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RESEARCH ARTICLE

Prevalence and Associated Factors of *Cryptosporidium* spp and *Cyclospora cayetanensis* in Izmir Province, Turkey

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Summary

Cyclospora cayetanensis and *Cryptosporidium* spp are the two emerging coccidian parasites causing human infections by inhabiting the intestinal tract and can either remain asymptomatically, or can cause endemic and epidemic diarrhea in both children and adults. In the present study, the prevalence of *C. cayetanensis* and *Cryptosporidium* spp were assessed in an epidemiological study in Izmir province, along with risk factors related to infection. A total of 873 individuals from both the city centre and counties were included in the study, and stool samples were examined with formalin ethyl acetate and Kinyoun acid-fast stained preparations. The prevalence of *C. cayetanensis* and *Cryptosporidium* spp were found to be 3.0% and 0.8%, respectively. Lower socio-economic conditions, lack of health insurance, consumption of tap water, eating in common places were all found to be associated with higher positivity for *C. cayetanensis* and *Cryptosporidium* spp. Abdominal pain was the prominent complaint of infected individuals. Despite relatively low prevalence rates, the presence of *C. cayetanensis* and *Cryptosporidium* spp in Izmir province requires further assessments of intestinal parasites. Public health measures that may prevent the local risk factors of infection should be improved and implemented.

Keywords: *Cryptosporidium spp, Cyclospora cayetanensis, Prevalence*

İzmir'de *Cryptosporidium* spp ve *Cyclospora cayetanensis*'in Görülme Sıklığının ve İlişkili Faktörlerin Araştırılması

Özet

Cyclospora cayetanensis ve *Cryptosporidium* spp, gerek çocuklarda gerekse yetişkinlerde endemik veya epidemik diyarelere neden olan ve son yıllarda önemleri giderek artan koksidian protozoonlardır. Ayrıca her iki parazit toplumda asemptomatik olarak da bulunabilmektedir. Araştırmamızda İzmir'in merkezi ve bazı ilçelerinde *C. cayetanensis* ve *Cryptosporidium* spp.'un görülme sıklığının araştırılması ve epidemiyolojik risk faktörleri açısından değerlendirilmesi amaçlanmıştır. İlçelerden seçilecek kişi sayısı nüfuslarına orantılı olarak belirlenmek üzere toplam 873 kişi çalışmaya dahil edilmiştir. Kişilerle görüşülerek bir anket formu doldurulmuştur. Çalışma grubundan alınan dışkı örneklerine, formaldehit etil asetat konsantrasyon ve kinyoun asit fast boyama yöntemleri uygulanmıştır. *C. cayetanensis* sıklığı %3.0 ve *Cryptosporidium* spp. sıklığı %0.8 olarak saptanmıştır. İlçelerin sosyoekonomik şartları kötü olan yerleşim yerlerinde, sosyal güvencesi olmayan kişilerde, içme ve kullanım suyu olarak şebeke suyu tüketenlerde ve dışarıda yemek yeme alışkanlığı olanlarda daha yüksek oranda *C. cayetanensis/Cryptosporidium* spp. pozitifliği saptanmıştır. Pozitif kişilerde en sık saptanan semptom karın ağrısı olarak belirlenmiştir. Çalışmamızda İzmir ve ilçelerinde düşük oranda da olsa bu parazitlerin saptanmasının toplum için bir kaynak oluşturabileceği ve enfeksiyon açısından risk faktörlerinin düzeltilmesinin gerekli olduğu kanısına varılmıştır.

Anahtar sözcükler: *Cryptosporidium spp, Cyclospora cayetanensis, Prevalans*

INTRODUCTION

Cryptosporidium spp and *Cyclospora cayetanensis* are two intracellular protozoa which may cause gastro-intestinal complaints in both immunocompromised and immunocompetent individuals, ranging from asymptomatic infection, intermediate diarrhea, and severe enteritis to biliary tract involvement in immunocompromised

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individuals [1,2].

The incidence of *Cryptosporidium* spp is reported to be 1-3% and 7-10% in immunocompetent patients with diarrhea in developed and developing countries, respectively [1]. Studies on different patient groups in Turkey revealed an incidence rate between 0 and 35.5% [3]. *C. cayetanensis* has been reported since early 1980s, and the number of cases has been on the rise after the application of sensitive diagnostic methods [2]. Large scale studies revealed that incidence rates of *C.cayetanensis* in immunocompetent patients in an endemic area and individuals in control group are 0-13% (mean, 1.7%) and 0-4.2% (mean, 0.4%), respectively. The incidence rates of immunocompromised patients with diarrhea range between 0-36% (mean, 4.5%) [4].

Diagnosis of *Cryptosporidium* spp and *C.cayetanensis* mostly relies on the microscopic identification of parasites in permanent stained smears, such as modified Kinyoun's acid fast and Ehrlich-Ziehl-Neelsen stains of concentrated stool samples. Formalin ethyl acetate concentration method is commonly preferred for stool samples [5,6].

The aim of the present study is to assess the incidences and risk factors associated with *Cryptosporidium* spp and *Cyclospora cayetanensis* in Izmir province.

MATERIAL and METHODS

Study Population

This cross sectional study was carried out in Izmir province between September 2005 and April 2006. According to the 2005 census, the total number of dwellers in Izmir was 3.370.866. A total of 16 clusters (8 from urban and 8 from rural areas with 30-60 km distance to city centre) was chosen randomly from 28 counties of the province *(Fig. 1)*. The size of the sample in each stratum (county) was calculated in proportion to the population with Epi-Info 5.0. This lead to a sample size of 773 persons with a confidence interval of 95%, a sample error of 2% and design effect of 2, when the estimated prevalence of *C. cayetanensis* [4] was considered as 4.2%. A total of 873 persons were participated in the study, according to the population of the counties *(Table 1)*.

Data Collection

The questionnaires were completed with face-to-face interviews and stool samples from each individual were collected in house visits. The questionnaires contained questions about personal and socio-demographic features such as age, sex, marital status, education and employment, total income and environmental conditions such as social security, hand-washing habits, source of drinking and non-drinking water and presence of sewage system.

Parasitological Examination

Stool samples of 873 individuals were concentrated with formalin ethyl acetate and smears of concentrated sediment were stained with modified Kinyoun's acid-fast stain and examined under x1000 magnification [7].

Statistical Analyses

The assessment of study data was done with SPSS 13.0, using t test and chi-square analyses. The relationships between the incidences of the parasitic infections and personal and environmental conditions were assessed.

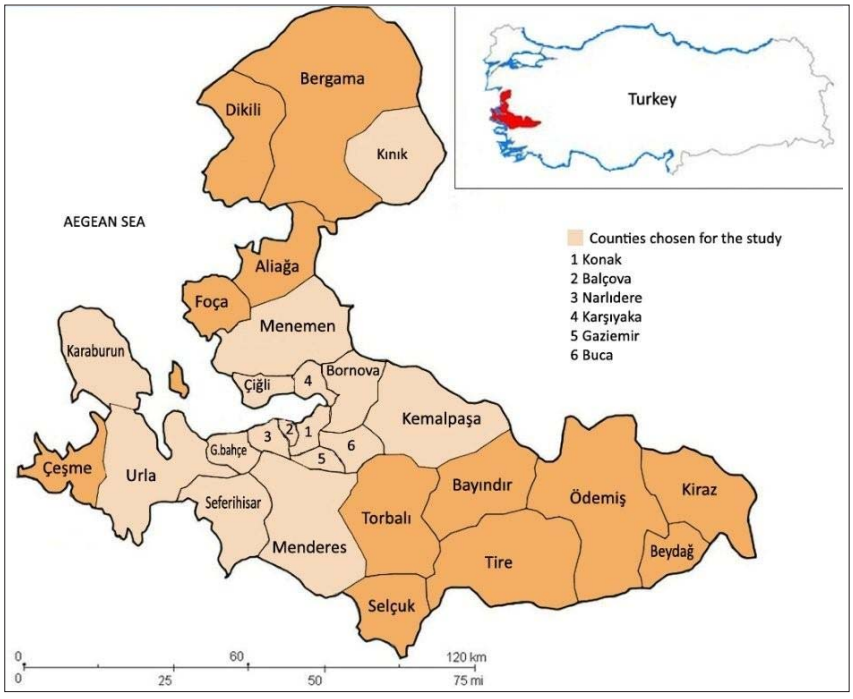

Fig 1. Map of Izmir province and 16 counties chosen for the study

Şekil 1. Çalışmanın yapıldığı İzmir ili ve 16 ilçesinin haritası

Table 1. *Distribution of the participants according to their places of inhabitance (n=873)*
Tablo 1. *Çalışmaya katılan tüm kişilerin ilçelere göre dağılımı (n=873)*

Counties Close to City Centre	No	(%)	Counties Distant to City Centre	No	(%)
Balçova	20	2.3	Güzelbahçe	5	0.6
Bornova	130	14.9	Karaburun	5	0.6
Buca	87	10.0	Kemalpaşa	36	4.1
Çiğli	17	1.9	Kınık	8	0.9
Gaziemir	4	0.5	Menderes	23	2.6
Karşıyaka	135	15.5	Menemen	48	5.5
Konak	316	36.2	Seferihisar	10	1.1
Narlıdere	22	2.5	Urla	6	0.7

RESULTS

Some sociodemographic features of the participants were shown in *Table 2*. As all the participants have not responded all questions in the inquiry form, the results were assessed after the responses. The most of the participants were female (59.1%), and the mean age was found to be 27.23±19.39, ranging between 1 month to 86 years. Most participants were primary school graduates living in the urban sites of Izmir and have health insurance.

Table 2. *Socio-demographic features of the participants (n=873)*
Tablo 2. *Çalışmaya katılan tüm kişilerin (n=873) sosyodemografik özellikleri*

Features		No	%
Sex	Female	516	59.1
	Male	357	40.9
Age Groups	0-5	87	10.1
	6-14	225	11.1
	15-24	128	14.8
	25-34	126	14.6
	35-44	129	14.9
	45-54	79	9.1
	55-64	41	4.7
	65+	49	5.7
Education Degree	No education	104	12
	No literacy	55	6.3
	Literate	42	4.8
	Primary school	358	41.1
	Secondary school	144	16.6
	High school	152	17.5
	University graduate	15	1.7
Duration of living in Izmir	Life-long	226	26.0
	0-1 years	78	9.0
	1-5 years	142	16.3
	5-10 years	130	15.0
	More than 10 years	293	33.7
Social Insurance	Yes	634	72.7
	No	238	27.3
Place of Inhabitance	Rural	22	2.5
	Urban	466	53.4
	Shanty house	385	44.1
Socioeconomic Level of the Neighborhood	Lower	369	42.3
	Higher	504	57.7

The incidences of *C.cayetanensis* and *Cryptosporidium* spp were 3.09% (n=27) and 0.80% (n=7) respectively, with a co-infection in one patient.

Selected sociodemographic features of the individuals infected with *C.cayetanensis* or *Cryptosporidium* spp were demonstrated in *Table 3*. There was no statistically significant relationship between the socio-ecenomic parameters and parasite prevalence. However, it was observed that parasite positivity was higher in participants living in lower socioeconomic conditions, without social insurance, using tap water for both drinking and washing, having no water in toilet, and frequently eating outside.

The leading symptom were abdominal pain, loss of appetite and fatigue in the last 6 months *(Table 4)*. There was no statistically significant difference in the incidence of complaints between the parasite-positive and parasite-

Table 3. *Socio-demographic features of individuals infected with C. cayetanensis/Cryptosporidium spp*
Tablo 3. *C.cayetanensis/Cryptosporidium spp pozitif kişilerin sosyodemografik özellikleri*

Features		**No**	**%** *
Sex	Female	19	3.7
	Male	13	3.6
Age Groups	0-5	4	4.6
	6-14	7	3.1
	15-24	5	3.9
	25-34	4	3.2
	35-44	8	4.7
	45-54	2	2.5
	55-64	3	7.3
	65+	1	2.0
Duration of living in Izmir	Life-long	7	3.1
	0-1 years	2	2.6
	1-5 years	3	2.1
	5-10 years	6	4.6
	Over 10 years	14	4.8
Monthly income	Less than 1000 TL **	29	7.3
	Over 1000 TL	2	4.5
Social insurance	Yes	20	3.2
	No	12	5.0
Place of inhabitance	Rural	11	3.0
	Urban	21	2.4
Socioeconomic level of the neighborhood	Shanty house	-	-
	Lower	21	4.5
	Higher	11	2.9
Source of water	Tap	32	3.7
	Well	-	-
Buying drinking water	Yes	6	2.1
	No	26	4.4
Sewer system	Present	32	3.7
	Absent	-	-
Running water in toilet	Tap water	31	3.8
	No running water	1	9.1
Hand hygiene practices	Present	31	3.9
	Absent	1	1.4
Frequent eating outside	Var	17	4.8
	Yok	1	1.4

** Percentage reflects the line, ** 1000 TL = 600 USD = 450 EURO*

Table 4. *Distribution of symptoms and frequencies of both C. cayetanensis / Cryptosporidium spp positive and negative individuals in the study*

Tablo 4. *Araştırmadaki C.cayetanensis/Cryptosporidium spp pozitif ve negatif kişilerin semptom sıklık dağılımları*

Symptoms	Parasite-negative (%)	Parasite-positive (%)
Abdominal pain	28.2	34.4
Loss of appetite	20.4	21.9
Fatigue	22.4	21.9
Diarrhea	15.6	19.3
Nausea	18.4	18.8
Fever	14.8	18.8
Weight loss	12.2	15.6

negative individuals.

It was noted that 453 of the 841 (53.9%) individuals in parasite-negative group and 15 of 32 (46.9%) in parasite-positive group were asymptomatic during the assessments.

DISCUSSION

The incidence of *Cryptosporidium* spp was reported as 1-3% in immunocompetent patients with diarrhea in developed countries, whereas the incidence of *C. cayetanensis* was found to be 4.2% in community-based studies [1,4]. The incidence of *C. cayetanensis* in Turkey was assessed in regional studies on patients with diarrhea and found to be 0.4-0.5% [8,9], whereas *Cryptosporidium* spp incidence in Turkey in similar studies was 4.5% and 5.2% [10,11]. Our community-based screening revealed incidence rates of 3.1% and 0.8% for *C. cayetanensis* and *Cryptosporidium* spp, respectively.

Both infections are generally more common among crowded communities where life conditions are relatively less hygienic [1,4]. Our results confirmed these data; presenting that parasites were more common in crowded communities with lower socio-economic levels, lower income and no social security.

Our research was conducted in autumn and winter, when precipitation is relatively high. Both parasites have been reported more common in wet seasons worldwide [1,4,12].

The transmission of *Cryptosporidium* spp and *C. cayetanensis* relies on the ingestion of oocysts with food and water resources. Both parasites are resistant to environmental conditions, chlorination of water and filtration [1,4]. In the present study, most parasite-positive individuals reported using tap water instead of bottled water and eating outside frequently. Thus, to prevent any risk of epidemics in the region, it is suggested to improve the disinfection of water resources and filtration of drinking water in the houses.

Asymptomatic infection of both *Cryptosporidium* spp and *C. cayetanensis* infections, are found to be important for epidemics [13]. In a prospective study in New York, the prevalence of asymptomatic infection caused by *Cryptosporidium* spp was found to be 6.4% and 22% in immunocompetent and immunocompromised children, respectively [14]. In two different studies conducted in Venezuela, asymptomatic infections with *Cryptosporidium* spp and *C. cayetanensis* were identified in 71.4% (15/21) and 84.6% (11/13) of the cases, respectively [15,16]. The incidence of asymptomatic carriers may relatively be higher in regions with lower socio-economic status leading to higher infection risk [17]. Our study data is consistent with the literature: almost half of the infected individuals (46.9%) were found to be asymptomatic during the study.

The prominent symptoms in an American study that reviewed cyclosporiasis cases diagnosed between 1997 and 2008, were abdominal pain (82.2%), fatigue (75.2%) and loss of appetite (65%) [18]. Abdominal pain, diarrhea, fatigue and loss of appetite were reported as the most common symptoms in cryptosporidiosis [17]. In the present study, leading symptoms were also found to be abdominal pain, loss of appetite and fatigue, which is also consistent with other studies.

This study is important as it is a large-scale, community-based trial investigating the incidence of *Cryptosporidium* spp and *C. cayetanensis* in Izmir. Presence of both parasites even in low rates in the province may threaten the public health, which requires evaluation and elimination of improvement in risk factors associated with infection.

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