An Epidemiological and Diagnostic Study of Cyclospora Cayetanensis Parasite in Anbar Province - Iraq

An Epidemiological and Diagnostic Study of Cyclospora Cayetanensis Parasite in Anbar Province - Iraq

S S Shahatha*, S A Alkubaisy, M O Mousa

Center of Desert Studies, University of Anbar, Anbar, Iraq

*Corresponding author's e-mail: suad.alfahdawi@uoanbar.edu.iq

Abstract: This study was conducted to find out the prevalence of *Cyclospora cayetanensis* in humans, animals, and vegetables in Anbar province- Iraq. The parasite was diagnosed by examining the samples microscopically, by examining 560 stool samples (262 males and 298 females) that were collected from patients attending hospitals (Ramadi, Fallujah, and Haditha), and its health centers. The results showed that the total infection rate was 12.1%, and no significant difference was recorded between the infection of both sexes. The highest rate of infection was 25.8% in the age group (1-9) years, with significant differences ($P \leq 0.05$) from the rest of the age groups. The infection rate in the rural was 14.5% higher than in the urban 8.4%. The highest rate was recorded at 41.5% in April and the lowest at 2.5% in November, while the summer months did not record any infections. The study also included an examination of 188 samples of animal feces (48 sheep, 50 cows, 47 goats, and 43 dogs), the parasite was not diagnosed in any of the examined animals. This is the first study in the country to investigate the parasite in goats. The study also included the investigation of the *C. cayetanensis* parasite in five types of vegetable leaves (garden cress, radish, leek, green onions, and purslane). Where it is observed the presence of Oocyst in vegetables washing water by a percentage (6, 7.8, 7.2, 4.4, and 3.2) Oocyst/ liter respectively.

1. Introduction

Cyclospora cayetanensis is a parasitic protozoan that causes cyclosporiasis or gastroenteritis, which is severe in people with weak immunity but rarely causes death [1]. The parasite was discovered in the 1990s. Despite its recent discovery, research points to its global widespread, especially in tropical and subtropical regions [2]. The parasite belongs to class Sporozoa and subclass coccidia [3]. Species belonging to this genus infect many mammals, reptiles, and birds, but humans are the only host of the species *C.cayetanensis* [4]. This parasite is transmitted to humans by the ingestion of the mature Oocyst with food and contaminated water [5]. *C.cayetanensis* Oocysts are circular in shape and range from 8-10 microns, each Oocyst has two sporocyst, each containing two sporozoites, and the envelope of Oocyst is wrinkled [6]. The parasite causes many symptoms, including watery diarrhea, muscle pain, weight loss, fever, and long-term complications such as reactive arthritis [7].

Several studies were conducted to investigate this parasite, for instance, ogren *et al.,* [8] found that the ratio was 5.3% in Venezuela. Besides, the Centers for Disease Control and Prevention (CDC) has conducted several studies in the United States, where 372 cases were recorded in 16 states by eating salad contaminated with the parasite in 2013. Moreover, In South Carolina, 200 cases were recorded in

2014 and the number reached 316 cases in 2015, but in 2016 the number of infections was 384 [9]. Besides, Edwinson [10] indicated a 1.8 % infection rate in North Dakota.

As for studies in Iraq, Al-Samarrai [11] found that the infection rate was 1.2% in Tikrit province, and a study by Abdullah [12] reported an infection rate of 2.2% in the province of Diwaniyah. Also, Al-Morshedi [13] recorded an infection rate of 1.9% in Babylon province. Furthermore, Salman *et al*., [14] found an infection rate of 0.49 % in Kirkuk province in 2016, the researcher Sulaimani [15] mentioned a 4% infection rate in Sulaymaniyah province in the same year.

Since this parasite was recently discovered in the world, and due to the lack of studies focusing on this parasite in Anbar province, this study was conducted to find out the prevalence of infection among the population of this province and its diagnosis, as well as in some animal species. Moreover, this study includes the investigation of the parasite in some types of vegetables, to identify sources of infection, to plan health services programs to limit the spread of parasitic infections that may cause health and economic damage.

2. Materials and Methods

2.1. Collecting and examining human stool samples

560 stool samples were collected from patients coming to hospitals Ramadi, Falluja, and Haditha and health centers from age groups (1-69) years for both genders. The information for each patient was recorded and the samples were examined using two methods:

2.1.1. The modified Zell–Nelson method. A sample of stool was taken and brushed on the slide to make a thin smear and left to dry. It was fixed using absolute ethyl alcohol for 5 minutes and left to dry. Then, a carbol Fuxin was used for 60 minutes to dye the slide. After that, the samples were washed with tap water and the sulphuric acid (2%) was added for 20 seconds. The acid was removed by tap water then the slide was stained with 5% green malachite stain for 5 minutes. After that, it was washed with water and left to dry. Finally, the slide was examined with a microscope on (40x) and (100 x) [16].

2.1.2. UV fluorescent microscopy method. A sample of the stool was taken and mixed with a drop of normal saline solution on a slide and covered with a cover slide. Then, the slide was examined using a fluorescent microscope on the wavelength (340-380) nm [17].

2.2. Collection and examination of animal feces samples. A total of 188 animal feces were collected in Anbar province (48 sheep, 50 cows, 47 goats, and 43 dogs). Plastic bottles were used to keep the samples, which were examined in a floatation method with the saturated sugar solution [11]. The examination was applied by mixing 4-5 g of feces with 10-15 ml of distilled water, then was filtrated in several layers of gauze and placed in the centrifuge at 1000 cycle/min for 10 minutes.

After that, the floating part was removed and 10 ml of the saturated sugar solution was added to the sediment, and placed in a centrifuge at the same speed and the operation was repeated 3 times to collect as many Oocysts as possible. Then, a drop from the surface of the solution was taken and placed on a slide. The slide was dyed with the stain of modified Zell – Nelson as in the previous method and examined on (40x) and on (100x). After that, another drop of the solution was collected and put on another slide, and examined under a fluorescent microscope.

2.3. Collection and examination of water samples of washing vegetables

Fresh vegetable leaves were collected including (garden cress, radish, leeks, green onions, and purslane) in clean pots and washed with distilled water, 25 liters each time and the washing process was repeated 3 times. The washing water was collected each time separately, and water samples were filtered to investigate the presence of the Oocysts [18] using the filter apparatus and filter papers, the paper is divided into 4 sections and washed with 400 ml distilled water, the washing water was

distributed in 4 glass tubes of capacity 100 ml and placed in the centrifuge at a speed of 2000 cycle/minute for 10 minutes. The floating part was removed and the distilled water was added to the sediment and the previous operation was repeated. Then, equal amounts of Tween 80 (0.1%) and Sodium Dody coel sulphate 0.1% were added to the precipitate and put in a centrifuge at a speed of 200 cycles/minute for 10 minutes.

The floating part was removed and the previous operation was repeated and then the precipitate was washed with Phosphate buffer (PH: 7.2) twice and Placed in the centrifuge at the same speed for 10 minutes. The floating parts were removed and 10 ml of a saturated sugar solution was added to the precipitate to float the Oocysts and put in a centrifuge at a speed of 1000 cycle/minute for 10 minutes. The process was repeated again to collect the largest number of Oocysts. A drop from the surface of the solution was taken and examined under the fluorescent microscope، where Oocysts were counted on a Neubarchamber slide to measure the size of Oocysts using the ocular micrometer [19].

2.4. Statistical Analysis

The data were statistically analyzed using the chi-square test to determine the lowest significant difference LSD at the probability level ($P \leq 0.05$) using a set of ready-made statistical software SAS [20].

3. Results and Discussion

The present study is the first to focus on the occurrence of *Cyclospora cayetanensis* in humans, animals, and vegetables in Anbar province- Iraq. The present study showed that the overall prevalence was 12.1% (68/560) during the examination of stool samples collected from patients coming to some hospitals in the province. According to the gender, there are no significant differences ($P \leq 0.05$) between the infection rate of males (12.5%) and females (11.7%) (Table 1). This agrees with the study by Yazar *et al,* [21] in Turkey with 12.5 %, and was higher than that found by Chacin-Bonilla [8] in Venezuela where it was 5.3% and the study by Kaminsky *et al*, [22], where the rate was 1.3 % in Honduras, and a study by Abdullah [12] in Diwaniyah, where the rate amounted to 2.2%, and lower than that found in the study by Giangaspero *et al.,* [23] in southern Italy which amounted to 27.5%. The difference in this ratio with previous studies was due to several reasons including study areas, the number of samples examined, and methods of diagnosing parasites.

The high rate of infection in this study compared to previous studies in the provinces of the country is due to the deterioration of the health and service conditions due to the bad conditions in the previous three years, particularly, the war and displacement, as well as the lack of clean drinking water. It is also caused by water pollution, which plays a big role in the spread of infection and this is confirmed by Alsqr *et al.,* [24]. Furthermore, it is also caused by a lack of attention to food hygiene especially, fruits and vegetables that are without being washed and have a significant role in the spreading infection. The results showed no significant differences in the incidence of infection between males and females, this agrees with studies were done by Wang *et al.,* [25] Which recorded an infection rate of 2.61% in males and 1.44% in females. This indicates that the chances of exposure are equal for both sexes through contaminated water and food. This was illustrated by Hussein [26] that sex and race did not affect parasitic infection.

Table 1. Infection rate of C. cayetanensis parasite in human according to gender.

Gender	Number of examined sample	Positive sample	Percentage %
Male	262	33	12.5
Females	298	35	11.7
Significant variations			Less than 0.001

The highest rate of infection was 25.8% in the age group (1-9) years and the lowest rate was 1% in the age group (30-39) years. The statistical analysis showed a significant difference ($P \leq 0.05$) for age (Table 2). This is consistent with the study by Yadav *et al.,* [27] in India. The reason is that this

parasite is one of the most prominent parasites in children, especially if accompanied by malnutrition and immunodeficiency, and poor ways of preparing food, and may be due to lack of health awareness among children, lack of attention to hygiene, eating and drinking from peddlers, unhealthy places and shops, playing in the streets, and direct contact with sources of infection of dust and water contaminated with Oocysts[8].

Table 2. Infection rate of C. cayetanensis parasite in human according to age.

Age group (year)	Number of examined sample	Positive sample	Percentage %
1-9	112	29	25.8
10-19	96	21	21.8
20-29	72	12	16.6
30-39	93	1	1.0
40-49	83	3	3.6
50-59	71	1	1.4
60-69	33	1	3.0
Total Significant variations	560	68	12.1 Less than 0.001

The infection rate in rural areas was 14.5% higher than in the urban areas 8.4%and with statistically significant differences ($P \leq 0.05$) (Table 3). This agrees with Al-Qobati [28]. This is due to the low cultural and social level, lack of health awareness among the rural population, lack of attention to hygiene, water pollution, and the lack of potable water. Many rural people depend on the water of the rivers and streams and eat vegetables directly from the farm without washing and disinfecting them. Also, direct contact with sources of infection from contaminated soil and water as well as swimming in the rivers cause the spreading of infection.

Table 3. Infection rate of C. cayetanensis parasite in human according to residence region.

Residence region	Number of examined sample	Positive sample	Percentage %
Rural	336	49	14.5
Urban	224	19	8.4
Total Significant variations	560	68	12.1 0.001

Table 4 shows the relationship between the infection rate and the months of the year, the highest infection rate was recorded in April (41.5%) and the lowest infection rate was recorded in November (2.5%), but there are no infections was recorded in the summer months. This result is in agreement with the study of Jiang *et al.* [29]. This may be due to Oocyst's need for moderate temperature which coincides with rainfall in the spring months to complete Oocyst ripening, where infection events, seasonal changes, and environmental factors play a major role in determining infestation rates that increase in spring [30].

The current study showed that no infection with this parasite was recorded in all the animals examined. This is consistent with the study of the Samurai [11]. Many studies have been conducted all over the world to diagnose the parasite in different types of animals, but none of them prove the existence of host reservoirs for the parasite and thus humans are the only host for this parasite [4].

Table 5 shows that the numbers of Oocysts of *C.caytanensis* in the washing water for five types of vegetables namely (garden cress, radish, Leeks, green onions, and purslane) are (6, 7.8, 7.2, 4.4, and 3.2) Oocyst/liter respectively, this corresponds to a study by Sim *et al.,* [31] in Korea, where they.

Table 4. Infection rate of C. cayetanensis parasite in human according to Months.

Months	Number of examined sample	Positive sample	Percentage %
January	58	4	6.8
February	61	2	3.2
March	55	16	29.0
April	65	27	41.5
May	50	12	24.0
June	48	-	0
July	32	-	0
August	29	-	0
September	44	3	6.8
October	46	2	4.3
November	40	1	2.5
December	32	1	3.1
Total	560	68	12.1
Significant variations			0.001

recorded an infection rate of 1.2% while examining six types of vegetables (chives, cherry tomatoes, winter cabbage, sprouts, berries, and perilla leaves), also, agree with a study Latif *et al*., [32] in Baghdad Province, they found an infection rate of 3.7%, during their examination of 54 vegetable samples (lettuce, parsley, and basil). Contamination of vegetables with Oocysts is attributed to washing them with polluted water or using human excrement to fertilize agricultural land, as washing vegetables with polluted water leads to the spread of infection. Hussein [26] mentioned that Oocysts resist the natural proportions of chlorine used in water treatment, Therefore, Oocysts remain stuck in the leaves of the vegetable even after repeated washing, this proves the role of food in the spread of infection

The Oocysts of the parasite dyed with Modified Zell-Nelson stain were spherical in shape, pale and red. Also, its dimensions were between 8 -10 microns (Figure 1). Some or all of the Oocysts may not be stained with carbol Fuxin and this will not differentiate them from the green color of the green malachite stain, while the Oocysts appeared in a shining ring when examined with a fluorescent microscope (Figure 2).

Table 5. Number of Oocysts C.cayetanensis parasites in the vegetable wash water.

Types of vegetables / Number of times Washing	Oocyst/liter				
	Garden cress	Radish	Leek	Green Onions	Purslane
First washing stage	3.2	4	3	2.8	2
Second washing stage	1.8	2.6	2.6	1	1.2
Third washing stage	1	1.2	1.6	0.6	0
Total	6	7.8	7.2	4.4	3.2

The total three stages are 75 liters.
The sample size at each washing stage is 25 liters.

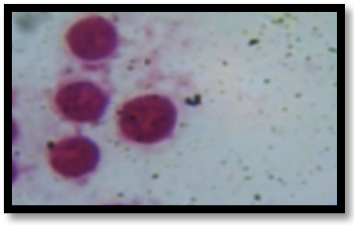

Figure 1. Oocysts of *C.cayetanensis* stained with Zell-Nelson modified 100x

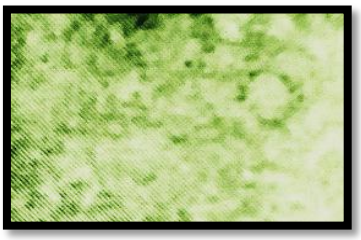

Figure 2. Oocysts of *C. cayetanensis* examined with fluorescent microscope 100x

4. Conclusion

This study concluded that infection of humans and vegetable species with *C. cayetanensis* parasite in Anbar province. Thus, the current study provides exploratory indicative data for future monitoring of parasitic diseases of medical importance, identification of risk factors, and minimization of economic losses to chart strategies to control these diseases. This is the first study in the province, in which the parasite is diagnosed in humans and these types of vegetables.

References

[1] Hadjilouka, A and Tsaltas, D 2020, *Cyclospora Cayetanensis*-Major Outbreaks from Ready to Eat Fresh Fruits and Vegetables. *Foods*, **9**(11), 1703.

[2] Rosser, JI and Blackburn, BG 2020, Pathogenic Intestinal Parasites in Transplant Recipients. Emerging Transplant Infections:*Clinical Challenges and Implications*, 1-55.

[3] Shahatha, SS 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.

[4] Li, J, Wang, R, Chen, Y, Xiao, L and Zhang, L 2020*,* Cyclospora cayetanensis infection in humans: Biological characteristics, clinical features, epidemiology, detection method and treatment. *Parasitology*, **147**(2): 160-170.

[5] Shahatha, SS 2019, Evaluation the efficiency of the fungus Metarhizium anisopliae as biocontrol agent for adults of hard ticks Hyalomma anatolicum. *Iraqi Journal of Veterinary Sciences*, **33**(2), 57-62.

[6] Li, J, Wang, Z, Karim. MR and Zhang, L 2020, Detection of human intestinal protozoan parasites in vegetables and fruits: a review. *Parasites & Vectors*, **13**(1): 1-19.

[7] Shahatha, SS and Saleh, TA 2018, An Epidemiological, Diagnostic, and Therapeutic Study of the Leishmania tropica Parasite in Iraq's Anbar Province. *Baghdad Science Journal,* **15**(4).392-400.

[8] Ögren, J, Dienus, O, Beser, J, Henningsson, AJ and Matussek, A 2020, Protozoan infections are under-recognized in Swedish patients with gastrointestinal symptoms. *Europan Journal Clinical Microbiology Infection Discese*, **39**(11): 2153-2160.

[9] Carter, R 2016, Foodborne outbreaks of Cyclosporiasis. Centers for Disease Control and prevention. *Retieved;* 30-June. U.S.

[10] Edwinson, A 2017, Glycan Triggers of Life Cycle Development in the Apicomplexan Parasite Cryptosporidium. Doctoral dissertation, *North Dakota State University*, 231p.

[11] Al-Samarrai, UIM 2008, Diagnostic and epidemiological study of the parasite Cyclospora cayetanensis in Tikrit and surrounding areas. *Ph.D. thesis, College of Education, University of Tikrit*. 331 P.

[12] Abdullah, SR 2014, Epidemiological survey on the prevalence of intestinal parasites in working in restaurants and food shops in Diwaniyah Governorate. *Al Qadsia pure scicence Journal* , **19**: (2).

[13] Al-Morshidi, KA and Al-Amari, MJ 2015, Detection of parasitic contamination in Hilla city drinking water/Babylon province/Iraq. *Advances in Natural and Applied Sciences*, **9**(3): 80-85.

[14] Salman, YJ, Al-Taee, ARA and Abid, AM 2016, Prevalence of Giardia lamblia among Iraqi displaced peoples in Kirkuk province. *International Journal Current Microbiology Appl Science,* **5**: 753-760.
[15] Sulaimani, SI 2016, Detection of Cyclospora cayetanensis Infections among Diarrheal Children Attending Pediatric Teaching Hospital in Sulaimani City. *Health Sciences*. **5**(3): 77-84.
[16] Khanaliha, K, Mohebali, M, Davoudi, S, Hosseini, OR, Tarighi, F, Rezaeian, T and Rezaeian, M 2015, Detection of emergence *Cyclospora cayetanensis* in A HIV+/AIDS patient with diarrhea from Tehran: a case report. *Iranian Journal public health*, **44**(6): 865-*868*.
[17] Garcia, LS and Procop, GW 2016, Diagnostic medical parasitology. 284-308.
[18] El-karamany, EM, Zaher TI and ei-Bahnasawy, MM 2005, Role of water in the transmission of cyclosporiasis in sharki Governorate., Egypt, *Journal Egypt social Parasitology*, **35**(3):953-962
[19] Hohweyer, J, Citeaux C, Travaillé E, Languet E, Dumètre A, Aubert, D and Loic, F *2016*, Simultaneous detection of the protozoan parasites Toxoplasma, Cryptosporidium and Giardia in food matrices and their persistence on basil leaves. *Food microbiology Journal*, **57**, 36-44.
[20] SAS. Statistical Analysis system 2012, users guide. statistical version 9th ed. New York: *SAS Institute Inc*; 5121 p.
[21] Yazar, S, Yalcin, S and Sahin, Í 2004, Human cyclosporiasis in Turkey. *World Journal Gastroenterol: WJG*, **10**(12): 1844.
[22] Kaminsky, RG, Lagos, J, Santos, GR and Urrutia, S 2015, Marked seasonality of Cyclospora cayetanensis infections: ten-year observation of hospital cases, Honduras. *BMC infectious diseases*. **16**(1): 66.
[23] Giangaspero, A, Marangi, M and Arace, E 2015, Cyclospora cayetanensis travels in tap water on Italian trains. *Journal water and health*. **13**(1): 210-216.
[24] Alsqr, IMM, Obeidi, SH and Ali, HM 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.
[25] Wang, KX, Li, CP, Wang, J and Tian, Y 2002, Cyclospore cayetanensis in Anhui, China. *World Journal of gastroenterology*, **8**(6), 1144.
[26] Hussein, SMF 2018, Detection of Parasitic Infections and Their Associated Risk Factors in Drinking Water at Basic Schools in Khartoum State-Sudan. *Doctoral dissertation, Sudan University of Science & Technology)*. *156*p.
[27] Yadav, P, Khalil, S, Mirdha BR, Makharia, GK and Bhatnagar, S 2015, Molecular characterization of clinical isolates of Cyclospora cayetanensis from patients with diarrhea in India. *Indian Journal medical microbiology*, **33**(3): 351-*356*.
[28] Al-Qobati, SA *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.
[29] Jiang, Y, Yuan, Z, Zang, G, Li, D, Wang, Y, Zhang, Y and Shen, Y 2018, Cyclospora cayetanensis infections among diarrheal outpatients in Shanghai: a retrospective case study. *Frontiers of medicine.* **12**(1): 98-103.
[30] Shahatha, SS 2019, An Epidemiological, Diagnostic and Therapeutic Study of Giardia lamblia in Anbar Province–Iraq. International Journal of Drug Delivery Technology, **9**(01), 39-45.
[31] Sim, S, Won J, Kim JW, Kim K, Park, WY and Yu, JR 2017, Simultaneous Molecular Detection of Cryptosporidium and Cyclospora from Raw Vegetables in Korea. *Korean Journal Parasitology.* **55**(2): 137-*142*.
[32] Latif, B, Al-Talib, H and Al-Akely, S 2020, Prevalence of Intestinal Protozoa among Humans, Animals and Vegetables in Baghdad, Iraq. *International Medical Journal,* **27**(2),136-140.