

## ***Cyclospora cayetanensis*: First Report of Prevalence and Risk Factors for Infections among Immunocompetent Children with Diarrhea, Sana'a, Yemen**

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### **Abstract**

**Objective:** The present study conducted for the first time to determine the prevalence and risk factors for infection with *C. cayetanensis* among immunocompetent children with diarrhea in Amanat al-Asimah Sana'a, Yemen.

**Methods:** Using cross-sectional approach, a total of 282 children, 157 (55.7%) males and 125 (44.3%) females, aged 1 -< 13 year were recruited from two Governmental hospitals. Relevant data were enrolled by means of questionnaire. Single fecal specimens were collected and modified Kinyoun's acid fast straining of formalin- ether concentrate feces were examined for the detection of *C. cayetanensis* oocysts.

**Results:** The rate of infection was found to be 4.6% (13 of 282). Age, eating of raw vegetables and fruits, and use of public and/or well water for drinking were the risk factors that significantly associated with infection ( $P < 0.05$ ). Children passed watery diarrheic stool (WDS) showed a higher percentage of infection, compared to those who passed soft diarrheic stool (SDS). Watery diarrhea was associated with a high rate of cyclosporiasis cayetanensis in spite of being not statistically significant. The risk of infection neither differed significantly with children's sex, food consumption habits nor residence.

**Conclusion:** Prevalence of *C. cayetanensis* infection among immunocompetent diarrheic children in Sana'a is a substantial. Age, eating of raw vegetables and fruits, and use of public and/ or well water for drinking were the risk factors that significantly associated with infection.

**Keywords:** *C. cayetanensis*; Prevalence; Risk Factors; Immunocompetent Children; Diarrhea; Sana'a

### **Introduction**

*Cyclospora cayetanensis* (*C. cayetanensis*) is an apicomplexan oocysts-forming protozoan parasite that infects the microvillus border of the gastrointestinal tract of the susceptible hosts [1]. It has emerged as an important human coccidian causing gastroenteritis and persistent diarrhea in both immunocompromised and immunocompetent individuals especially children [2-4]. It was first described in 1979 [5], and classified in 1994 [6]. The only stage seen in fresh stool specimen is unsporulated oocysts, which are non-infectious, and require 7 to 10 days outside host under ideal conditions (23 to 27°C), to sporulate and presumably become infectious [6,7]. The route of transmission to susceptible host is mainly through ingestion of food and/ or water contaminated with sporulated oocysts [1,8,9]. Contaminated raw fruits and vegetables were identified as further vehicles for the transmission of infection [1]. The coccidian has associated with traveler's diarrhea in adults [4,10]. Approximately 17 species of the genus *Cyclospora* are now recognized; one species; *C. cayetanensis* is the only causing agent of human cyclosporiasis [8,11]. The resistance of oocysts to chlorine level used in water treatment contributes greatly to spread of coccidian via drinking water [12]. Persons with no previous immunity as well as very young children are likely to

exhibit symptoms [13]. Protracted watery diarrhea lasts between few weeks to months is pronounced symptom reported in previously infected healthy individuals. Those may also experience abdominal cramps, flatulence, vomiting, weight loss, low grade fever, and fatigue [8,14]. Diagnosis of *C. cayetanensis* infection is based on the observation of non-sporulated oocysts measuring from 8 to 10  $\mu\text{m}$  in stool smears stained by modified Kinyoun's method( that used for detection of other acid fast coccidia) [15]. The disease is under- reported by physicians, and it is under- diagnosed in Yemeni hospital laboratories, besides the lacking studies of such problem. Characterization of epidemiology of cyclosporiasis cayetanensis may identify geographic and socio-demographic risk factors that may contribute to the disease. The objective of the current study was designed to assess the prevalence of infection with *C. cayetanensis* among immunocompetent children with diarrhea in Amanat al- Asimah Sana'a, Yemen, and to identify potential risk factors that could be associated with infection.

## Subjects and Methods

### Study subjects and sampling

This study was conducted during the period from June to December, 2014 in Amanat Al- Asimah Sana'a, Yemen. Using cross-sectional approach, a total of 282 children, 157(55.7%) males and 125 (44.3%) females, aged one to less than thirteen year were recruited. They were attended the pediatric clinics of two Governmental hospitals; Al-Sabeen (153 children), and Al-Gomhoree (129). Majority of subjects (52.5%) suffered from watery diarrhea. Information about each child were enrolled by means of questionnaire, filled with assistance of child's parents or companion when necessary. Requested data included; some socio-demographic and environmental data as, name, sex, residence (urban/rural), food consumption habits, eating of raw vegetables and fruits, drinking water source, stool consistency, and history of diarrhea episode was recorded. Subjects were apparently immunocompetent, they had no history of receiving immunosuppressive agents from their reports. Fresh single fecal specimen was collected from each subject in a sterile labeled plastic covered cup, about one ml of each specimen was placed in a labeled tight-bottle containing 3 ml of 10% formalin until being processed. To diagnose oocysts of *C. cayetanensis* and other acid fast coccidia, each specimen was subjected to formol- ether concentration technique. A thin smear was prepared from the sediment and stained by modified Kinyoun's acid fast straining method [15].

### Modified Kinyoun's acid-fast staining technique (Kinyoun's cold method)

After concentration of fecal specimen in formol-ether technique, a thin smear was prepared from one drop of each fecal sample sediment and it was fixed in absolute methanol for one minute. Smear was stained with cold Kinyoun's carbol fuchsin for 5 minutes, the stain was rinsed off in 50% ethanol for about 3 seconds and then the stain rinsed off in clean tap water. Smear was decolorized using 1% sulfuric acid until no more color flooded from the smear. Decolorizer was rinsed off in tap water. Smear was counter stained with 0.3% malachite green for about 1 minute. Counter stain was rinsed off in tap water and blot to dry. Smear was examined microscopically using high dry objective to identify the oocysts and oil immersion objective to see the internal morphology. In positive sample, oocysts of *C. cayetanensis* and *Cryptosporidium species* appeared as acid fast densely stained pink to red spherical structures measuring 8 - 10 and 4 - 6  $\mu\text{m}$  in diameters respectively against a green background [15].

### Data Analysis

The data in questionnaires were transcribed onto computer coding sheets, and statistically analyzed by MstatC program. The results were tested by Chi-square ( $X^2$ ) test for significance between categorical variables. A value of  $P < 0.05$  was considered significant.

### Ethical consideration

Ethical approval was yielded from the health authorities with agreeableness of each hospital principal. The purpose of research was explained to children and their parents or companions, and informed consent was obtained from each, prior to subjects inclusion in the study.

## Results

Out of 282 children's fecal specimens examined, 13 (4.6%) and 32 (11.3%) were positive for oocysts of *C. cayetanensis* and *Cryptosporidium species* respectively (Table 1). Two cases (0.7%) of thirteen infected with *Cyclospora* were co-infected with *Cryptosporidium* (Table

2). Percentage of infection increased significantly as the children's age decreased. The higher percentage of *C. cayetanensis* infection was among children aged 1- 4 years (8.7%) compared to 2.4% and 1.9% among those aged 5- 8 and 9- <13 years respectively with statistically significant difference; ( $X^2 = 6.68, P = 0.033$ ). Eating of raw vegetables and fruits was another risk factor for cyclosporiasis; ( $X^2 = 4.18, P = 0.040$ ). Moreover, children who drank public and/ or well water were associated with the higher risk of infection (10.9%) than those who drank sieved and tap water (2.1% and 4.2%) respectively; ( $X^2 = 7.37, P = 0.025$ ) (Table 3). Majority of children (52.5%) were suffered from watery diarrhea. Subjects passed watery diarrheic stool (WDS) showed a higher percentage of infection, compared to those who passed soft diarrheic stool (SDS). Watery diarrhea as well elevated the rate of infection despite being not statistically significant (Table 4). The risk of infection neither differed significantly with children's sex, food consumption habits nor residence (Table 3, 5).

Type of coccidian	Infection with coccidia (N = 282)	
	No.	%
<i>C. cayetanensis</i>	13	4.6
<i>Cryptosporidium spp</i>	32	11.3
Total	45*	15.9

**Table 1:** Distribution of infections with intestinal coccidia among immunocompetent Yemeni children with diarrhea according to type of coccidian.

\*2 Children had double coccidial infection (*Cyclospora* and *Cryptosporidium*).

Type of coccidian	Infection with coccidia (N = 282)	
	No.	%
<i>C. cayetanensis</i> (single)	11	3.9
<i>Cyclospora</i> and <i>Cryptosporidium</i> (mixed)	2	0.7
<i>Cryptosporidium spp</i> (single)	30	10.6
<i>Cryptosporidium</i> and <i>Cyclospora</i> (mixed)	2	0.7
Total	45	15.9

**Table 2:** Co-infections with *C. cayetanensis* and *Cryptosporidium* species among immunocompetent Yemeni children with diarrhea.

Variable	No. examined	<i>C. cayetanensis</i> infections		Chi-Square $X^2$ (P. value)
		No.	%	
Age groups in years				
1 - 4	104	9	8.7	6.68 (0.033)*
5 - 8	125	3	2.4	
9 - < 13	53	1	1.9	
Sex				
Males	157	7	4.5	0.01 (0.900)
Females	125	6	4.8	
Food consumption habits <sup>o</sup>				
Under-cooked	44	2	4.5	0.00 (0.990)
Sufficiently cooked	238	11	4.6	

Eating of raw vegetables and fruits <sup>‡</sup>				
Yes	83	8	9.6	4.18 (0.040)*
No	199	5	2.5	
Drinking water source				7.37 (0.025)*
Public and/or well water	46	5	10.9	
Tap water	143	6	4.2	
Sieved	93	2	2.2	
Total	282	13	4.6	

**Table 3:** Distribution of *C. cayetanensis* infections among immunocompetent diarrhoeic children according to socio-demographic variables.

\*P < 0.05, <sup>‡</sup>Vegetables as: broad beans, lettuce or salad, peas, basil and fruits as: berries, strawberries..etc...

<sup>◊</sup>Food including: fleshes, fishes, eggs, cereals, cedar...etc

Stool consistency	No. examined	<i>C. cayetanensis</i> infections		X <sup>2</sup> (P. value)
		No.	%	
WDS <sup>&amp;</sup>	148	10	6.8	2.36 (< 0.200)
SDS <sup>#</sup>	134	3	2.2	
Total	282	13	4.6	

**Table 4:** Association between *C. cayetanensis* infections and stool consistency.

<sup>&</sup>WDS: Watery Diarrheic Stool; <sup>#</sup>SDS: Soft Diarrheic Stool.

Residence	No. examined	<i>C. cayetanensis</i> infections		X <sup>2</sup> (P. value)
		No.	%	
Urban	242	11	4.5	0.02 (0.800)
Rural	40	2	5.0	
Total	282	13	4.6	

**Table 5:** Percentage of *C. cayetanensis* infections among immunocompetent diarrhoeic children according to residence.

## Discussion

Diarrheal disease is the second leading cause of death in children particularly those who under five years old, and is responsible for killing 1.5 million children every year. All over the world, there are about two billion cases of diarrheal disease annually, and mostly results from contaminated food and water sources with enteric pathogens. A number of agents are associated with human diarrhoea including major types of microorganisms: bacteria, viruses and parasites [16]. *C. cayetanensis* is a coccidian protozoan parasite that has emerged as an enteric pathogen associated with diarrheal illness in both unhealthy and healthy individuals [5,7,16]. For this reason the causes of infective diarrhoea illness should have to be properly investigated and treated.

In the current study, the prevalence rate of *C. cayetanensis* among immunocompetent diarrhoeic children was found to be 4.6%. Nearly similar rates, 4.4%, 5%, and 4% were reported from Cuba, Nepal, and Iraq respectively [17-19]. However, lower rates were stated from Kuwait, Mexico, and Honduras, 0.4%, 0.7%, and 1.3% respectively [20-22]. In contrast, higher rates of 29.4%, 17%, 12.1%, and 11% were recorded from Nigeria, Egypt, Nepal, and Saudi Arabia respectively [23-26]. The rates of infection may have differed according to multiple factors; including; type of the study, hospital or community based, socioeconomic, sanitary and environmental conditions. In addition to these, the parasitological methods applied to each study could influence the outcome of results [22].

In the present work, percentage of infection increased significantly as the age decreased. Thus, those aged 1 - 4 years represented the highest percentage of infection, whereas those aged 5 - 8 and 9 - < 13 years represented the lowest one ( $P = 0.033$ ). The rate of infection increased gradually as the age decreased and strong tendency was observed that, the younger the age the higher the infection rate. This proposed that, the higher rate of infection is restricted to the very young old, and young children are more likely to develop clinical symptoms [1,18]. The severity of symptoms and duration of infection tend to be milder after repeated infections, which could be suggestive of acquired immunity. Older children and adults may either be resistant to infections or have asymptomatic infections [1].

Concerning sex, percentages of infections among males and females were similar. In agreement with current findings, uniform sex distribution of cyclosporiasis cayetanensis had been reported by many studies, where males and females were equally susceptible [18,19,27,28].

Children with WDS showed a higher percentage of cyclosporiasis, compared to those with SDS with statistically insignificant association. Findings of the present work were supported by previous studies [22,24,29]. It has been suggested that, subjects with WDS may be bad-nourished and have a lower level of immunological resistance in whom replication of parasite is not prevented to some extent [1].

Regarding the source of drinking water, the majority of houses in Sana'a had tap water connection inside their houses but potable water was not available all the time, as a result, people tend to use of public taps and/ or wells as alternative sources of water supply. In result of the present work, use of public and/ or well water for drinking was statistically associated with infection ( $P = 0.025$ ). This is in accordance with another studies that recorded a high prevalence of cyclosporiasis. This was explained by the presence of fecal contamination of human excreta that may possibly contaminate the public water [1,14].

Eating of raw vegetables and fruits was identified as a further significant risk factor for cyclosporiasis in the current findings ( $P = 0.040$ ). This goes in line with previous surveys that reported; consumption of contaminated raw fruits and vegetables like raspberries, basil, snow peas, and lettuces were risk factors associated with infection. Raw vegetables and fruits are thought to be contaminated by *C. cayetanensis* oocysts originating from human feces [1,30-33].

In results of the current study, statistically insignificant association between the residence of children and cyclosporiasis doesn't indicate the ineffectiveness of this factor on the spread of infection. Percentage of infection among rural children was higher than urban one in our study. Prevalence of cyclosporiasis is usually higher in rural than urban communities that may attributable to the impact of environmental and personal conditions. Deficiency of sanitary facilities, close contact with animals, quality of water supply, unsafe human waste disposal, poor standards of personal hygienic practices, all of the above provoke increased exposure to infection [14,34]. The present result is consistent with previous findings carried out in Egypt, and China [27,35]. However, it was disagree with other studies done in Iraq, and Peru that reported nearly similar rates of infections among immunocompetent children from urban and rural areas [19,36].

In the present findings, *Cryptosporidium* spp. ranked the first coccidian detected and *C. cayetanensis* was the second. This is in agreement with a previous surveys stated in Nigeria, and Jordon [23,37]. In contrast, *C. cayetanensis* among Iraqi infected children was the most prevalent coccidian followed by *Cryptosporidium* [19]. This discrepancy may be ascribed to the impact of socio-economic, sanitary, and environmental factors [22].

## **Conclusion**

It can be concluded that, prevalence of *C. cayetanensis* infection among immunocompetent children with diarrhea in Sana'a is a substantial. Age, eating of raw vegetables and fruits, use of public and/ or well water for drinking were the risk factors that significantly associated with infection. Symptomatic infections represent a small part of cases, further studies should directed to asymptomatic infections in indigenous populations of all ages including older children and adults especially in rural areas. Moreover, estimation of seasonality as a risk factor for infection is recommended. Physicians should request a routine fecal examination for this coccidian in any case with diarrhea or gastrointestinal disturbances. Examinations of drinking water source and raw vegetables and fruits for the presence of oocysts are

recommended. Investigations of other microorganisms that have associated with diarrhea are also required. Improvements of environmental sanitation particularly proper sewage and safe water supply are needed. Public health measures, and health education programs should be go on to focus on the importance of hygienic practices.

This is the first report of prevalence and risk factors for gastrointestinal cyclosporiasis among immunocompetent Yemeni children with diarrhea.

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