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Incidence of Intestinal Coccidian Sporozoa Infection in Patients with diarrhea Attended to Al-Diwaniyah Teaching Hospital at Al-Qadisiyah Province / Iraq

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Abstract

The present study was conducted from April 2018 to May 2019 at Al-Diwaniyah teaching hospital (Al-Qadisiyah province/Iraq) to investigate the incidence of coccidian protozoa in patients suffering from gastrointestinal symptoms using conventional classical methods and staining technique. Stool samples were collected from 1136 patients to detect the coccidian sporozoa. The result shows overall of the patients, sporozoa infectionswere 470/ 1136(41.3 %)were positives. Wet amount and staining method were used to detect the intestinal coccidian oocysts and the results show that *Cryptosporidium parvum*was 237/1136 (20.8%), *Isospora belli* was128/ 1136 (11.2%) and *Cyclosporacayetanensis*was 105/1136 (9.2%).Concerning to social factors (age, gender, residence and other factor effect on the rate of the infection such as alcoholism, diabetes patients, chemotherapy patient) were recorded , significant elevation ($P<0.05$) were obtain statistically in groups.

Keywords: Intestinalcoccidian, Patient, Iraq, Al-Diwaniyah, Al-Qadisiyah.

Introduction :

Coccidian protozoa are one of the largest public health problems in both developing and developed countries, which produce many problems in the human body especially digestive system resulting in significant morbidities and mortalities (1,2).In developed countries, coccidian protozoa tend to be a neglected parasite among gastrointestinal infections compared to helminthes and other infectious agent (3). The intestinal coccidian diseases reported in humans include *Cryptosporidium parvum* ,*Isospora belli* and *Cyclosporacayetanensis*(4). Although, many countries joined under WHO have pledged to address the problem in group effort with multinational pharmaceutical companies who have committed to provide antiparasitic drugs free of charge or at reduced cost (5).

Several environmental, social, geographical and other factors can affect on distribution of intestinal coccidian sporozoa (6,7,8) . In addition, parasitic infections can inflict on health and social problems such as drinking of alcohol, small age, old age, chemotherapy patients and infection with other disease affected on the immune system such as diabetic patients(9). Chronic diarrhea with other gastrointestinal disorders such as abdominal pain, fatigue espiratory infections, which conceived that parasitic infections are the main pathogens to be considered (10).

Today, a classical methods are still more applicable at field because as they simple, fast and cheap (11). In Iraq, many studies have shown that the prevalence of intestinal parasites among different regions is still wide spread (12). Therefore, the present study aims to investigate the incidence of intestinal coccidian sporozoa and diagnose the species of them among diarrheal patients in Al-

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Qadisiyah province; with detection the association of infection rates to social factors among infected patients.

Materials and methods:

Samples: This study was conducted during the period from April 2018 to May 1019 at Al-Diwaniyah teaching hospital (Al-Qadisiyah province / Iraq). Of different ages, sexes, and residence; a total of 1136 diarrheal patients were collected with a history abdominal pain and/or mixed with blood.Fecal samples were collected into disposable plastic containers that labeled with certain number and adding 10% of formalin solution as conservation solution . Related data concerned to age, gender, residence and other factors such as drinking of alcohol, history of diabetes and chemotherapy were recorded.

Diagnostic methods: In this study fecal samples were examined at laboratory to detect the presence of intestinal coccidian oocysts using classical conventional methods such as a direct saline/Iodine wet mount (13) and Acid fast stain /Modified Ziehl-Neelsen stain method (14).

Results:

The results of this study showed that a total of 1136 stool samples included in, out of which 470 microscopically infected with intestinal coccidian sporozoa. Wet amount and staining method were used to detect the intestinal coccidian and the results shows protozoon's oocysts in *Cryptosporidium parvum* was 237/1136 (20.8%), *Isospora belli* was 128/ 1136 (11.2%) and *Cyclosporacayetanensis*was 105/1136 (9.2%). Table 1; figer 1

Table 1: Number and percent of cases according to type of parasitic infestation

Parasite	Number	Percentage	P value
Cryptosporidium parvum	237	20.8%	< 0.005
Isospora belli	128	11.2%	
Cyclosporacayetanensis	105	9.2%	
Total	470	41.3%	

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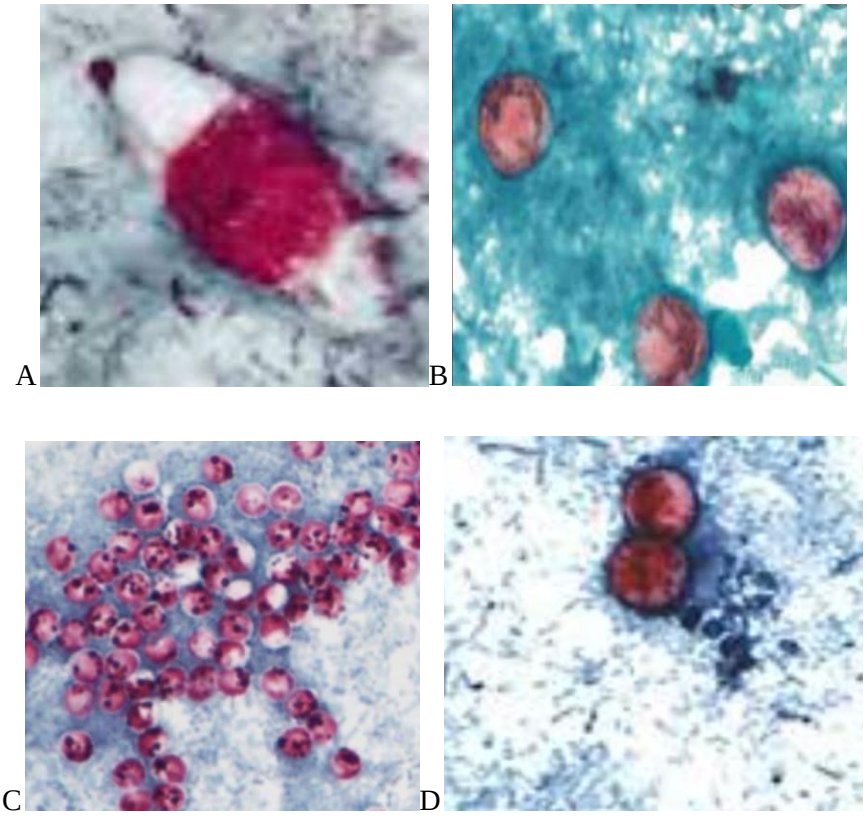

Figure 1 :(A)*Isospora belli*unsporulatedoocysts(B) *Isospora belli*sporulated oocysts (C) *Cryptosporidium parvum*oocysts (D)*Cyclosporacayetanensis* oocysts.Modified acid-fast stain.X100

Related to the gender, male patients were significantly more liable for intestinalcoccidian infection, in whom the number of infected patients with *C .parvum* and *I. beli* were 176/237 and 89/128 respectivelywhile in female patients wasmore liable for *C. cayetansis* and the microscope was recorded a number of this parasite 77/105. Table 2.

Table2:Number and percent of cases according to type of parasitic infection based on gender

Characteristic	Parasitic infestation	Gender				P
		Male	percent	Female	Percent	
Cryptosporidium parvum	237	176	74.2%	61	25%	< 0.005 *
Isospora belli	128	89	69.5%	39	30.4%	
Cyclosporacayetanensis	105	28	26.6%	77	73.3%	
Total	470	293	62.3%	177	37.6%	

Data were expressed as either mean standard error of mean or number (%); * significant at P ≤ 0.05; ** highly significant at P ≤ 0.01

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Also the patients living in rural area were highly significantly more percent to parasitic infection than those from urban areas. Table 3

Table3 :Number and percent of cases according to type of parasitic infection based on residency

Characteristic	Parasitic infestation	Residency				P
		Rural	Percent	Urban	Percent	
Cryptosporidium parvum	237	196	82.7%	41	17.2%	< 0.001 **
Isospora belli	128	94	73.4%	34	26.5%	
Cyclosporacayetanensis	105	91	86.6%	14	13.3%	
Total	470	381	81.0%	89	18.9%	

Data were expressed as either mean standard error of mean or number (%); * significant at P ≤ 0.05; ** highly significant at P ≤ 0.01

Based on the age the result shows that the higher rate in *C .parvum* and *I. belli* was in the age group over 70 years old, while *C. cayetanensis*recorded the higher rate the age group(41-50 and 51-60) there were a statistically differences among the patients

Table4 :Number and percent of cases according to type of parasitic infestation based on age group.

Age group	Parasitic infestation	Species of the parasite						P
		C. parvum	%	*I. belli*	%	*C. cayetanensis*	%	
≥ 12	11	5	2.1%	4	3.1%	2	1.9%	No sig.
13-20	17	9	3.7%	8	6.2%	0	0%	No sig.
21-30	57	24	10.1%	14	10.9%	19	18.0%	No sig.
31-40	65	32	13.5%	16	12.5%	17	16.1%	No sig.
41-50	73	30	12.6%	21	16.4%	22	20.9%	*
51-60	87	45	18.9%	20	15.6%	22	20.9%	No

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								sig.
61-70	78	44	18.5%	20	15.6%	14	13.3%	No sig.
≤ 71	82	48	20.2%	25	19.5%	9	8.5%	*
Total	470	237	50.4%	128	27.2%	105	22.3%	*

Data were expressed as either mean standard error of mean or number (%); * significant at P ≤ 0.05; ** highly significant at P ≤ 0.01

In this study several factor might be effect on the rate of the infection also studied such as (alcoholism, diabetes patients, chemotherapy patient) and the result showed cryptosporidiosis higher(14%) in the alcoholism while isosporiosis and Cyclosporiasis showed higher in the chemotherapy patient(7.4%and 9.1%) respectively. Table 5

Table5:Number and percent of cases according to type of parasitic infestation based on factor affected on the immune system.

factor	Parasitic infestation	Species of the parasite						P
		C. parvum	%	*I. belli*	%	*C. cayetanensis*	%	
alcoholism	96	66	14%	23	4.8%	7	1.4%	**
Diabetes type 1	86	50	10.6%	20	4.2%	16	3.4%	**
Diabetes type 2	106	47	10%	33	7%	26	5.5%	*
chemotherapy patients	112	34	7.2%	35	7.4%	43	9.1%	*
Non	70	40	8.5%	17	3.6%	13	2.7%	*
Total	470	237	50.4%	128	27.2%	105	22.3%	*

Data were expressed as either mean standard error of mean or number (%); * significant at P ≤ 0.05; ** highly significant at P ≤ 0.01

Discussion:

An intestinal sporozoa considered as opportunistic parasite and pathetic protozoa that cause least effect in the infected healthy man but have sever effect on infected old and young ages and also in the immunocompromized patients (15). These coccidian parasites can invade the small intestinal

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epithelium and completes its life cycle in the cytoplasm of the cell, the Oocysts (diagnostic stage) are sheet out in the feces and be mature outside the body into infected stage, then containing sporozoites, each with sporocysts (16).

The results of present study reportedthat C. *parvum* highest infection among the three coccidian sporozoa. Many previous studies carried out in Iraq and other neighboring and other adjacent countries have reported wide ranges of variation in prevalence of intestinal sporozoa as followings; 25% (17) and 23.5% (18) in Iraq, 10.66% (19) and 38% (20) in Iran, 32.2% (21) and 21.8% (22) in Kingdom of Saudi Arabia, 25% (23) and 36% (24) in Turkey, 47% in Lebanon (25), 10.2% in Qatar (26). Relatively, the stable prevalence of coccidian sporozoa. In Iraq compared to previous studies could be attributed to absence of awareness and health education; influences of contaminated environments particularly water resources, and low practical and general improvements to health services and sanitary conditions.

The current study reported the infectious rate of the coccidian protozone parasite with the gender, age and resident and the result range between significant and highly significantand , it was found that the infectious rate was more common in rural than urban and this result may be due to people living in rural areas cought be lack sanitary water supplies, direct-contact with animals, and live close to sources of parasites in social and environmental conditions that predispose to intestinal parasitic infection. The results wereagreedwith other study (27, 28, 29, 30).

The present study suggests that risk factors for high rate ofcoccidian sporozoa infection and these factors may include a wider variety of conditions than have ever been documented (31). In addition to immunocompromised conditions that include alcoholism, diabetes type1, and 2 chemotherapy patients various immunocompetent conditions can also be the risk factors for severity of the disease such factors include poor hygiene, malnutrition (32,33).

The current study reported cryptosporidiosis higher(14%) in the alcoholism while isosporiosis and Cyclosporiasis showed higher in the chemotherapy patient(7.4% and 9.1%) respectively,. Certain factors were recognized to play important roles such as the life style of infested person, malnutrition, presence of co-existing conditions or diseases that inhibit immune response, level of immune response at time of infestation, type/strain and number of infested parasites, and nature of waste products .The present showed chronic alcoholism considered as arisk factor for development of chronicdiarrhea.

Intestinal parasitic infestations still continue to be the most common cause of chronic infections in communities living in resources poor countries situated in the tropics and sub-tropics (34). Despite the attempts for controlling or reducing the prevalence of intestinal parasites in affected regions, these pathogens remained show remarkable resilience to maintain a stable population in their hosts (35). It poorly understood to what extent intestinal parasites can contribute to the clinical symptoms of anemia, abdominal pain and/or diarrhea.

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In addition, low-income countries, overcrowded, hot and humid weather, poor sanitation, and contaminated food and water can act as predisposing factors (36). Several studies demonstrated the association between infection with intestinal parasites and under-nutrition that increase the susceptibility to infection (37)

Conclusion

In view of the incidence of intestinal coccidian protozoan among Iraqi community patientsand the factor that affected on the percent of the infection , preventive measures must be taken due to great risk population by increasing the knowledge about personal and community health and hygiene. In addition, further studies are required to demonstrate the actual effects for intestinal parasites on values of risk factor and immunocopromised patients and investigate the correlation between each species of parasites with the types of disease.

References:

1- D. S. Lindsay, J. P. Dubey, and B. L. Blagburn (1997) "Biology of Isospora spp. from humans, nonhuman primates, and dome- stic animals," Clinical Microbiology Reviews, vol. 10, no. 1, pp. 19–34.

2- E. Ng, E. K. Markell, R. L. Fleming, and M. Fried (1984) "Demon- stration of Isospora belli by acid-fast stain in a patient with acquired immune deficiency syndrome," Journal of Clinical Microbiology, vol. 20, no. 3, pp. 384–386.

3- Barta JR, Thompson RC.(2006). What is Cryptosporidium? Reappraising its biology and phylogenetic affinities. Trends Parasitol. ;22:463-468.

4- Mannheimer SB, Soave R.(1994) Protozoal infections in patients with AIDS, Cryptosporidium, isosporiasis, cyclospoiasis and microsporidiosis. Infect Dis Clin North Am.;8(2):483-498.

5- Escobedo AA, Almiral P, Alfonso M, Cimerman S, Rey S, Terry SS. (2009). Treatment of intestinal protozoan infections in children. Arch Dis Child.; 94(6):478-482.

6- Adamu H, Endeshaw T, Teka T, Kifle A, PetrosB.(2006) The prevalence of intestinal parasites in pediatric diarrheal and immunocompromised patients in the Islamic Republic of Iran. non- diarrheal patients in Addis Ababa hospitals, with special emphasis on opportunistic parasitic infections and with insight into the demographic and socio- economic factors. Ethiop J Health Dev. 2;20(1):39-46.

7- Al Braiken FA, Amin A, Beeching NJ, Hommel M, Hart CA.(2003) Detection of Cryptosporidium amongst diarrheic and asymptomatic children in Jeddah, Saudi Arabia. Annals Trop Med & Parasitol;97(5):505- 510.

8- Escobedo AA, Almiral P, Alfonso M, Cimerman S, Rey S, Terry SS.(2009) Treatment of intestinal protozoan infections in children. Arch Dis Child.; 94(6):478-482.

9- Meamar AR, Rezaian M, Zare-Mirzaei A, Zahabiun F, Faghihi AH, Oormazdi H, Kia EB.(2009). Severe diarrhea due to Isospora belli in a patient with thymoma. J MicrobiolImmunol Infect; 42: 526-529.

10- Ud Din N, Torka P, Hutchison RE, Riddell SW, Wright J, GajraA.(2012) Severe Isospora (Cystoisospora) belli diarrhea preceding the diagno- sis of human T-cell-leukemia-virus-1-associated T-cell lympho- ma. Case Repts Infect Dis; 2012: 640104.

11- Atambay M, Bayraktar MR, Kayabas U, Yilmaz S, Bayindir Y. (2007). A rare diarrheic parasite in a liver transplant patient: Isospora belli. Transplant Proceed; 39: 1693-1695.

12- Gruz F, Fuxman C, Errea A, Tokumoto M, Fernandez A, Velas- quez J, Nagel C, Ruf A, Mariño E, Nachman F, Rumbo M, Gon- dolesiG.(2009). Isospora belli infection after isolated intestinal trnsplant. Transpl Infect Dis; 12: 69-72.

13- Polman, K., Becker, S.L., Alirol, E., Bhatta, N.K., Bhattarai, N.R., Bottieau, E., and Horié, N.S. (2015). Diagnosis of neglected tropical diseases among patients with persistent digestive disorders (diarrhoea and/or abdominal pain≥ 14 days): a multi-country, prospective, non-experimental case–control study. *BMC infectious diseases*, *15*(1), 1-13.

14- Curry A, Smith HV. Emerging pathogens: Isospora, Cyclospora and microsporidia. Parasitology 1998; 117: S143-S159.

15- R. Soave and W. D. Johnson (2018). "Cryptosporidium and Isospora belli infections," Journal of Infectious Diseases, vol. 157, no. 2, pp. 225–229.

16- D. S. Lindsay, J. P. Dubey, and B. L. Blagburn(2018). "Biology of sporozoa spp. from humans, nonhuman primates, and dome- stic animals," Clinical Microbiology Reviews, vol. 10, no. 1, pp. 19–34, 1997.

17-Hussein, R.A., Shaker, M.J., and Majeed, H.A. (2011). Prevalence of intestinal parasitic infections among children in Baghdad city. *Journal of the college of basic education*, *17*(71), 139-147

18-Arani, A. S., Alaghehbandan, R., Akhlaghi, L., Shahi, M., and Lari, A. R. (2008). Prevalence of intestinal parasites in a population in south of Tehran, Iran. *Revista do Instituto de Medicina Tropical de São Paulo*, *50*(3), 145-149.

19-Daryani, A., Hosseini-Teshnizi, S., Hosseini, S.A., Ahmadpour, E., Sarvi, S., Amouei, A., and Sharif, M. (2017). Intestinal parasitic infections in Iranian preschool and school children: A systematic review and meta-analysis. *Actatropica*, *169*, 69-83.

19- Al-Shammari, S., Khoja, T., El-Khwasky, F., and Gad, A. (2001). Intestinal parasitic diseases in Riyadh, Saudi Arabia: prevalence, sociodemographic and environmental associates. *Tropical Medicine & International Health*, *6*(3), 184-189.

20- Wakid, M.H. (2010). Fecal occult blood test and gastrointestinal parasitic infection. *Journal of parasitology research*, 2010 (434801), 1-4.

21- Doni, N.Y., Gurses, G., Simsek, Z., and Zeyrek, F.Y. (2015). Prevalence and associated risk factors of intestial parasites among children of farm workers in the southeastern Anatolian region of Turkey. *Annals of Agricultural and Environmental Medicine*, *22*(3) 438-442.

22- Quihui-Cota, L., Morales-Figueroa, G. G., Javalera-Duarte, A., Ponce-Martínez, J. A., Valbuena-Gregorio, E., and López-Mata, M.A. (2017). Prevalence and associated risk factors for Giardia and Cryptosporidium infections among children of northwest Mexico: a cross-sectional study. *BMC public health*, *17*(1), 1-10.

23- Osman, M., El Safadi, D., Cian, A., Benamrouz, S., Nourrisson, C., Poirier, P., and Wawrzyniak, I. (2016). Prevalence and risk factors for intestinal protozoan infections with Cryptosporidium, Giardia, Blastocystis and Dientamoeba among schoolchildren in Tripoli, Lebanon. *PLoS neglected tropical diseases*, *10*(3), 1-17.

24- Abu-Madi, M. A., Behnke, J. M., &Doiphode, S. H. (2010). Changing trends in intestinal parasitic infections among long-term-residents and settled immigrants in Qatar. *Parasites & vectors*, *3*(1), 1-13.

25- Aly, N.S., and Mostafa, M.M. (2010). Intestinal parasitic infection among children in the Kingdom of Saudi Arabia. *Australian Journal of Basic and Applied Sciences*, *4*(9), 4200-4204.

26- Mehraj, V., Hatcher, J., Akhtar, S., Rafique, G., and Beg, M.A. (2008). Prevalence and factors associated with intestinal parasitic infection among children in an urban slum of Karachi. *PloS one*, *3*(11), 1-6.

27- Bones AJ, Jossé L, More C, Miller CN, Michaelis M, TsaousisAD(2019). Past and future trends of *Cryptosporidium* in vitro research. ExpParasitol [;196(November 2018):28-37.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 3430 - 3438
Received 05 March 2021; Accepted 01 April 2021.

28- Bouzid M, Hunter PR, Chalmers RM, Tyler KM. *(2013).Cryptosporidium* pathogenicity and virulence. ClinMicrobiol Rev.;26(1):115-34.

29- Ludington JG, Ward HD.(2015). Systemic and Mucosal Immune Responses to *Cryptosporidium*-Vaccine Development. Curr Trop Med Reports. 2;2(3):171-80.

30- Silva-Díaz H, Fernández-Valverde D, Hernández-Córdova G, Failoc-Rojas VE.(2017). InfecciónporCystoisospora belli enpacientes con VIH: análisis de casos con diferenteevolucióńclínica. Rev Chil infectología;34(4):347-51.

31- Sangaré I, Bamba S, Cissé M, Zida A, Bamogo R, Sirima C, et al.(2015). Prevalence of intestinal opportunistic parasites infections in the University hospital of Bobo-Dioulasso , Burkina Faso. Infect Dis Poverty;1-6.

32- Adamu H, Wegayehu T, PetrosB.(2013). High Prevalence of Diarrhoegenic Intestinal Parasite Infections among Non-ART HIV Patients in Fitche Hospital, Ethiopia. PLoS One.;8(8):e72634.

33-Alemu A, Shiferaw Y, Getnet G, Yalew A, Addis Z.(2011). Opportunistic and other intestinal parasites among HIV/AIDS patients attending Gambi higher clinic in Bahir Dar city, North West Ethiopia. Asian Pac J Trop Med;4(8):661-5.

34- Casmo V, Lebbad M, Maungate S, Lindh J.(2018) Occurrence of *Cryptosporidium* spp. and *Cystoisospora belli* among adult patients with diarrhoea in Maputo, Mozambique. Heliyon.;4(9):1-13.

25- Shehata AI, Hassanein F, Abdul-ghaniR.(2019). Opportunistic parasitoses among Egyptian hemodialysis patients in relation to CD4 + T-cell counts : a comparative study. BMC Infect Dis.;19(1):480.

31. Zhou Y, Lv B, Wang Q, Wang R, Jian F, Zhang L, et al.(2017). Prevalence Characterization and Molecular of Cyclosporacayetanensis, Henan, China. Emerg Infect Dis.;17(10):1887-90.