

Full Length Research Paper

Evaluation of demographic, clinic and treatment features of patients and a cross-sectional survey of cyclosporiasis in patients with diarrhea in Southeastern Turkey

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Accepted 27 December, 2011

In this research, we aimed at reporting the results of a cross-sectional epidemiological scanning performed on an outbreak of cyclosporiasis, occurring in a family and patients' socio-demographic epidemiological, clinical, diagnostic and therapeutic features, after detecting *Cyclospora oocysts* on stool sample of a person admitted to gastroenterology polyclinic. Scanning was performed in the neighborhood of the patient. The investigation group consisted of 75 individuals with diarrheal occurring from neighbor and family of patients. A questionnaire was performed for information on epicrisis of diarrheal persons and the samples were collected in stool containers. The samples were examined with native-lugol, sedimentation and modified acid fast staining methods. The stools were cultured in Salmonella-Shigella agar medium to investigate their bacteriological properties. The different vegetables from the mobile market place (peddler) founded in the neighborhood and water samples from house were collected to detect the infection source. Parasitosis (single or mix parasite) were encountered in 20 out of 75 persons in the examined samples (26.6%) and *C. oocysts* were detected in 13 out of 75 persons (17.3%). Out of the total number of patients having cyclosporiasis, none has immunodeficiency and chronic diseases. All cases were determined in the month of July. Oocysts were detected in six different families. Bacteria were not cultivated in stool cultures and occult blood was negative. The agent was not encountered in green vegetables, though water samples were examined to detect infection resource. Examination of the samples for *Cyclospora* was not neglected in diarrhea individuals; as such an examination was performed for the source of transmission of the infection. *Cyclospora* may generate family infection in individuals and if detected in one individual of a family, all the family individuals were examined for this infection.

Key words: *Cyclospora cayetanensis*, diarrhea, familial transmission, Southeastern Turkey.

INTRODUCTION

Cyclospora cayetanensis is a gradually increasing causative agent for diarrhea with high morbidity and mortality. While it causes mild or moderate, self limited diarrhea in immunocompetent individuals, severe

intestinal symptoms and longstanding diarrhea are observed in immunocompromised individuals (Shields and Olson, 2003). Oocysts of *C. cayetanensis* which are excreted through human stool measures approximately 8 to 10 µm, round and stained variably with acid-fast and are immature when excreted with stool (Albert et al., 1994).

This parasite was first reported by Eimer in 1870 and included in *Cyclospora* genus by Scheneider in 1881.

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The first case in the human was reported from Papua New Guinea in 1979 (Ashford, 1979). In our country, this agent was first reported in a HIV positive subject in 1998 (Koc et al., 1998). Studies concerning the epidemiology of this parasite in our country are quite limited.

Cyclosporiasis that contaminates with food and water is an important community health problem worldwide (Ortega and Sanchez, 2010). The first water outbreak due to cyclosporiasis was reported from a hospital in Chicago, USA (Huang et al., 1995). Afterwards, *Cyclospora oocysts* were detected in three out of five water resources used as drinking water in Guatemala (Dowd et al., 2003), in drinking water and gray water in Peru (Zerpa et al., 1995; Sturbaum et al., 1998), in water of an artesian well in Haiti (Lopez et al., 2003), in unrefined drinking water in Nepal (Hoge et al., 1993), in swimming pool water and different water supplies in Egypt (Youseff et al., 1998) and the infection was reported to be related with drinking these water. It was associated with vegetable consumption in Nepal (Sherchand and Cross, 2001), Peru (Ortega et al., 1997) and Jordan (Nimri et al., 2003) and lettuce consumption in Egypt (Abou el Naga IF 1999). Cyclosporiasis cases associated with basil, lettuce, raspberry, mesclun and pea consumption were reported from America, Canada and Europe (Herwald, 2000; Brockmann et al. 2001; CDC 2004; Hoang et al., 2005; Doller et al., 2002; Lopez et al., 2001).

In this research, a cross-sectional screening study and clinical, diagnostic and therapeutic features of a family in Diyarbakır presenting with cyclosporiasis outbreak are reported.

MATERIALS AND METHODS

This study was carried out in Diyarbakır in 2010. After a patient who was admitted to Gastroenterology Outpatient Clinic of Dicle University Research and Training Hospital had been diagnosed as cyclosporiasis, a cross-sectional screening was performed towards the source due to presence of similar complaints in the same family and neighbors. After required formal consent had been obtained from Provincial Directorate of Health, three health centers in Huzurevleri Neighborhood in which these subjects live were interviewed. Neighbors of the first patients were visited; the individuals with complaints of diarrhea, abdominal pain, fatigue were invited to the health center. A total of 75 patients who were admitted to health centers in two weeks (July 2010) constituted the study group.

After the patients who were invited to the health center with diarrhea had been informed about the research, a structured questionnaire including questions about socio-demographic features, addresses, history of travel and disease was applied. Samples were collected using stool container. Stools were brought to parasitology laboratory within one hour and inoculated onto proper mediums and examined in terms of parasitology.

Specimens were examined with native lugol, midi Parasep® Faecal Parasite Concentrator (Diasys Company), modified acid-fast staining methods. Stool culture was evaluated by inoculating onto Salmonella Shigella agar medium. Formations measuring 8 to 10 µm that were suggested to belong to *Cyclospora* in native lugol method were examined with modified acid-fast staining

and variably stained (dark red, pink or colorless). *C. cayetanensis* oocysts were detected. Native preparations were examined under fluorescent microscope with x40 objective using filters 380 to 420 wavelength and those formations were detected to show auto fluorescence.

15 specimens from each vegetable (lettuce, parsley, cucumber, watercress, rucola, fresh mint, and fresh onion) were taken from the mobile market place (peddler) founded in the neighborhood in order to determine the source of the infection and these specimens were investigated for *Cyclospora*. Each vegetable was washed in a large case and stored in the water. This fluid was transferred to centrifugation tubes, after centrifugation, the sediment was examined with direct examination and staining with acid-fast.

Water specimens were taken from tap water of the houses that the subjects live. Specimens were analyzed for *C. oocysts* and the amount of chloride in the water was measured. Water specimens were taken into 5 L of clean plastic cans and brought to the laboratory. Water specimens were filtered from vacuum pump filtration device including 0.45 µm cellulose acetate membrane filter. Particulate on the filter was washed with 20 ml of same water sample and centrifuged and the sediment was put on the microscope slide. 3 preparations were prepared for each specimen. These preparations were stained with modified acid-fast staining and examined.

In the statistical analysis of obtained data, Odds ratio and Pearson's qui-square (χ^2) test were used for risk assessment.

RESULTS

Mean number of the residents in the visited 56 houses was 5.9 and a total of 300 residents were living in those houses. Of the houses, 44.6% were garden houses, 55.4% were apartment houses. City water was being used in all of them. Toilet, washing stand and soap were present in all except one and all toilets were connected to sewerage system. There was the history of diarrhea in 75 of 300 residents (22.7%). There was no history of travel in patients who were detected to have *Cyclospora*. Of 75 patients with diarrhea of whom stool specimens were examined, 50.6% were males and 49.3% were females with mean age of 17.0±17.6 years. Single or multiple parasitosis was detected in 28 % (21 subjects) and *C. oocysts* were detected in 17.3% (13 subjects). Symptoms of patients with cyclosporiasis and socio-demographic information of patients with cyclosporiasis were indicated in Tables 1 and 2 respectively.

Of the *Cyclospora* positive patients, 69.2 % (9 subjects) were females and 30,7 % (4 subjects) were males. The parasitosis (single or mix parasite) were encountered in 26.6 % (in 20/ of 75 person of examining samples) Eight different parasite species (pathogen or non-pathogen) [*C. cayetanensis* 17.3%, *Giardia intestinalis* 6.6%, *Entamoeba histolytica/Entamoeba dispar* 4%, *Entamoeba coli* 4%, *Blastocystis hominis* 4%, *Enteromonas hominis* 1.3%, *Hymenolepis nana* 1.3%] and 4 mixed parasite infections were detected in the subjects whose stool specimens were examined.

Complaints of the *Cyclospora* positive patients were evaluated. Subjects who are negative for *Cyclospora* constituted control group. Complaints of fatigue, fever, anorexia and abdominal pain were found higher in the

Table 1. Symptoms of patients with cyclosporiasis.

No	Diarrhea	Type of diarrhea	How many times a day	Span (day) of diarrhea	Weakness	Anorexia	Abdominal pain	Nausea	Vomiting	Loss of weight	Fever
1	+	Aqueous	2-3	15	+	+	+	-	-	-	-
2	+	Aqueous, Greenish color	5-6	10	+	+	+	+	-	+	+
3	+	Aqueous	3-4	2	+	+	+	+	+	-	+
4	+	Aqueous	2-3	2	+	+	+	+	+	-	-
5	+	Aqueous	5-6	6	+	+	+	-	-	-	+
6	+	Aqueous	3-4	6	+	+	+	+	+	-	+
7	+	Much Aqueous, Yellow-Green	3-4	15	+	+	+	+	-	+	+
8	+	Aqueous	2-3	4	+	+	+	+	-	+	+
9	+	Aqueous	4-5	7	+	+	+	+	+	+	+
10	+	Much Aqueous, Yellow-Green	5-6	10	+	+	+	+	+	+	+
11	+	Aqueous	5-6	9	+	+	+	+	-	-	+
12	+	Aqueous	2	10	+	+	+	-	-	-	-
13	+	Aqueous /Mucus	4-5	8	+	+	+	-	-	-	+

patients with *Cyclospora* compared to control group and the difference was found statistically significant. No difference was found between groups in terms of nausea, vomiting and weight loss (Table 3).

6 subjects were initially detected to have *Cyclospora*. Afterwards, family members of these subjects were screened. Among 6 different families, oocysts were detected in 5 out of 8 members of a family, in 2 out of 8 family members, in 3 out of 6 family members, in 1 out of 8 family members, in 1 out of 6 family members and in 1 subject who lives alone (a total of 13 cases).

Cross-table was formed in order to determine the risks towards the source of the infection in *Cyclospora* positive subjects. Subjects who are negative for *Cyclospora* constituted the control group. *Cyclospora* contamination risk was found 3.5 fold higher in the subjects who eat out compared to the ones who do not eat out

($p=0.012$) (Table 4). Risk was found 1.2 fold higher in the ones who live in flat homes compared to the ones who live in apartment houses however the difference was not statistically significant ($p=0.76$). There were no immunocompromised or chronic patients among the cases. No pathologies were detected in systemic examinations of *Cyclospora* positive patients. On laboratory examination, whole blood count, urine analysis, hepatic and renal function tests were found normal. Bacteria were not cultivated in stool cultures, occult blood test was negative. ELISA test for HIV was found negative.

Causative agent was not found in green vegetables and water specimens. Trimethoprim/sulfamethoxazole 160/240 mg bid was administered for two weeks in *Cyclospora* positive patients, complaints were found to be resolved on controls after treatment and parasites were not found in stool examinations. Patients diagnosed of other parasites were treated with appropriate

drugs.

DISCUSSION

Cyclospora infections epidemiologically appeared in 3 different types: sporadic cases in endemic areas, as the result of travel to an endemic area or water or food borne outbreaks in non-endemic areas. Contamination and infection rates are quite high in tropical and subtropical areas like Nepal, Haiti and Peru. Water and food borne outbreaks are also seen in developed countries (Warren, 2009). *Cyclospora* infections have been reported sporadically also in our country in the recent 10 years (Turk et al., 2004; Yazar et al., 2009; Doğan and Saglik, 2010; Tasbakan et al., 2010; Cicek et al., 2011). *Cyclospora* was reported in 23 out of 4986 stool specimens in the study of Turgay et al. (2007) from Izmir and in 20 out of 1876 stool specimens in the study of Ozdamar et al. (2010)

Table 2. Socio-demographic information of patients with Cyclosporiasis.

No	Gender	Age	Other parasites	Travelling	Eating outside the home	Type of house	Resource of drinking water	Is there water tank	Person number in home	Type of toilet	Place of hand washing in toilet
1	F	49	-	-	-	Apartment	Tap drinking	-	8	Connected to the drains	+
2	F	54	-	-	-	Apartment	Tap drinking	-	6	Connected to the drains	+
3	F	34	-	-	-	Apartment	Tap drinking	-	6	Connected to the drains	+
4	F	14	-	-	-	Apartment	Water drinking	-	6	Connected to the drains	+
5	F	56	-	-	-	Single-storey house	Tap drinking	-	8	Connected to the drains	+
6	M	23	<i>Giardia intestinalis</i> , <i>Hymenolepis nana</i>	-	+	Single-storey house	Tap drinking	-	8	Connected to the drains	+
7	F	18	-	-	+	Single-storey house	Tap drinking	-	8	Connected to the drains	+
8	F	20	<i>E. histolytica</i> / <i>E. dispar</i> , <i>Chilomastix mesnili</i> , <i>Blastocystis hominis</i>	-	+	Single-storey house	Tap drinking	-	8	Connected to the drains	+
9	F	15	<i>Entamoeba coli</i>	-	+	Single-storey house	Tap drinking	-	8	Connected to the drains	+
10	F	11	-	-	-	Single-storey house	Tap drinking	-	8	Connected to the drains	+
11	F	22	<i>Blastocystis hominis</i> , <i>Entamoeba coli</i> , <i>Enteromonas hominis</i>	-	+	Single-storey house	Tap drinking	-	8	Connected to the drains	+
12	M	51	-	-	-	Single-storey house	Tap drinking	-	6	Cesspool	-
13	M	33	-	-	+	Apartment	Bottle/Tap drinking	-	1	Connected to the drains	+

from Istanbul. In this study, oocysts were found in 13 out of 82 stool specimens. These case reports and researches reported from different provinces indicate the presence of the disease, not only the ones associated with travel.

The season that the parasite is encountered has been reported as the rainiest seasons in many countries. The agent has been reported to be

seen more common in rainy and warmer seasons in Guatemala (Bern et al., 1999), Honduras (Kaminsky, 2002), Jordan (Nimri, 2003), Nepal (Kimura et al., 2005) and Indonesia (Fryauff et al., 1999). On the other hand, it has been reported more common in less rainy, drier and colder months of the year in Haiti (Eberhard et al., 1999) and Peru (Ortega et al., 1993). In Turkey, in the

studies carried out in Izmir (Turgay et al., 2007) and Istanbul (Ozdamaret et al., 2010), cyclosporiasis cases have been detected in hot and dry season. All cases detected by us were seen in July.

That region is rainless in summer. July and August are the hottest months of the year. Besides, this city lived the hottest season of

Table 3. Evaluating of symptoms according to *Cyclospora* positive and negative.

Symptoms		Cyclospora positive person		Cyclospora negative person		Total	P
		No	%*	No	%*		
Weakness	Positive	13	100.0	31	50.0	44	0.0001
	Negative	0	0	31	50.0	31	
Fever	Positive	10	76.9	22	35.5	32	0.006
	Negative	3	23.1	40	64.5	43	
Anorexia	Positive	13	100.0	26	41.9	39	0.0001
	Negative	0	0	36	58.1	36	
Abdominal pain	Positive	13	100.0	23	37.1	36	0.0001
	Negative	0	0	39	62.9	39	
Nausea	Positive	10	76.9	30	48.4	40	0.07
	Negative	3	23.1	32	51.6	35	
Vomiting	Positive	6	46.2	30	48.4	36	0.8
	Negative	7	53.8	32	51.6	39	
Loss of weight	Positive	5	38.5	11	17.7	16	0.09
	Negative	8	61.5	51	82.3	59	

*Percentages are column percentage.

recent years in 2010. Groundwork for natural gas was done during the period that infections were detected. There was water cut for 2 to 3 days in the area of infection. These environmental conditions may have increased the risk for contamination and spread. Our cases are different from cyclosporiasis cases in other countries in terms of season and similar to those reported from Izmir and Istanbul, Turkey in as all are seen in dry and hot summer.

Cyclosporiasis outbreaks in America, Canada and Europe were reported to be related with raspberry, basil, lettuce, greens and pea consumption. Almost all of food borne outbreaks were reported to be seen during the first months of warm and rainy summer and most of them were reported to be related with imported food (Shields and Olson, 2003; CDC, 2004; Ho et al., 2002; Anonymous, 2004; Petry et al., 1997; Herwaldt, 2000). In our province, green vegetables like rucola, cress, parsley, green mint, green radish, lettuce and fruits like strawberry, mulberry are grown in this season and consumed much. Due to the absence of travel history in cases, the presence of the infection in that region is certain and the source of the infection is probably food or water. However methods for oocyst investigation in water and food are conventional and current modern methods have not been used thus we could not diagnose the source of infection. Contamination of cyclosporiasis with food is a great threat for food production and a great

problem for community health worldwide.

Cyclospora infection presents with anorexia, nausea, vomiting, meteorism, fatigue, abdominal pain, diarrhea, mild fever and weight loss. Infection is more serious in non-endemic areas, children and immunocompromised individuals. Diarrhea is usually watery and accompanied by nausea, vomiting, abdominal pain and anorexia (Ortega and Sanchez, 2010; Warren, 2009; Connor et al., 1999; Herwaldt and Ackers, 1997). All of our cases had diarrhea, abdominal pain, anorexia and fatigue. 9 out of 13 cases had vomiting, 5 had vomiting, 5 had weight loss and 10 had mild fever, fatigue, fever, anorexia and abdominal pain were more frequent among the *Cyclospora* positive patients and the difference was statistically significant. There was no difference between two groups in terms of nausea, vomiting and weight loss (Table 3). Direct contamination between humans is not possible as sporification of *C. cayetanensis* occurs out of the host (42). *Cyclospora* infection may affect all family due to using common food and water as contamination with water and food is common. In our study, family members of *Cyclospora* positive individuals were screened. In 6 different families, oocysts were determined in 5 of 8 family members, in 2 of 8 family members and in 3 of 6 family members. Thus when oocyst is detected in one member of the family, the remaining should be investigated for *Cyclospora*.

In our research contamination risk for *Cyclospora* was

Table 4. Relation between eating outside the home with Cyclospora positive.

Eating outside the home	Cyclospora (+)		Cyclospora (-)		p	Odds ratio 95 CI (%)
	No	%	No	%		
Yes	6	46.2	8	12.9	0.012	3.5 (1.49-8.58)
No	7	53.8	54	87.1		

found 3.5 fold higher in the ones who have the history of eating out compared to the ones who do not eat out ($p=0.012$). Probability of serving food and salads under non-hygienic conditions is high when eaten out due to easy contamination of oocysts with food. Thus eating out increased the risk of contamination.

In conclusion *Cyclospora* infections have been reported sporadically in our country in the recent 10 years. Causes for reporting *Cyclospora* as sporadic cases in our country may be clinicians' not adequately recognizing the infection, not applying diagnostic laboratory methods and lack of experienced microscopists. Cases and researches reported from different provinces indicate the presence of this infection in our country. Examinations for *Cyclospora* should not be neglected in individuals with diarrhea and investigations aimed at the source of the infection should be done. *Cyclospora* may lead to familial infections, thus all family members should be screened when it is seen in one. We consider that the infection may be treated early and spread may be prevented by this way.

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