

Identification and prevalence of *Cyclospora* spp. isolated from samples in Al-Muthanna province

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Abstract

The purpose of this study was to determine the prevalence of the parasite *Cyclospora* spp. in residents of AL-Muthanna province. This parasite's prevalence in Samawah is currently unknown. As a result, the study conducted from October 2021 to April 2022, stool samples from 138 people who had diarrhea and other symptoms, The range of ages was less than five years to thirty-four years. All feces samples were inspected under a microscope, by looking at 148 stool samples, 73 of which were taken from men and 75 from women, who were general hospital patients. The findings revealed that the overall infection rate was 46.61% and that there were no significant variations in the infection rates of the two sexes. The age range (5-9) had the greatest infection rate at 28.9855%. The urban infection rate was 73.91% greater than the rural infection rate of 26.09%. The greatest rate, 39.13%, was reported in March, and the lowest, 2.899%, was recorded in December.

Keywords: *parasite, protozoan, samples, infection, cyclospora.*

INTRODUCTION

Protozoan parasites are especially dangerous since they are responsible for more than 58 million instances of pediatric diarrhea each year (1). Chronic to severe diarrhea, occasionally accompanied by flatulence, abdominal cramping, nausea, vomiting, anorexia, exhaustion, low-grade fever, and weight loss, is a hallmark of intestinal protozoan infections (2, 3). Protozoan parasites called *Cyclospora* spp. belong to the family Eimeriidae, order Eimeriorina, class Sporozoa, and phylum Apicomplexa. The only *Cyclospora* species known to infect people is *Cyclospora* spp (4,5). Human cyclosporiasis, a digestive disorder, is brought on by *Cyclospora* spp., a developing food- and water-borne pathogen. The parasite is

indigenous to tropical and subtropical areas, although the predominance of these areas is heavily influenced by environmental elements as weather and rainfall patterns (6). The primary method of spreading the parasite is by a foodborne infection with *C. spp* (7). contamination in fresh food The main sources of infection include foods that are difficult to fully clean and that are eaten without being processed to inactivate or eliminate the oocysts, like berries and leafy greens. Foods that have been thoroughly cooked or pasteurized before ingestion have not been linked to disease (8). On the other hand, water that has been polluted with feces may act as a means of spreading the *C. spp* infection. In endemic locations, the source of drinking water has been identified as a risk factor for

cyclosporiasis (9,10). Both immunocompetent and immunocompromised individuals can contract *Cyclospora* spp. It is more severe in immunocompromised hosts, especially in HIV-infected patients, for whom it is a significant cause of diarrhoea, weight loss, abdominal discomfort, and nausea (11,12). Symptoms may linger from a few days to a month or longer in persons with robust immune systems, although the majority recover without therapy (13). This study was carried out to determine the prevalence of infection among the people of this province and its diagnosis because this parasite was newly identified in the world and because AL-Muthanna province hasn't had any studies specifically on it. In contrast, this is the first research done in the province in question.

Materials & Methods

Stool samples

Between October 2021 and April 2022, 148 stool samples were taken from patients at the general hospital, including both sexes, who experienced diarrhea cases with varying degrees of abdominal pain. The survey's goal was to learn more about the cyclospora parasite, which causes cyclosporiasis in humans.

Microscopic Examination

The cyclospora parasite was concentrated using the sedimentation method (10 min at 500). The following methods can be used for a microscopical examination of the sediment:

1-Wet mounts by the Novex – Holland compound microscope (40x and 100x).

2-Staining of samples:

Ziehl-Nelsen stain, produced by Ismailia Company for laboratory medical supplies in Egypt, was used according to the steps shown below:

1- Prepare a slide by taking a stool sample (2 mg) and distributing it on the glass slide and leaving it to dry and fix it using heat.

2- Dip the slide in carbolfuchsin solution for 5 minutes.

3- Wash the slide with distilled water.

4- Dip the slide in sulfuric acid H₂SO₄ at a concentration of 20% for one minute.

5- Wash with distilled water to remove excess sulfuric acid.

6- Dip the slide in methylene blue for one minute.

7- The slide is washed with distilled water to remove the excess dye and placed in the slide holder until it dries and then examined.

Stained smears (using modified acid-fast stain). The staining of parasites may be more varied with a fast-modifying acid (12,13)

Analytical statistics

The current study's results were statistically analyzed utilizing the SPSS program and a t-test with a (P0.05) significance level.

Results & Discussion

The current study is the first to concentrate on the prevalence of *Cyclospora* species among residents of the Al-Muthanna province (figure 1). The oocysts of this parasite are able to get out when there is a counter debris stain of feces. Parasite oocysts vary in color from pink to deep purple in spots, while others may be stainless. According to an acid-fast stain, the parasite's oocysts have the following main characteristics: range (8 to 10 m) might not be spherical; some might appear collapsed or off-center. Additionally, they occasionally have a wrinkled wall and may contain granules. This staining technique is the easiest to use and most effective practically. The samples can be stored for a longer amount of time until the diagnosis is made when using this staining

technique. However, a misdiagnosis may result in misunderstandings and inconsistent staining for older samples.

By reviewing all the sources associated with this parasite, which is regarded as one of the causes of diarrhea in humans and is significant medically, it has been determined that no cases of this parasite (*Cyclospora* spp.) have been documented in Iraq, particularly in the city of Samawah, Muthanna province (2, 5, 8, 10), On the one hand, this is true, and the measurements and characteristics of the parasite in the current study are consistent with those noted by (14), therefore it is thought to be the first discovery of this parasite in Iraq's Al-Muthanna area.

The analysis of stool samples taken from patients visiting a general hospital in the province revealed that the overall prevalence was 46.61% (69/148)(Table 1). The high infection rate in this study is a result of deteriorating health and service conditions, a shortage of clean drinking water, and other factors. According to Alsqret al., it is also a result of water pollution, which is a major factor in the spread of sickness (15). Additionally, it is brought on by a disregard for food cleanliness, particularly with regard to fruits and vegetables that are not washed and play a large part in the transmission of infection (17). It may also be the cause of people's increased interest in keeping domestic animals, such as cats and dogs, which have been confirmed to be a vector medium (18). This concurs with a study conducted in Diwaniyah by Abdullah (19), where the rate was 2.2%. The study also agreed with a study

conducted in southern Italy by Giangaspero et al. (20), where the rate was 27.5%. This relative amount differed from earlier research for a variety of reasons, including the study locations and techniques of parasite diagnosis (Turki, et al., 21, Mezher, et al. 22, Hawel, and Alasadiy, 23). Moreover, how many samples were analyzed.

Males (46.58%) and females (46.67%) both have similar infection rates based on gender (Table 1). This is in line with several research, such as the ones by Shakir, Al-Asadiy, (24), Wang et al., (25), Shahatha, et al.(17), and Al-Asadiy et al., which found no appreciable differences in the infection rate between males and females (26, 27). This explains why both sexes have an equal likelihood of being exposed by tainted food and drink.

Figure (1): Explain the parasite Cyclospora spp.

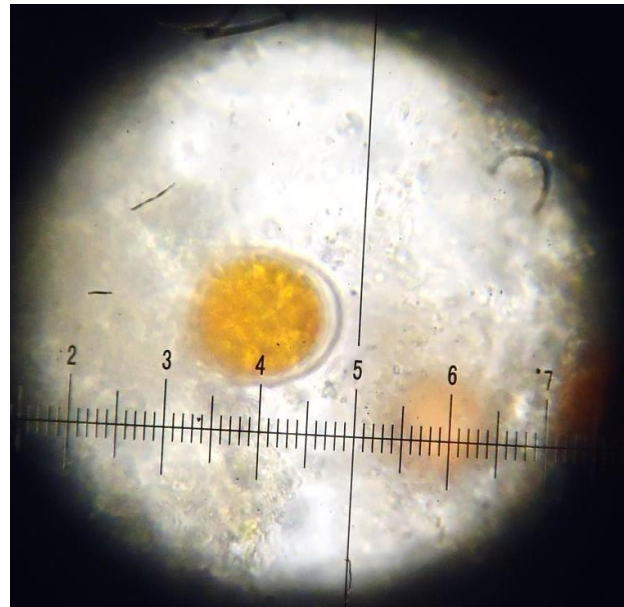


Table 1 shows the percentage of Cyclospora spp. infections by gender.

Gender	Number of samples	Number of patients	Percentage (%) from total patients/gender	Percentage (%) from infected patients
Males	73	34	46.58	49.28
Females	75	35	46.67	50.72
Total	148	69	46.61	100

Infection rates ranged from 4.34783% in the age group (30-34) years to 28.9855% in the age group (5-9) years (Table 2). This study supports a number of other studies that found

that young children are the ones that suffer the disease's effects the most, including (26,28,29,30).

Table 2 shows the distribution of infected samples by age group of children under five.

Age groups (years)	The total number of samples examined	infected samples	
		No.	%
<5	15	9	13.0435
5-9	40	20	28.9855
10-14	30	13	18.8406
15-19	13	8	11.5942
20-24	10	6	8.69565
25-29	30	10	14.4928
30-34	10	3	4.34783
Total	148	69	100

Urban areas had an infection rate that was 73.91% greater than rural areas' 26.09% (Table 3). This is a result of the lifestyle differences between the city and the countryside, where there is a significant urban reliance on eating out, particularly in recent years. Since children are the most affected,

urban children's attendance at nurseries, kindergartens, and schools is thought to contribute to the spread and transmission of infection among young children. The findings of this study conflict with those of Al-Qobati (31).

Table 3 shows the percentage of Cyclospora spp. infections based on where the samples were collected.

Location of Samples Collection	Patients	
	Number	Percentage
Rural	18	26.09
Urban	52	73.91
Total	69	100%

The highest infection rate was observed in March (39.13%) and the lowest infection rate was reported in December (2.899%), according to Table 4's association between the infection rate and the months of the year. This finding is consistent with research by Jiang et al. (32) and Shahatha et al (17). This might be

because oocyst ripening requires moderate temperatures and rainfall in the spring months, and infection episodes, seasonal shifts, and environmental factors all have a significant impact on infestation rates, which rise in the spring (33).

Table 4 shows the percentage of infections with Cyclospora spp. by month.

Months	The total number of samples examined	infected samples	
		No.	%
October 2021	10	5	7.246
November	15	3	4.348
December	9	2	2.899
January 2022	7	2	2.899
February	29	10	14.49
March	43	27	39.13
April	35	20	28.99
Total	148	69	100

Observations and suggestions

We draw the conclusion that Cyclospora spp. have spread among humans in Al-Muthanna province, where this study is the first of its kind. As a result, we advise carrying out additional research to determine the parasite's level of spread in some types of animals and some vegetables, to pinpoint the infection's sources, and to establish health service programs to control the spread of parasitic infections that could harm people's health and the economy.

Reference

1. WHO/PAHO Informal Consultation on Intestinal Protozoal Infections .(1991).(Mexico), World Health Organization. Division of Communicable Diseases, Pan American Health Organization. WHO/PAHO Informal Consultation on Intestinal Protozoal Infections, Mexico, 21–23 October 1991. Geneva: World Health Organization, 1992; Published online: 1992
2. Giangaspero, A.; Gasser, R.B. Human cyclosporiasis. Lancet Infect Dis. (2019),19:e226–36.

3. Ryan, U.;Hijjawi, N. ;Feng, Y. & Xiao, L. (2018).Giardia: an under-reported foodborne parasite. Int J Parasitol.;49:1–11.
4. Shahatha, S.S.(2019). An Epidemiological and Therapeutic Study of the Cryptosporidium parvum Parasite in Some Farm Animals in Anbar Province/Iraq. Journal of Global Pharma Technology, parasite. 10)11):147-152
- Ortega, Y.R.; Gilman, R.H.; Sterling, C.R. A new coccidian parasite (Apicomplexa: Eimeriidae) from humans. J. Parasitol. 1994, 80, 625–629. [CrossRef] [PubMed]
5. ThivyaNaganathan; Annette O’Connor ;Jan, M. ;Sargeant , Karen Shapiro , Sarah Totton;Charlotte Winder & Amy, L. Greer . .(2021).The prevalence of Cyclospora cayetanensis in water: a systematic review and meta-analysis Epidemiology and Infection 150, e15, 1–9. <https://doi.org/10.1017/S0950268821002521> .
6. Butler, A.J.; Thomas, M.K.&Pintar, K.D.M.(2015).Expert elicitation as a means to attribute 28 enteric pathogens to foodborne, waterborne, animal contact,

- and person-to-person transmission routes in Canada. *Foodborne Pathog. Dis.*, 12, 335–344.
7. Dawson, D. (2005). Foodborne protozoan parasites. *Int. J. Food Microbiol.*, 103, 207–227.
 8. Tandukar, S.; Ansari, S.; Adhikari, N.; Shrestha, A.; Gautam, J.; Sharma, B.; Rajbhandari, D.; Gautam, S.; Nepal, H.P. & Sherchand, J.B. (2013). Intestinal parasitosis in school children of Lalitpur district of Nepal. *BMC Res. Notes*, 6, 449.
 9. Bhandari, D.; Tandukar, S.; Parajuli, H.; Thapa, P.; Chaudhary, P.; Shrestha, D.; Shah, P.K.; Sherchan, J.B. & Sherchand, J.B. (2015). Cyclospora infection among school children in Kathmandu, Nepal: Prevalence and associated risk factors. *Trop. Med. Health*, 43, 211–216.
 10. Chacín-Bonilla, L. (2010). Epidemiology of Cyclospora cayetanensis: A review focusing in endemic areas. *Acta Trop.*, 115, 181–193.
 11. Giangaspero, A., & Gasser, R. B. (2019). Human cyclosporiasis. *The Lancet Infectious Diseases* Volume 19, Issue 7, July, Pages e226–e236.
 12. CDC-Centers for Disease Control and Prevention. (2019). CDC – Cyclosporiasis - Treatment. Available at <https://www.cdc.gov/parasites/> (Accessed 5 July 2020).
 13. Alasadiy, Y. D. K. (2022). Evaluation of parasitic infection in two species of fishes from Euphrates river passing through Al-Muthanna province. First International Scientific Conference Al-Ayen University IOP Conf. Series: Journal of Physics: Conf. Series 1279 (2019) 012030 IOP Publishing doi:10.1088/1742
 14. Centers for Disease Control and Prevention. (2022). Cyclosporiasis, DPDx. <https://www.cdc.gov/>
 15. Alsqr, I. M. M.; Obeidi, S. H. & Ali, H. M. (2015). Investigation on Giardia lamblia and other parasites in tap water as a potential source of movement in some areas of Baghdad. *Iraqi Journal of science*, 56 (1B): 337–344.
 16. Key points for laboratory diagnosis of cyclosporiasis. (2022). Cyclospora cayetanensis. www.DPDx, Laboratory Identification of Public Health Concern.
 17. Shahatha, S. S. Alkubaisy, S. A. & Mousa, M. O. (2021). An Epidemiological and Diagnostic Study of Cyclospora Cayetanensis Parasite in Anbar Province – Iraq. *IOP Conf. Series: Earth and Environmental Science* 904 (2021) 012026.
 18. Almeria, S.; Hediye, N. C. & Dubey, J. P. (2019). Cyclospora cayetanensis and Cyclosporiasis: An Update. *Microorganisms*, 7, 317; doi:10.3390/microorganisms7090317
 19. Abdullah, S. R. (2014). Epidemiological survey on the prevalence of intestinal parasites in working in restaurants and food shops in Diwaniyah Governorate. *Al Qadsia pure science Journal*, 19: (2).
 20. Giangaspero, A.; Marangi, M. & Arace, E. (2015). Cyclospora cayetanensis travels in tap water on Italian trains. *Journal water and health*. 13(1): 210–216.
 21. Turki, N. M.; Mallah, M. O. & Alasadiy, Y. D. K. (2016). First isolation of G. lamblia genotyping by Multiplex-PCR assay for detection of mixed infection in Human Stool in AL-Muthanna province–Iraq, AL-Muthanna *Journal of Pure Sciences (MJPS)* VOL.(3), NO.(2), PP1–10.

22. Mezher, J.O.; Alasadiy, Y. D.K. and Ali, G. B.(2017). Molecular Detection for Cutaneous Leishmaniasis In Al-Muthanna Province , AL-Muthanna Journal of Pure Sciences (MJPS) VOL.(4), NO.(1), DOI: 10.18081/2222-4223/017-6/119-128.
23. Hawel, N. H. & Alasadiy, Y.D.K.(2018). Immunologic al Diagnosis for Trichomonas in Women in AlMuthanna Province AL-Muthanna Journal of Pure Sciences (MJPS) VOL.(5), NO.(1), pp1-9.
24. Shakir ,A.M. & Al-Asadiy, Y.D.K.(2018). Detection of parasites infecting Coptodon zillii and Planilizaabu caught from Euphrates river and their records as new hosts for three identified parasite species. Al-Muthanna J. For Agric Sci., 6(4), pp. 76-83.
25. Wang, K.X.; Li, C.P.; Wang, J. & Tian, Y.(2002). Cyclospore cayetanensis in Anhui, China. World Journal of gastroenterology, 8(6), 1144.
26. Alasadiy, Y.D.K.(2021). Prevalence Of Parasitic Infection In Fresh Water Fish Farm In Samawah City. Indian J Ecology 2021) 48 Special Issue (13): 72-76
27. Alasadiy, Y.D.K. ; Mukdad R.M. & Alhasnawi, A. N.(2022). A Comparative Study of Parasitic Infections in Domestic and Wild Pigeons in Iraq. Archives of Razi Institute, Vol. 77, No. 2 (2022) 709-715 Copyright © 2022 by Razi Vaccine & Serum Research Institute DOI: 10.22092/ARI.2022.357105.1976.
28. Bhandari, D.; Tandukar, S.; Parajuli, H.; Thapa, P.; Chaudhary, P.; Shrestha, D.; Shah, P.K.; Sherchan, J.B. & Sherchand, J.B.(2015). Cyclospora infection among school children in Kathmandu, Nepal: Prevalence and associated risk factors. Trop. Med. Health, 43, 211–216.
29. Kaminsky, R.G.; Lagos, J.; Raudales Santos, G. & Urrutia, S.(2016). Marked seasonality of Cyclospora cayetanensis infections: Ten-year observation of hospital cases, Honduras. BMC Infect. Dis., 16, 66.
30. Eabaid, F, AlaAll., Alasadiy Y.D.K. & Gatea, E.A.(2020). COMPARATIVE STUDY OF SOME DIAGNOSTIC METHODS FOR ENTAMOEBA HISTOLYTICA (SUBJECT REVIEW) Biochem. Cell. Arch. Vol. 20, No. 1, pp.557-561, DOI : 10.35124/bca.2020.20.1.557.
31. Al-Qobati, S.A.(2017). Cyclospora cayetanensis: First Report of Prevalence and Risk Factors for Infections among Immuno- Competent Children with Diarrhea, Sana'a, Yemen. EC Microbiology., 10, 11-18.
32. Jiang, Y.; Yuan, Z.; Zang, G.; Li, D.; Wang, Y.; Zhang, Y. & Shen, Y.(2018). Cyclospora cayetanensis infections among diarrheal outpatients in Shanghai: a retrospective case study. Frontiers of medicine. 12(1): 98-103.
33. Shahatha, S.S.(2019). An Epidemiological, Diagnostic and Therapeutic Study of Giardia lamblia in Anbar Province–Iraq. International Journal of Drug Delivery Technology, 9(01), 39-45.