

Diagnosis of *Cyclospora Cayetanensis* in Diabetic Patients

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ABSTRACT: This study was carried out at Wasit Province / Iraq in cooperation with AL-Karamah Teaching Hospital. Samples were collected from many places at Wasit Province. It included 46 stool samples from Iraqi patients. The study conducted during period from September to October, 2023 to investigate the prevalence of *Cyclospora caytanensis*. Only 18 stool sample were positive for *C. Cayetanensis*. These samples were collected from the urban and rural areas of Wasit Province. All samples were examined microscopically using the direct wet smear and staining with modified Ziehl – Neelsen stain. The result showed that the positive samples of *C. cayetanensis* infection among patients were 18. With regard to parasitic infection, the highest infection rate was 15(83.3%) recorded for the group (51-60) years old. Also, the males reported higher 14 (77.7%) than the females 4 (22.2%). According to the residence, rural areas were reported 8 (44.4%) and urban areas are 10 (55.5%) with significant differences. The purpose of this study is to detection the infection with cyclospora in immunocompromised patient.

Keywords: Microscope, *Cyclospora cayetanensis*, Ziehi-Neesen stain.

1. INTRODUCTION

Cyclospora cayetanensis is a coccidian parasite that causes a human-specific gastrointestinal disease called cyclosporiasis. It has a direct fecal–oral transmission cycle. And the transmission occurs when sporulated oocysts of the parasite are ingested through consumption of contaminated food or water (1- 3). After ingestion of oocysts, symptoms of cyclosporiasis begin within an average of 7 days (ranging from 2 days to ≥ 2 weeks post ingestion. Although the course of the infection can be more severe in immunosuppressed patients, cyclosporiasis is normally self-limiting. Infected individuals shed unsporulated oocysts; once outside the host, the oocysts can sporulate and become infectious within 7–15 days, depending on ideal environmental factors (4, 5).

Cyclosporiasis is becoming a significant public health concern in food production. The detection of *Cyclospora cayetanensis* in produce should be considered a possible risk to public health (6). Oocysts of this parasite have already been detected in fresh produce items such as lettuce, parsley, green onion, cucumber, celery, tomato, spinach, basil, blueberries, and raspberries, among others, in many surveillance studies worldwide (2,7,8,9,10). Oocysts of *C. cayetanensis* have also been

identified in farm workers and food handlers, which reinforces the need for the development, implementation, and monitoring of on-farm control measures in endemic areas (4, 11). Over the last few years, outbreaks and sporadic cases of cyclosporiasis associated with the consumption of fresh produce were reported in Latin America (4), Europe (12, 13), and North America (12, 14, 15).

The aim of study:

- 1-detection of infection with *cyclospora cayentanensis* in immunocompromised patient at wasit province.
- 2-Study of some related variable with *cyclospora* infection

2 MATERIALS AND METHODS

2.1 Materials:

Normal saline, Modified Ziehl-Nelsen stain, glass slide, microscope, stick wood, plastic container.

2.2 Methods:

2.3 Stool specimens' collection:

A total of 46 stool samples were obtained randomly from patients who suffering from diarrhea in Al karamah Teaching Hospital at Wasit province from September to October, 2023. Before analyzing the fecal samples, the special questionnaire form was prepared to denote full information from each patient which was relevant to various epidemiological factors that might be responsible for parasite infection which included patient name, age, gender, and region. The stool samples were collected in clean universal screw cap bottles from patients; it placed in dry plastic container and transported to the laboratory for microscopically examination.

Identification of Intestinal parasite in collected sample:

Fecal samples were collected from diarrheal patients in clean screw capped tubes. Socio-demographic data were collected simultaneously with sample collection such as gender, age, and residence area. Outside hospital collected samples were transported to the laboratory within two hours for immediate microscopic examination. After collection, the stool samples placed in dry plastic container and transported to the laboratory for microscopically examination (16).

Small portion of each stool sample mixed with a drop of 0.9% normal saline (NaCl) on a glass slides making a wet smear were and leave the sample to air dry after that staining with Modified Ziehl-Neelsen was used on thin fecal smears for coccidian protozoa oocyst detection by 100 X oil immersion (17). *Cyclospora*, *Cryptosporidium*, and *Cystoisospora* oocyst walls contain acid-fast lipids (18), which is a common property and makes acid-fast staining relevant for the screening of these three parasites in a single test. Although modified acid-fast staining can be used to identify these organisms, in *Cyclospora*, variable levels of dye uptake may result in ghost cells, or poorly stained cells, along with well stained ones (20,18 ,19) .There have been minor variations in modified acid-fast stains to improve *Cyclospora* detection. One of the favored modifications is to use 1% H₂SO₄ as a decolorizer without alcohol (18).

2.3 Statistical analysis:

Descriptive analysis was used with percentage to express the positive sample of cyclospora cayetanensis.

3 RESULTS AND DISCUSSION

A total of 46 stool sample of diabetic patients were examined by microscope. The results appeared that 18(39.1%) of feces samples were positive for parasite of *Cyclospora cayetanensis* among diarrheal cases that staining by modified Ziehl- Neelsen stain.

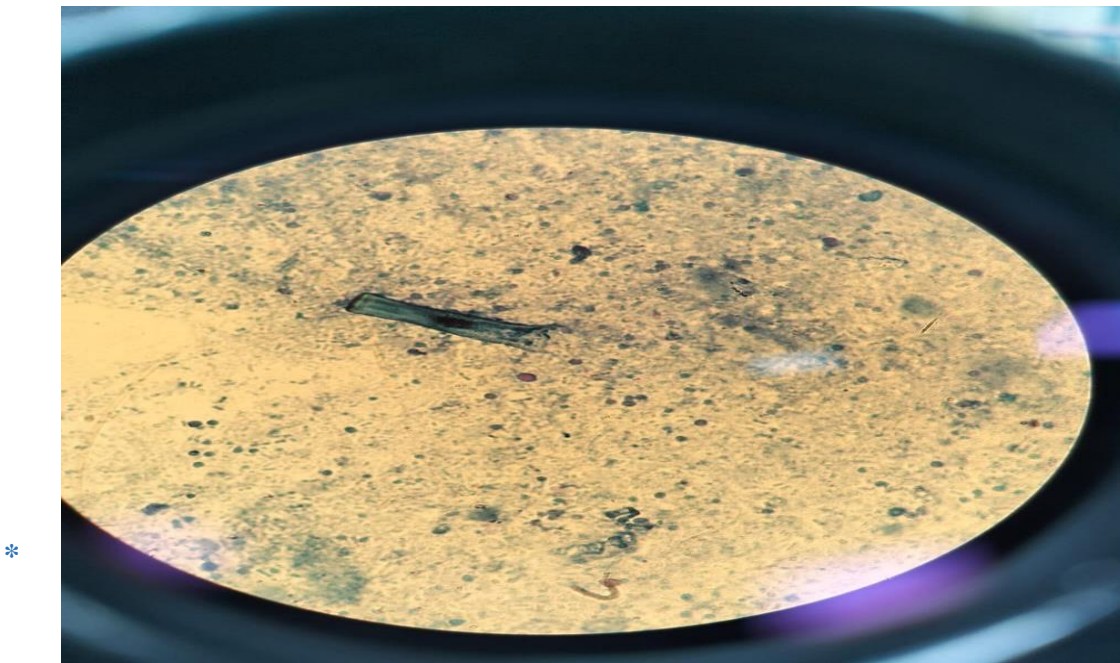


Figure 1: Oocyst of *Cyclospora Cayetanensis*

Table 1. Distribution of *C. Cayetanensis* infection according to age groups and residency

Age groups (Years)	No. of examined sample	No. of <i>C. cayetan ensis</i> infection	Urban %	Rural %
20-30	8	1(5.5%)	0	1 (5.5%)
31-40	4	1(5.5%)	1(5.5%)	0(0.0%)
41-50	10	0	0 (0.0%)	0 (0.0%)
51-60	15	10(55.5%)	6 (33.3%)	4 (22.2%)
61-70	8	5(27.7%)	3 (16.6%)	2(11.1%)
71-80	1	1(5.5%)	0 (0.0%)	1(5.5%)

Table (1) shows the total number of people examined and the infection rates for parasite according to the age groups and residence under study. The total infection rate with *C. Cayeteaensis* was 39 %. It was found that the highest rate of infection with *C. Cayeteaensis* was within the age group (51-60) years, it reached 21.7% and the lowest infection rate was within the age group (41-50) years. The coccidian parasites are important pathogens. Many physicians remain unaware of their clinical importance (24). *Cyclospora* has now been identified worldwide in the feces of both immunocompetent and Immunocompromised patients with diarrhoea (28-29). Several studies have documented the fact that *C. cayetanensis* is diarrheal causing agent (21, 22, 23, 25, 26, 27). The prevalence of infection in the current study is high compared to results of previous studies. The reason for the difference may be attributed to many reasons, the most important of which are the service and environmental situation, as well as public hygiene, attention to personal culture, and the difference in the standard of living and social. The stool examined samples were collected from different age groups, starting from 20 to 70 years. From the data observed that there was no significant difference between urban (55.5%) and rural (44.5%) 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(35,36). This result agreed with the results of (37,38), but it was disagreed with other studies carried out by (32) in Egypt and (39) 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 (33).

Table 2. Distribution of *C. Cayetanensis* infection according to age groups and gender

Age groups (Years)	No. of examined sample	No. of positive sample	Positive male sample	Positive female sample
20-30	8	1(5.5%)	1(5.5%)	0(0.0%)
31-40	4	1(5.5%)	1(5.5%)	0(0.0%)
41-50	10	0(0.0%)	0(0.0%)	0(0.0%)
51-60	15	10(55.5%)	9(50%)	1(5.5%)
61-70	8	5(27.7%)	3(16.6%)	1(5.5%)
71-80	1	1(5.5%)	2 (11.1%)	0(0.0%)

Table (2) shows the total number of people examined and the infection rates for parasite according to the age groups and gender under study. The total infection rate with *C. Cayetanensis* It was found that the highest rate of infection with *C. Cayeteaensis* was in male and the lowest infection rate in female. This result disagreed with the results of (30) in Nepal, (31) in Anhui, China and (32) in Egypt, but the result agreed with results of (33) in Alexandria, Egypt and by (34) in Kathmandu, Nepal.

Table 3. Distribution of *C. Cayetanensis* infection according to age and diarrheal groups

Age groups (Years)	No. Of examined sample	No. of positive sample	Diarrheal sample	Non
20-30	4	1(5.5%)	1(5.5%)	0(0.0%)
31-40	8	1(5.5%)	0(0.0%)	1(5.5%)
41-50	10	0(0.0%)	0(0.0%)	0(0.0%)
51-60	15	10(55.5%)	8(44.4%)	2(11.1%)
61-70	8	5(27.7%)	5(27.7%)	0(0.0%)
71-80	1	1(5.5%)	1(5.5%)	0(0.0%)

The coccidian parasites are important pathogens. Many physicians remain unaware of their clinical importance (24). Cyclospora has now been identified worldwide in the feces of both immunocompetent and immunocompromised patients with diarrhoea (28-29). Several studies have documented the fact that *C. cayetanensis* is a diarrhoea causing agent (21, 22, 23, 25, 26, 27). The incidence of infection in the current study is high compared to results of previous studies. The reason for the difference may be attributed to many reasons, the most important of which are the service and environmental situation, as well as public hygiene, attention to personal culture, and the difference in the standard of living and social.

4 CONCLUSIONS

In conclusion, the purpose of this study was to determine the prevalence of *Cyclospora cayetanensis* in patients with diabetes. It was carried out in cooperation with Al-Karamah Teaching Hospital in Wasit Province, Iraq. 18 of the 46 stool samples taken from diarrhea patients were positive for *C. cayetanensis* under a microscope using a modified Ziehl-Neelsen stain and a direct wet smear. The investigation showed that different age groups had variable infection rates, with the 51–60 age group showing the highest frequency (83.3%). Infection rates were greater in males (77.7%) than in females (22.2%). Furthermore, the infection rate in urban regions was found to be 5.5% higher than in rural areas (44.4%). These results highlight the need to comprehend the epidemiology of *C. cayetanensis* in certain demographic groups, especially in patients with diabetes, and the requirement for efficient control and prevention methods. Addressing the growing public health problems linked to *Cyclospora* infections will require further investigation and observation, particularly in light of the disease's link to tainted food and water supplies.

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