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Detection of *Cyclospora cayetanensis* Infections among Diarrheal Children Attending Pediatric Teaching Hospitalin Sulaimani City

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ABSTRACT

The present study aimed to investigate the Cyclospora infection among children attended Pediatric Teaching Hospital in Sulaimani City and to determine its prevalence among other intestinal parasites. The study started from the 1st of Jun. to the 1st of Dec. 2014. Three hundred stool samples were collected from children aged between6 months to 14 years old from both genders who attended the hospital. Data was collected using a questionnaire form including information about gender, age, location…..etc. Stool samples were examined by direct wet mount and modified acid-fast stain as a standard method. Using Modified Ziehl-Neelsen stain method revealed that 12(4.0%) was positive for Cyclospora oocysts, with no significant difference in the total rate of infection. Theinfection rate in males was (3.9%) while in females was (4.1%),with no significance difference between genders and the rate among children in urban area was (4.1%) while in rural areas was (3.3%). According to the age group children from (6 months - 2 years) of age showed the highest (5.6%) prevalence rate, while the lowest rate of infection(3.9%) was recorded among children aged between (3-5 years), with no significant difference between the rate of infection and the age groups. Also by applying direct wet mount method the rate of infection was (3.0%), which became in the second level of infection after Entamoeba histolytica (10.3%). Cyclosporias is founded as an endemic case in Pediatric Teaching Hospitalin Sulaimani City for the first time, were modified acid-fast stain was the most reliable technique for its diagnosis, and must be considered as one of the most important cause of diarrhea among children.

Keywords: Stool samples, Children, *Cyclospora* oocysts, Sulaimani, Iraq**.**

INTRODUCTION

*Cyclospora*is obligate intracellular apicomplexan protozoan parasites that infect the mucosal epithelium of the small intestine or bile duct of a variety of hosts, mostly vertebrates[1].

In recent years, *Cyclospora cayetanensis* has consider as an important human pathogen that causes diarrhea in both immuno-compromised and immunocompetent hosts. In the later, diarrhea is usually prolonged, but self-limited, while in immuno-compromised, it may be prolonged and severe [2]. It differs significantly from all other *Cyclospora* species not only in its host but also in its oocyst stage, which is much smaller and spherical rather than oblong [3, 4].

The first published report of *C. cayetanensis* in humans was by Richard Ashford at 1979[5].It was described under the genus *Cyclospora*[6].Details about this coccidian, complete morphologic description and it's taxonomic status on the basis of in-vitro sporulation, mechanicalexcystation and transmission electron microscopy[3].

The route of transmission is by ingestion of contaminated water and food products with sporulated oocysts, especially vegetables that are the most implicated source for the spread of cyclosporiasis[2]. It is unlikely transmitted directly between individuals. The infection dose is presumed to be low [7].

In recent years, several studies have shown that *C. cayetanensis* is distributed worldwide,[8, 9] its prevalence is considerably higher in developing countries than in Europe and North America. It has caused a number of sporadic cases and epidemic outbreaks diarrheal illness [10, 11].Outbreaks have been linked to contaminated water and various types of fresh products. Females and males are equally susceptible to *Cyclospora* infection; it can cause illness that varies significantly with age and condition of the host.

MATERIALS AND METHODS

The present study was carried out from1stJun. 2014 to 1st Dec. 2014. Three hundred stool samples were collected from children aged between 6 months to 14 years old who attending the Pediatric Teaching Hospital. Stool samples were obtained randomly from in-patients and out-patients, excluding of diarrheal and abdominal pain cases.

The stool samples were collected in a dry, clean screw capped plastic container. Patient's name, number and date of collection were written on the container. Questionnaire form was prepared to each patient. The specimens were transported to the laboratory as soon as possible.

Stool examination: Each stool specimen was examined microscopically by:

(a) Direct smear examination (saline and iodine smear).
The color, consistency, and presence of blood, mucus, ova and the parasites were recorded. Stool specimen was then inspected by using direct wet smear technique using saline and iodine solution for the presence of oocysts of *Cyclospora* the slides then examined under (40X)power[12].

(b) Staining method using modified Ziehl–Neelsen(MZN).
A smear of stool specimen was prepared by an applicator stick, and spread by rolling the stick over the middle part of the slide. Let to dry then fixed with absolute methanol, by adding few drops (2-3 drops) and let to dry again then stained with modified cold Ziehl-Neelse[13]. The slides then examined by using the oil immersion lens (100X).

Cyclospora oocysts were identified by morphological criteria, and also other pathogenic parasites found in the samples.

Data analysis
Appling SPSS (Version16) for Statistical analysis. Descriptive statistics (numbers and percentage) were calculated for all variables, as well as analytical statistics was performed to observe the relations between the variables, and variables were calculated by using the Chi-squaretest.

RESULTS

From the results out of 300 stool specimens examined,12 (4.0%) was positive with oocysts of *Cyclospora* by using MZN staining (Figure 1).

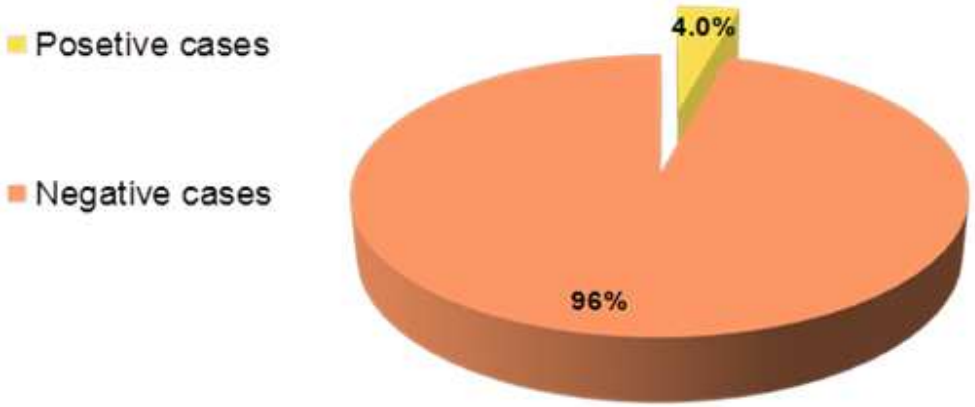

Figure 1: The rate of *Cyclospora* infection in Pediatric Teaching Hospital by MZN

The prevalence rate of *Cyclospora* infection in males was lower (3.9%) than females (4.1%), with no significant difference in the total rate of infection between both gender (Table 1).Also there was no significant difference between the rate of infection among children in urban (4.1%) and rural (3.3%) areas by MZN method (Table 2).

From the relationship between *Cyclospora* infection and age groups it was found that children between (6 months-2years) of age recorded the highest (5.6%) rate while the lowest rate of infection (3.9%) was in (3-5 years) age group with no significant difference between the rate of infections and age groups, alsono rate of infections was recorded among (6-8 years), (9-11 years) and (12-14 years) age groups (Table 3).

Modified cold Ziehl-Neelsen stain showed that the oocysts were about 8µm, spherical in shape, stained with pink-red color against green (blue) background color. (Figure 2 A&B). The result of using direct wet mount method in examination recorded a rate of (3.0%) of *Cyclospora* infection that came in the second level of infection after *Entamoeba histolytica*10.3% (Table 4). The result of using these two microscopic methods indicated that the higher rate of infection was 4.0% by modified cold Ziehl-Neelsen stain then direct wet mount method with a prevalence rate of 3.0%, with no significant difference between both methods (Table 5).

Table (1): The number of examined sample and the rate of infection with *Cyclospora* according to the gender of children in Pediatric Teaching Hospital (n= 300).

Characteristics		Exam. samples	The Result of ZN test		The Result of ZN test		P-value
			Negative		Positive		
		No.	No.	%	No.	%	
Gender	Male	153	147	96.1	6	3.9	0.9436
	Female	147	141	95.9	6	4.1	
Total		300	288	96	12	4.0	

Table (2): The number of examined sample and the rate of infection with *Cyclospora* according to the residency of children in Pediatric Teaching Hospital (n= 300)

Characteristics		Exam. samples	The Result of ZN test		The Result of ZN test		P-value
			Negative		Positive		
		No.	No.	%	No.	%	
Place	Urban	270	259	95.9	11	4.1	0.4
	Rural	30	29	96.7	1	3.3	
Total		300	288	96	12	4.0	

Table (3): The relationship between *Cyclospora* infection and theage of children

Characteristics		Exam. Samples	The Result of ZN test Negative		The Result of ZN test Positive		P-value
		No.	No.	%	No.	%	
Age	0.5-2	142	134	94.4	8	5.6	0.5054
	3 – 5	102	98	96.1	4	3.9	
	6 – 8	44	44	100	0	0	
	9 – 11	08	08	100	0	0	
	12 – 14	04	04	100	0	0	
Total		300	288	96	12	4.0	

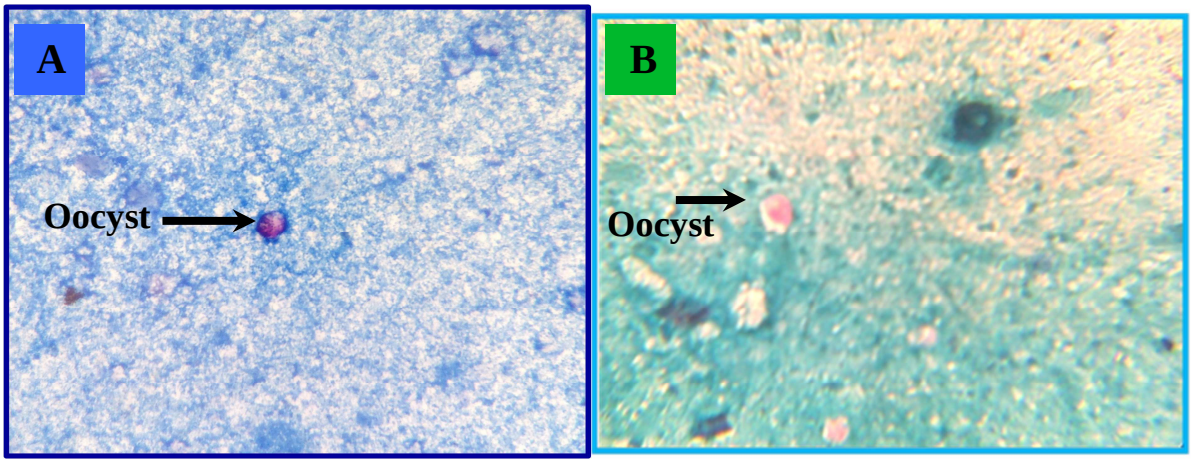

Figure (2): Oocyst of *Cyclospora* by Modified cold Ziehl-Neelsen stain with magnification 1000X

Table (4): Type, species and the rate of infection of parasites (by direct wet mount) in children in Pediatric Teaching Hospital in Sulaimani

No. and Type of Parasites	Species of Parasites	Total	
		No.	%
Protozoa (Single infection)	*Entamoeba histolytica*	31	10.3
	Cyclospora	9	3.0
	Cryptosporidium	5	1.7
	Giardia lamblia	2	0.7
	Entamoeba coli	1	0.3
	Blastocystis hominis	2	0.7
Protozoa (double infection)	*E.histolytica*& *Blastocystis hominis*	2	0.7
Total Exam. Samples **(300)**		52	17.4

Table (5): Comparison between two methods for diagnosis of *Cyclospora* infection in Pediatric Teaching Hospital

The tests	Exam. samples	The Result of the tests Negative		The Result the tests Positive		P-value
	No.	No.	%	No.	%	
Ziehl –Neelsen stain	300	288	96	12	4.0	0.5051
Direct wet mount	300	291	97	9	3.0	

DISCUSSION

The coccidian parasites are important pathogens. Many physicians remain unaware of their clinical importance[14].*Cyclospora* has now been identified worldwide in the feces of both immunocompetent and immunocompromised patients with diarrhoea [15-19]. Several studies have documented the fact that *C. cayetanensis* is a diarrhoea causing agent[3, 4, 20, 10, 21, 22].

The present study findings revealed that *Cyclospora* infection was common among diarrheic children attending the Pediatric Teaching Hospital in Sulimani City, and this may be due to contaminated food and water with the oocysts of this parasite. This result was in accordance with previous studies done in Egypt [23-26] in Jordan and [27- 29] in Guatemala. From reviewing the results for *Cyclospora* it was found that the lower prevalence rates were reported in Tanzania [30], [31] in Thailand,[32] in Cuba, in India [33-35] in Mexico, [36] in china and by[37] in Albania. While higher prevalence rates were reported in Venezuela [38-39] in Egypt this may be because of many factorssuch asnumber of patient's samples in different screening studies, differences in the geographical distribution, ages of patients, also variation in diagnostic methods used, socioeconomic conditions, immunological status, personal hygiene and the modifying of temperature between the seasons in different locations the studies.

The results also showed no significant difference between males (3.9%) and females (4.1%) infection with *Cyclospora*, although the rate of infection in females was relatively higher than the rate of males, this may be due to that both genders can be exposed to *Cyclospora* oocysts equally and both male and female have the same sensitivity to infection especially at the early stages of their lives. This result agrees with the results of[40] in Nepal,[36]in Anhui, China and[41] in Egypt, but the result disagree with results of[42]in Alexandria, Egyptand by [43] inKathmandu, Nepal.

From the data observedthat there was no significant difference between urban (4.1%) and rural (3.3%) infection with *Cyclospora* , where the rate of infection in urban area was relatively higher than the rural area this may be due to the use of the same sources of drinking water by panties in urban area, though tap water was implicated as the most likely source of contamination, lack of adequate sanitation, and the presence of animals in the household are associated with increased risk of *Cyclospora* infections[27, 44].This result agreed with the results of [45, 46], but it was disagree with other studies carried out by [41]in Egypt and [47]in China which most of the infected children were living in rural areas, this may be because of personal hygiene and living environmental conditions. In rural areas, simple toilets, deficiency of sanitary facilities and diffusing feces contamination were commonly recorded, and most people were unaware of health knowledge and good hygiene habits [42].

Although all age groups can infect this disease, the most vulnerable age group seems to be less than 1 year to 15 years of children[48, 19, 49, 50].From the data children of (6 month to 2 years) age group showed the highest (5.6%) rate of infection while the lowest rate of infection (3.9%) was reported in children of (3-5 years) age groups with no infection rate among (6-8 years), (9-11 years) and (12-14 years) age groups and there was no significant difference between the rate of infection and the age groups. This may be because of the development of partial immunity in elder age groups protect them from being infected with the coccidia also they are more aware of their personal hygiene, but not from re-infection[47]. This result agrees with the results of studies done by [3, 41, 42, 40, 27, 51, 2, 52, 53] suggesting either that protective immunity develops with repeated exposure to *cayetanensis* early in life or there may be differential exposure risk in older individuals.

The prevalence rate of *Cyclospora* was 3.5% in children aged 1.5 to 4 years old and 3.8% in children aged 5 to 9 years, according to surveillance studies[27].Similar results were obtained from the case-control studies, where the highest prevalence was in children aged 1.5 to 9 years (11.6%) [54].The prevalence rate among children > 11 years was 0%, suggesting either that protective immunity develops with repeated exposure to *cayetanensis* early in life or there may be differential exposure risk in older individuals[2, 51, 52].

From using the two methods it was clear that modified acid-fast stain was the only stain capable of detecting the coccidians in clinical specimens [55]. The data of comparing the two methods used in detection of *Cyclospora* showed that MZN method recorded the higher rate in detecting oocysts than direct wet mount, this may be due to the amount of stool specimen used and to the two stains that had been used in MZN that gave red and green (or blue) according to availability of the malachite green (or methylene blue) stain this gives chance to differentiate the parasite from the debris.

In the present study, the prevalence rate of *Cyclospora* became in the second level of infection after *E. histolytica* rate, and this showed the degree of importance of it among other intestinal parasite which causes diarrhea. This result agreed with [56] who reported that *E. histolytica* as the most prevalent protozoan pathogen in Lagos but disagree with[57] in which *Cryptosporidium* is the most prevalent protozoan pathogen and *Cyclospora* detected as a newer emerging diarrheal pathogen and its rate was higher than the rate of *E. histolytica*.

CONCLUSION

The results indicated that *Cyclospora* oocysts are endemic in Sulimani city and it is an important etiological agent of diarrhoea. However, it is the first study done here but the good result was obtained because it was done in dry hot season though all positive cases detected were recorded in June to August. More detailed and specific studies needed to provide more information about this causative agent of diarrhoea in this country.

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REFERENCES

[1] Lainson R. The genus Cyclospora: (Apicomplexa), with a description of Cyclospora schneiderin. sp. in the snake from Amazonian Brazil. Mem. Inst. Oswaldo. Cruz. 2005; 100: 103–115.
[2] Herwaldt BL. Cyclospora cayetanensis: a review, focusing on the outbreaks of cyclosporiasis in the 1990s. Clin Infect Dis.2000; 31: 1040–1057.
[3]Ortega YR, Sterling CR, Gilman RH, Cama VA, Dı´az F. Cyclospora species–a new protozoan pathogen of humans. N Engl J Med.1993; 328: 1308–1312.
[4]Ortega YR, Gilman RH, Sterling CR (1994) A new coccidian parasite (Apicomplexa: Eimeriidae) from humans. J Parasitol.1994; 80: 625–629.
[5]Ashford RW. Occurrence of an undescribed coccidian in man in Papua New Guinea. Ann Trop Med Parasitol.1979; 73: 497–500.
[6]Ortega YR, Sterling CR, Gilman RH, Carna VA, Diaz F. Cyclospora cayetanensis: a new protozoan pathogen of humans. Abstract 289 in Proceedings of the 41st Annual Meeting of the American Society of Tropical Medicine and Hygiene. J Am Soc Trop Med Hyg (Suppl.) 1992: 210.
[7]Jackson GJ, Leclerc JE, Bier JW, Madden JM. Cyclospora – still another new food borne pathogen. Food Technol. 1997; 51, 120.
[8]Sterling CR, Ortega YR. Cyclospora: An Enigma worth Unraveling. Emerg. Infect. Dis. 1999; 5(1):48-53.
[9]Brown GH, Rotschafer JC. Cyclospora: review of an emerging parasite. Pharmacotherapy.1999; 19:70–75.
[10]Clarke SC, McIntyre M. Human infection with Cyclospora cayetanensis. Rev. Med. Microbiol. 1996b; 7: 143-150.
[11]Pollok RC, Bendall RP, Moddy A, Chiodini PL, Churchill DR. Traveller's diarrhea associated with cyanobacterium-like bodies. Lancet. 1992; 340, 556–557.
[12]World Health Organization (WHO). Basic laboratory methods in medical parasitology. WHO Geneva: 1991; 11-12, 20, 102 pp.
[13]Fayer R, Xiao L. Cryptosporidium and cryptosporidiosis. 2nd edn., CRC Press, London :2008; 560pp.
[14]Julie A. Ribes, John P. Seabolt, Sue B. Overman. Point prevalence of Cryptosporidium, Cyclospora and Isospora infections in patients being evaluated for diarrhea. Am J Clin Pathol. 2004;122:28-32.
[15]Hart AS, Ridinger MT, Soundarajan R, Peters CS, Swiatlo AL, et al. Novel organism associated with chronic diarrhoea in AIDS. Lancet.1990; 335: 169– 170.
[16]Taylor DN, Houston R, Shlim DR, Bhaibulaya M, Ungar BLP, Echeverria P. Etiology of diarrhea among travelers and foreign residents in Nepal. JAMA 1988; 260: 1245-8.
[17]Serpentini A, Dutoit E, Camus D. Cyclospora cayetanensis: review of an emerging intestinal pathogen. Ann Biol Clin Paris :1999 Nov-Dec; 57(6): 677-83.
[18]Kansouzidou A, Charitidou C, Varnis T, Vavatsi N, Kamaria F. Cyclospora cayetanensis in a patient with travellers' diarrhoea: case report and review. J. Travel. Med.2004; 11(1): 61-3.
[19]Ghimire TR. Cyclosporiasis in HIV and Non- HIV patients: A study in Kanti Children's Hospital, Maharajgunj and Sukra Raj Tropical and Infectious Disease Hospital, Teku, Kathmandu, Nepal. Dissertation submitted in partial fulfillment of Master's Degree in Zoology (Parasitology), Central Department of Zoology, Tribhuvan. University, Kirtipur, Kathmandu, Nepal 2004.
[20]Clarke SC, McIntyre M. The incidence of in stool sample submitted to a District General Hospital. Epidemiol. Infect.1996a; 117: 189-193.

[21]Nassef NE, El-Ahl SA, El-Shafee OK, Nawar M. Cyclospora: a newly identified protozoan pathogen of man. J Egypt Soc Parasitol 1998;28:213–9.
[22]Fryauff DJ, Krippner R, Prodjodipuro P, Ewald C, Kawengian S, Pegelow K, Yun T, et al. Cyclospora cayetanensis among expatriate and indigenous populations of West Java, Indonesia. Emerg. Infect. Dis.1999; 5: 585-588.
[23]Abou el Naga IF. Studies on a newly emerging protozoal pathogen: Cyclospora cayetanensis. J Egypt Soc Parasitol .1999;29:575–86.
[24]Osman GA, Makled KM, El-Shakankiry HM, Metwali DM, Abdel- Aziz SS, Saafan HH. Coccidian parasites as a cause of watery diarrhoea among protein energy malnourished and other immunocompromised Egyptian children. J Egypt Soc Parasitol 1999; 29(3): 653-68.
[25]Rizk H, Soliman M. Coccidiosis among malnourished children in Mansoura, Dakahlia Governorate, Egypt. J Egypt Soc Parasitol.2001; 31: 877-86.
[26]Nimri LF. Cyclospora cayetanensis and other intestinal parasites associated with diarrhea in a rural area of Jordan. Int Microbiol. 2003; 6: 131–135.
[27]Bern C, Hernandez B, Lopes MB, Arrowood MJ, Alvarez De Mejia M, Merida AM De, Hightower AW, Venczel L, Herwaldt BL, Klein RE 1999. Epidemiologic studies of Cyclospora cayetanensis in Guatemala. Emerg Inf Dis 5: 766-774.
[28]Bern C, Hernandez B, Lopez MB, Arrowood MJ, de Mérida AM, Klein RE. The contrasting epidemiology of Cyclospora and Cryptosporidium among outpatients in Guatemala. Am J Trop Med Hyg 2000;63:231–5.
[29]Pratdesaba RA, Gonzalez M, Piedrasanta E, Merida C, Contreras K, Vela C. Cyclosporacayetanensis in three populations at risk in Guatemala. J. Clin. Microbiol.2001; 39: 2951-3.
[30]Cegielski JP, Ortega YR, McKee S, Madden JF, Gardo L, Schwartz DA. Cryptosporidium, Enterocytozoon and Cyclospora infections in pediatric and adult patients with diarrhea in Tanzania. Clin. Infect. Dis.1999; 28: 314-21.
[31]Manatsathit S, Tunsupasawasdikul S, Wanachiwanawin D, Setawarin S, Suwanagool P, Prakasvejakit S, et al. Causes of chronic diarrhoea in patients with AIDS in Thailand: a prospective clinical and microscopical study. J. Gastroenterology.1996; 31: 533 7.
[32]Escobedo AA, Nunez FA. Prevalence of intestinal parasites in Cuban Acquired Immunodeficiency Syndrome (AIDS) patients. Acta. Trop. 1999; 72: 125-30.
[33]Kumar SS, Ananthan S, Saravanan P. Role of coccidian parasites in causation of diarrhoea in HIV infected patients in Chennai. Ind. J. of Med. Research.2002; 116: 85-9.
[34]Mohandas K, Sehgal R, Sud A, Malla N. Prevalence of intestinal pathogens in HIV seropositive individuals in Northern India. Jpn. J. Infect. Dis. 2002; 55: 83-4.
[35]Diaz E, Mondragon J, Enrique R, Bernal R. Epidemiology and control of intestinal parasites with nitaxoxanide in children in Mexico. Am. J. Trop. Med. and Hyg. 2003; 68: 384-5.
[36]Wang KX, Li CP, Wang J, Tian Y. Cyclospore cayetanensis in Anhui, China. World J Gastroenterol.2002; 8: 1144–1148.
[37] Jelinke T, Lotze M, Eichenlaun M, Loscher T. Prevalence of infection with Cryptosporidium parvum and Cyclospora cayetanenesis. Gut.1997; 41: 801-4.
[38]Devera R, Blanco Y, Cabello E. High prevalence of Cyclospora cayetanensis among indigenous people in Bolivar State, Venezuela. Cad. Saude. Publica.2005; 21 (6):1778-84 (English Abstract).
[39]El-Nazer M, Sayed M, Hamza I, Shaheen M. Intestinal opportunistic spore forming infections in non diarrheic immuno-compromised patients. Egypt J. Med. Sci.1998; 19(2):459-67.
[40] Hoge CW, Echeverria P, Rajah R, Jacobs J, Malthouse S, Chapman E,et al. Prevalence of Cyclospora species and other enteric pathogens among children less than 5 years of age in Nepal. J. Clin. Microbiol, 1995; 33:3058–60.
[41]Abdel-Wahab A M, El-Sharkawy, Sonia G, Rayan H, Hussein EM.Detection of Cyclospora cayetanensis Infections among Diarrheal Children Attending Suez Canal University Hospital. PUJ.2008; 1(1):37–46.
[42]Naguib M, Massoud, Doaa ES, Ahmed R. El-Salamouny. Prevalence of Cyclospora cayetanensis among symptomatic and asymptomatic immune-competent children less than five years of age in Alexandria, Egypt. Alexandria J. of Med. 2012; 48: 251–259.
[43]Ghimire TR, Sherchan JB. Human Infection of Cyclospora cayetanensis: Review on its Medico-biological and Epidemiological Pattern in Global Scenario. J. Nepal Health Research Council: Vol.4 No.2; 2008.
[44]Zerpa R, Uchima N, Huicho L. Cyclospora cayetanensis associated with watery diarrhoea in Peruvian patients. J Trop Med Hyg.1995; 98: 325–329.
[45]Perez CG, Cordova Paz SO, Vargas VF, Velasco S, Jr BL, Sempere MM, et al. Prevalence of enteroparasites and genotyping of Giardia lamblia in Peruvian children. Parasitol. Res.2008; 103:459– 465.

[46]Cordova Paz SO, Vargas VF, Gonzalez VA, Perez CG, Velasco S, Jr, Sanchez-Moreno M, et al. Intestinal parasitism in Peruvian children and molecular characterization of Cryptosporidium species. Parasitol. Res.2006; 98:576–581.
[47]Ortega YR, Sterling CR, Gilman RH. Cyclospora cayetanensis. Adv. Parasitol.1998; 40: 339-418.
[48]Sherchand JB, Cross JH. Cyclospora cayetanensis in Nepal: A study of epidemiological and microbial aspects. J. Nepal Health Research Council, 2003; 3:1-8.
[49]Ghimire TR, Mishra PN. Intestinal parasites and haemoglobin concentration in the people of two different areas of Nepal. J.Nepal Health Research Council. 2005; 3(2): 1-7.
[50]Ghimire TR, Mishra PN, Sherchand JB. The seasonal outbreaks of Cyclospora and Cryptosporidium in Kathmandu, Nepal. J. Nepal Health Research Council.2005; 3(1): 39-48.
[51]Madico G, McDonald J, Gilman RH, Cabrera L, Sterling CR. Epidemiology and treatment of Cyclospora cayetanensis infection in Peruvian children. Clin Infect Dis.1997; 24: 977–981.
[52]Ortega YR, Roxas CR, Gilman RH, Miller NJ, Cabrera L, Taquiri C. Sterling CR. Isolation of Cryptosporidium parvum and Cyclospora cayetanensis from vegetables collected in markets of an endemic region in Peru. Am. J. Trop. Med. Hyg.1997; 57: 683–686.
[53]Hoge CW, Shlim DR, Ghimire M, Rabold JG, Pandey P, Walch A. Placebo-controlled trial of co-trimoxazole for Cyclospora infections among travelers and foreign residents in Nepal. Lancet (North American Edition).1995; 345: 691-3.
[54] Ortega YR, Roxana, Sanchez . Update on Cyclospora cayetanensis, a Food-Borne and Waterborne Parasite. Clinical Microbiology Reviews, Jan.2010; Vol. 23, No. 1: 218–234.
[55]Eberhard ML, Pieniazek NJ, Arrowood MJ. Laboratory diagnosis of cyclospora infections. Arch pathol Lab Med 1997 Aug; 121(8): 792-797.
[56]Aderounmu A O. (1999). The incidence of intestinal parasites and malaria in patients reporting to Lagos University Teaching Hospital between 1991 and 1999. (M. Sc. dissertation, University of Lagos).
[57]Alakpa, GE, Fagbenro-Beyioku AF. Cyclospora cayetanensis and Intestinal Parasitic Profile in Stool Samples in Lagos, Nigeria. Acta. Protozool. 2002; 41: 221 – 227.