acid-fast staining, which can also help avoid invasive procedures such as colonoscopy and intestinal biopsies in cases of persistent diarrhea.^{25,26} We would also like to point out the fact that the data we have presented here shows the actual burden of this parasitic infection and perhaps less than the real one due to parasitic oocysts are shed intermittently, which may not necessarily correspond to periods of clinical symptoms. Correspondingly, oocysts may not be eliminated during the first stage of infection when the asexual stage of the life cycle predominates and clinical symptoms become apparent.²⁷

CONCLUSION

The study highlights the high prevalence of *coccidian* parasites among immunocompetent school children in Yemen. The clinicians in Yemen need to be aware that *coccidian* parasites are a potential cause of childhood diarrhea even in immunocompetent children.

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CONFLICT OF INTEREST

"No conflict of interest associated with this work".

REFERENCES

- 1- Bera P, Das S, Saha R, Ramachandran VG, Shah D. *Cryptosporidium* in children with diarrhea: A hospital-based study. Indian Pediatr. 2014;51:906–8.
- 2- Kaur R, Rawat D, Kakkar M, Uppal B, Sharma VK. Intestinal parasites in children with diarrhea in Delhi, India. Southeast Asian J Trop Med Public Health. 2002;33:725-9.

- 3- Gupta S, Narang S, Nunavath V, Singh S. Chronic diarrhoea in HIV patients: prevalence of *coccidian* parasites. Indian J Med Microbiol. 2008;26(2):172-5.
- 4- Ryan ET, Wilson ME, Kain KC. Illness after international travel. N Engl J Med. 2002; 347(7):505-16.
- 5- Ud Din N, Torka P, Hutchison RE, Riddell SW, Wright J, Gajra A. Severe *Isospora* (*Cystoisospora*) *belli* diarrhea preceding the diagnosis of human T-cell-leukemia-virus-1-associated T-cell lymphoma. Case Rep Infect Dis. 2012; 2012:640104 .
- 6- de Oliveira-Silva MB1, de Oliveira LR, Resende JC, Peghini BC, Ramirez LE, Lages-Silva E, Correia D. Seasonal profile and level of CD4+ lymphocytes in the occurrence of *cryptosporidiosis* and *cystoisosporidiosis* in HIV/AIDS patients in the Triângulo Mineiro region, Brazil. Rev Soc Bras Med Trop. 2007; 40(5):512-5.
- 7- Baqai R, Anwar S, Kazmi SU. Detection of *Cryptosporidium* in immunosuppressed patients. J Ayub Med Coll Abbottabad. 2005; 17(3):38-40.
- 8- Certad G, Arenas-Pinto A, Pocaterra L, Ferrara G, Castro J, Bello A, *et al.* *Isosporiasis* in Venezuelan adults infected with human immunodeficiency virus: clinical characterization. Am J Trop Med Hyg. 2003; 69(2):217-22.
- 9- Dwivedi KK, Prasad G, Saini S, Mahajan S, Lal S, Baveja UK. Enteric opportunistic parasites among HIV infected individuals: associated risk factors and immune status. Jpn J Infect Dis. 2007; 60(2–3):76-81.
- 10- UNICEF. High Incidence rate of Severe Acute Malnutrition (SAM) among children in Yemen. Available from: <https://www.unicef.org/.../malnutrition-amongst-children-yemen-all-t>. Last Accessed 2019 August 11.
- 11- Wang L, Xiao L, Duan L, Ye J, Guo Y, Guo M, *et al.* Concurrent infections of *Giardia duodenalis*, *Enterocytozoon bieneusi*, and *Clostridium difficile* in children during a cryptosporidiosis outbreak in a pediatric hospital in China. PLoS Negl Trop Dis. 2013; 7(9): e2437.
- 12- Rodriguez-Morales AJ, Castañeda Hernandez DM. Protozoa: *Cystoisospora belli* (Syn. *Isospora belli*). In: Encyclopedia of Food Safety; Editors: Motarjemi Y, Moy GG, Todd ECD, editors. Academic Press; 2014:45-8.
- 13- Lee JK, Song HJ, Yu JR. Prevalence of diarrhea caused by *Cryptosporidium parvum* in non-HIV patients in Jeollanam-do, Korea. Korean J Parasitol. 2005; 43(3):111-4.
- 14- Nahrevanian H, Assmar M. *Cryptosporidiosis* in immunocompromised patients in the Islamic Republic of Iran. J Microbiol Immunol Infect. 2008;41(1):74-7.
- 15- Hamedi Y, Safa O, Haidari M. *Cryptosporidium* infection in diarrheic children in southeastern Iran. Pediatr Infect Dis J. 2005; 24(1):86-8.
- 16- Sangani GS, Mirjalali H, Farnia S, and Rezaeian M. Prevalence of intestinal *coccidial* infections among different groups of immunocompromised patients. Iran J Parasitol 2016;11(3):332-338.
- 17- Peletz R, Mahin T, Elliott M, Montgomery M, Clasen T. Preventing *cryptosporidiosis*: the need for safe drinking water. Bull World Health Organ. 2013; 91(4):238-8A.
- 18- Cho EJ, Yang JY, Lee ES, Kim SC, Cha SY, Kim ST, *et al.* A Waterborne Outbreak and Detection of *Cryptosporidium Oocysts* in Drinking Water of an Older High-Rise Apartment Complex in Seoul. Korean J Parasitol. 2013; 51(4):461-6.
- 19- Olusegun AF, Okaka CE, Luiz Dantas Machado R. *Isosporiasis* in HIV/AIDS Patients in Edo state, Nigeria. Malays J Med Sci. 2009; 16(3):41-44.
- 20- Lindsay DS, Dubey J, Blagburn BL. Biology of *Isospora* spp. from humans, nonhuman primates, and domestic animals. Clin Microbiol Rev. 1997; 10(1):19-34.
- 21- Kumar SS, Ananthan S, Lakshmi P. Intestinal parasitic infection in HIV infected patients with diarrhoea in Chennai. Indian J Med Microbiol. 2002;20(2):88-91.
- 22- Assis DC, Resende DV, Cabrine-Santos M, Correia D, Oliveira-Silva MB. Prevalence and genetic characterization of *Cryptosporidium* spp. and *Cystoisospora belli* in HIV-infected patients. Rev Inst Med Trop Sao Paulo. 2013; 55(3):S0036-46652013000300149.
- 23- Forthal DN, Guest SS. *Isospora belli* enteritis in three homosexual men. Am J Trop Med Hyg. 1984; 33(6):1060-4.
- 24- Lumb R, Hardiman R. *Isospora belli* infection. A report of two cases in patients with AIDS. Med J Australia. 1991; 155(3):194-6.

25- Fathy MM, Abdelrazek NM, Hassan FA, El-Badry AA. Molecular copro-prevalence of *Cryptosporidium* in Egyptian children and evaluation of three diagnostic methods. Indian Pediatr. 2014;51:727-9.

26- Shoaib S, Tauheed S, Hafiz A. Frequency of *Cryptosporidium* in childhood diarrhoea – Importance of modified acid fast technique. J Ayub Med Coll Abbottabad. 2003;15:3-5.

27- Mirdha BR, Kabra SK, Samantray JC. *Isosporiasis* in children. Indian Pediatr 2002;39:941-4.

Table 1: The prevalence of *coccidian* intestinal parasites among 228 children in Al-Torbah city in Yemen.

<i>Coccidian</i> parasites	Number	percentage
<i>Cryptosporidium</i> species	173	75.9
<i>Cyclospora</i> species	104	45.6
<i>Isospora belli</i>	4	1.75

Table 2. The prevalence rate of *Cryptosporidium* species in different sex and age of tested children.

Characters	Positive <i>Cryp spp</i>		OR	95%CI	χ^2	P
	No	%				
Sex						
Male n=109	75	68.8	0.4	0.22-0.8	5.7	0.01
Female n=119	98	83.3	2.1	1.1-3.9	5.7	0.01
Age groups						
6-10 years n=92	72	78.2	1.2	0.6-2.3	0.47	0.48
11-15 years n=136	101	74.3	0.8	0.4-1.5	0.47	0.48

Table 3. The association between *Cryptosporidium* species infections and income and water sources for tested children.

Characters	Positive <i>Cryp spp</i>		OR	95%CI	χ^2	P
	No	%				
Income						
Low n=83	63	75.9	1.0	0.5-1.8	0.0	0.99
Moderate n=145	110	75.8	0.99	0.5-1.8	0.0	0.99
Water sources						
Wells n=7	6	85.7	1.9	0.2-16.4	0.3	0.53
Subterranean water n=113	87	77	1.1	0.6-2	0.15	0.69
Rain n=41	33	80.5	1.3	0.5-32	0.5	0.44
Springs n=25	23	92	4	1.0-17	4	0.04
Treatment water n=7	5	71.4	0.78	0.14-4.1	0.07	0.77
wells+ subterranean water n=15	9	60	0.4	0.1-1.3	2.2	0.13
rain+ subterranean water n=20	10	50	0.27	0.1-0.7	8	0.004

Table 4. The prevalence rate of *Cyclospora* species in different sex and age of tested children.

Characters	Positive <i>Cyclospora spp</i>		OR	95%CI	χ^2	P
	No	%				
Sex						
Male n=109	56	51.4	1.5	0.9-2.6	2.7	0.09
Female n=119	48	40.3	0.6	0.3-1.0	2.7	0.09
Age groups						
6-10 years n=92	44	47.8	1.16	0.6-1.9	0.3	0.58
11-15 years n=136	60	44.1	0.8	0.5-1.4	0.3	0.58

Table 5. The association between *Cyclospora* infections and income and water sources for tested children.

Characters	Positive <i>Cyclospora</i>		OR	95%CI	χ^2	P
	No	%				
Income						
Low n=83	42	50.6	1.37	0.79-2.3	1.3	0.25
Moderate n=145	62	42.8	0.7	0.4-1.25	1.3	0.25
Water sources						
Wells n=7	3	42.8	0.8	0.19-4	0.02	0.88
Subterranean water n=113	53	46.9	1.1	0.65-1.8	0.15	0.69
Rain n=41	16	39	0.72	0.3-1.4	0.87	0.34
Springs n=25	11	44	0.9	0.4-2.1	0.02	0.86
Treatment water n=7	1	14.3	0.19	0.02-1.6	2.8	0.09
wells+ subterranean water n=15	9	60	1.7	0.6-5.1	1.17	0.29
rain+ subterranean water n=20	11	55	1.5	0.6-3.8	0.7	0.37

Table 6. The prevalence rate of *Isoospora belli* in different sex and age of tested children.

Characters	Positive <i>Isoospora belli</i>		OR	95%CI	χ^2	P
	No	%				
Sex						
Male n=109	2	1.8	1.1	0.15-7.8	0.007	0.9
Female n=119	2	1.7	0.9	0.12-6	0.007	0.9
Age groups						
6-10 years n=92	2	2.2	1.5	0.2-10	0.15	0.69
11-15 years n=136	2	1.47	0.6	0.009-4.8	0.15	0.69

Table 7. The association between *Isoospora belli* infections and income and water sources for tested children.

Characters	Positive <i>Isoospora belli</i>		OR	95%CI	χ^2	P
	No	%				
Income						
Low n=83	2	2.4	1.7	0.2-12.7	0.32	0.52
Moderate n=145	2	1.4	0.5	0.07-4	0.32	0.52
Water sources						
Wells n=7	0	0	0.0	undefined	0.12	0.74
Subterranean water n=113	2	1.76	1	0.14-7.3	0.003	0.98
Rain n=41	1	2.4	1.5	0.1-15	0.1	0.7
Springs n=25	1	4	2.7	0.27-27	0.8	0.36
Treatment water n=7	0	0	0.0	undefined	0.8	0.36
wells+ subterranean water n=15	0	0	0.0	undefined	0.28	0.59
Rain+ subterranean water n=20	0	0	0.0	undefined	0.39	0.53