REVIEW ARTICLE

A Systematic Review of the Neglected Parasite *Cyclospora cayetanensis* in Türkiye from 2004 to 2023

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Abstract

Cyclospora cayetanensis is a parasitic pathogen causing gastroenteritis, transmitted through contaminated food and water, and is prevalent in tropical and subtropical regions. Since C. cayetanensis requires different methods like acid-fast staining for detection, studies on its prevalence are insufficient. This study aimed to determine the prevalence of C. cayetanensis in Türkiye over the last two decades. A comprehensive literature search was conducted in databases Google Scholar, PubMed, Scopus, Mendeley, Web of Science, YOKtez, TR Dizin, and Türk MEDLINE Index for relevant English and Turkish publications from 2004 to 2023. Out of 184 identified articles, 47 met the inclusion criteria, of these, 41 were patient samples and 6 on water samples. C. cayetanensis prevalence averaged 32.7% in water samples and 2.71% in patient samples. Cyclospora was most frequently detected in the Aegean Region and Izmir province. The most frequent diagnostic method in the studies was the Kinyoun Acid-Fast Staining method (91.4%). Studies conducted in Türkiye have been limited to regions where expert parasitologists are available. High rates of positivity were detected in water samples. To accurately determine the prevalence in Türkiye, laboratory conditions must be provided to detect C. cayetanensis in every province.

Keywords: Cyclospora cayetanensis, Türkiye, Systematic reviews, Neglected diseases

INTRODUCTION

Cyclospora cayetanensis is a coccidian parasite that causes gastrointestinal disorders and prolonged diarrhea in humans. It causes serious diarrhea, especially in immunocompromised individuals, the elderly, and children. Furthermore, *C. cayetanensis* holds significant epidemiological importance in food and waterborne diarrhea outbreaks and endemic diseases such as traveller's diarrhea^[1,2].

Worldwide, approximately 780 million people do not have access to safe drinking water and 2.5 billion people do not have access to improved sanitation. Parasitic infections that develop due to this condition cause many diseases such as diarrhea, malnutrition, and growth retardation ^[3]. According to World Health Organization (WHO) 2019 data, diarrhea ranks second among preventable and treatable diseases among deaths in children under five years of age. Diarrhea affects approximately 1.7 billion children globally each year and causes the death of approximately 370,000 children under the age of five ^[4,5]. A systematic review including 195 countries reported that coccidian protozoa play a significant role in the etiology of fatal diarrhea ^[6].

Among coccidian parasites, *C. cayetanensis* is not well known and is often neglected. The reasons for this situation may include insufficient awareness of the disease by clinicians, the lack of appropriate diagnostic methods applied in laboratories, and the absence of parasitological evaluation conducted by expert personnel^[7]. Diagnosis of *C. cayetanensis* is conducted using acid-fast staining. Since *C. cayetanensis* cannot be detected by the native-Lugol method and there are no commercial serological kits that can be used in its diagnosis, data regarding its prevalence and incidence in risk groups do not reflect reality^[8].

Incorrect and incomplete diagnosis also delays treatment. Trimethoprim/sulfamethoxazole (TMP/SMX) are the most commonly preferred drugs for treating cyclosporiasis. There is currently no effective alternative treatment

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protocol defined for patients who do not respond to standard treatment or who are allergic to sulfa drugs. Due to differences in treatment protocols compared with other parasites and causative agents of diarrhea, especially in immunosuppressed individuals, prolonged diarrhea that does not heal related to cyclosporiasis has been observed ^[9].

This manuscript was prepared according to the guidelines outlined by the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA)^[10].

This article aims to investigate the prevalence of *C. cayetanensis*, a neglected parasite, in Türkiye in the last twenty years and to reveal the factors affecting its prevalence in a systematic manner.

MATERIAL AND METHODS

Search Strategy

For this systematic review, different combinations of the name of the parasite and the terms "Türkiye" were searched together in six international and three national databases in both English and Turkish. In the screening, electronic searches were made using the keywords "Cyclospora cayetanensis - C. cayetanensis - Cyclospora -Cyclospora sp., cyclosporiasis" and "Turkey", "Cyclospora cayetanensis - C. cayetanensis - Cyclospora sp., siklosporiyaz" and "Türkiye".

Searching platforms for the present study included the following: Google Scholar, PubMed, Scopus, Mendeley, Web of Science, YOKtez (*https://tez.yok.gov.tr/ UlusalTezMerkezi/*), TR Dizin (*https://trdizin.gov.tr*) and Türk MEDLINE.

YOKtez is the official website of the Turkish Higher Education Council, where master's, doctoral, and medical specialty theses are published. TR Dizin was created by the National Academic Network and Data Center (Ulakbim) of the Scientific and Technological Research Council of Türkiye (TÜBİTAK). It consists of journals on the basic subjects of health sciences, veterinary medicine, science, dentistry, pharmacy, engineering, and social and human sciences. TürkMedline is a database that collects articles in scientific and periodical health sciences journals published in Türkiye.

Study Selection, Inclusion, and Exclusion Criteria

This research includes cross-sectional studies that can reveal the prevalence of *C. cayetanensis* according to regions and years in Türkiye. The study included original articles, case reports, and theses published between January 2004 and December 2023, which could reveal the prevalence of *C. cayetanensis* in Türkiye. Articles that did not have epidemiological and statistical data, reviews, drug studies, unpublished data, and studies that did not contain suitable and reliable data were excluded from this review. The studies obtained because of the screening were uploaded to the EndNote program and then screened for duplicate publications in different journals. In addition, the publications were transferred to Excel and re-examined to check for duplicates (*Fig. 1*).

Study Selection

The selection of articles to be included in the research consists of two stages:

1. Scanning of titles and abstracts.

2. Scanning of the full texts.

Two independent reviewers worked at both stages of determining the article to be included. The inclusion and exclusion criteria for the study were determined according to the answers to the questions given below. Articles that received the approval of both referees were included in this study.

The following questions were used for title/abstract screening:

1) Is there a study that reveals the prevalence of *C*. *cayetanensis* in Türkiye according to the title/abstract?

2) Is this study an original paper, thesis, or case report?

If the scanned sections met the inclusion criteria, the full text of the article was obtained and reviewed.

The second stage of screening involved a full-text review using questions that required a 'Yes' or 'No' response. Any article that received a "No" response to any question was excluded from the review. All publications that were not excluded at this level were included in the systematic review.

The following questions were used in the second stage of screening:

1) Does the full text provide information on the prevalence of *Cyclospora* in Türkiye?

2) Is the region/city where the study in the full text was conducted clearly?

3) Are the methods used for data collection appropriate?

4) Are the data in the full text consistent with each other?

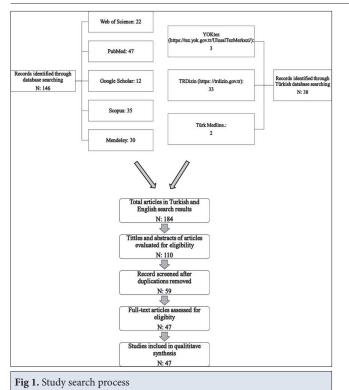
5) Are full-text findings appropriate for statistical data?

Data Items: Study-Level Data

The data from the studies published in the specified databases were examined comprehensively. For each article that met the inclusion criteria, the following data were collected: reference, study design, study location, methods applied, publication year, patient population, number of patients, and outcome information.

Statistical Analysis

The information extracted from the studies was entered



into the CMA (Comprehensive Meta-analysis V3) software. The heterogeneity among the studies were assessed using the I² test, and the results were analyzed based on the heterogeneity determined by the random

effects model. The presence of publication bias was investigated using the Egger test and Funnel plot. A metaregression analysis was conducted to explore the factors contributing to the observed heterogeneity among the included studies.

RESULTS

Among the 184 studies retrieved from databases covering 2004 to 2023 (20 years), 47 met the eligibility criteria for inclusion in this systematic review. Of the 184 articles obtained, 74 were excluded from the study because they were related to various scientific fields other than medicine. 51 studies were excluded because they were duplicates across various search engines and 12 studies were excluded because they did not provide epidemiological data such as drug resistance and sequence analysis. Data revealing the summaries of the studies obtained through this review are given in *Table 1*.

In the systematic review of 28 studies involving a total of 54.,043 human, the I² heterogeneity test showed high heterogeneity (I²: 97.2). Based on this, the random effects method was used to analyze the results. The meta-analysis indicated a prevalence of *C. cayetanensis* in 2.3% (95% CI: 1.6-3.4) of human in Türkiye (*Fig. 2*). Furthermore, the presence of publication bias in the studies was assessed using the Egger test, indicating such bias's existence (P-value = 0.0003). The funnel plot of the distribution

Table 1. The mai	n characteristics of 4	6 studies include	ed in the present review					
Study Type	Sample Type	City	Diagnostic Method	Year	Total Samples Size (n)	Positive Samples (n)	Prevalence (%)	Reference
OR	Н	Erzurum	MAF	2004	4322	1	0.02	[11]
OR	Н	İzmir	Con, MAF, Tric	2006	4986	23	0.46	[12]
OR	Н	İzmir	Con, KAF	2007	3925	75	1.91	[13]
OR	Н	İzmir	KAF	2007	191	9	4.71	[14]
OR	Н	İzmir	MAF	2007	554	11	1.99	[15]
OR	Н	Kars	Con, MAF	2008	138	1	0.72	[16]
OR	Н	İstanbul	MAF, PCR, Flor	2010	1876	20	1.07	[17]
OR	Н	Diyarbakır	KAF	2012	75	13	17.33	[18]
OR	Н	Van	Con	2012	6267	7	0.11	[19]
OR	Н	Eskişehir	Con, MAF	2012	225	1	0.44	[20]
OR	Н	İzmir	Con, MAF, Tric	2012	5073	187	3.69	[21]
OR	Н	İzmir	Con, KAF	2012	873	27	3.09	[22]
OR	Н	İstanbul	MAF, PCR	2013	91	2	2.20	[23]
OR	Н	İzmir	PCR	2014	53	14	26.42	[24]
OR	Н	Van	Con, MAF, Tric	2015	5985	7	0.12	[25]
OR	Н	Malatya	KAF, Flor	2015	2281	129	5.66	[26]
OR	Н	İstanbul	KAF, MAF, PCR	2017	115	3	2.61	[27]
OR	Н	Van	Con, KAF	2018	150	8	5.33	[28]

Study Type	Type Sample Type City		Diagnostic Method	Year	Total Samples Size (n)	Positive Samples (n)	Prevalence (%)	Reference	
OR	Н	İzmir	Con, KAF	2018	65	2	3.08	[29]	
OR	Н	Van	KAF	2018	150	4	2.67	[30]	
OR	Н	İzmir	Con, MAF, Tric	2019	10.726	828	7.72	[31]	
OR	Н	İzmir	PCR	2020	62	6	9.68	[32]	
OR	Н	Van	MAF, PCR	2021	200	24	12.00	[33]	
OR	Н	Malatya	Con KAF	2021	1057	52	52 4.92		
OR	Н	İzmir	KAF	2022	529	21	3.97	[35]	
OR	Н	Diyarbakır	KAF, Tric	2022	3624	15	0.41	[36]	
OR	Н	Aksaray	PCR	2023	232	8	3.45	[37]	
OR	Н	İzmir	Con, KAF, Tric	2023	4518	51	1.13	[38]	
OR	W	Ankara	KAF	2008	173	47	27.17	[39]	
OR	W	Giresun	KAF, Tric	2016	300	112	37.33	[40]	
OR	W	Ordu	KAF, Tric	2017	228	56	24.56	[41]	
OR	W	Samsun	KAF, Tric	2017	144	96	66.67	[42]	
OR	W	Denizli	KAF, Tric	2022	84	5	5.95	[43]	
OR	W	Iğdır	MAF, PCR	2023	69	11	15.94	[44]	
CR	Н	Kayseri	MAF	2004	6	6	100.00	[45]	
CR	Н	İzmir	Con, KAF, Flor	2006	1	1	100.00	[46]	
CR	Н	Ankara	MAF	2006	1	1	100.00	[47]	
CR	Н	Ankara	MAF	2006	5	5	100.00	[48]	
CR	Н	Malatya	Con, MAF	2008	1	1	100.00	[49]	
CR	Н	Ankara	Con	2009	1	1	100.00	[50]	
CR	Н	Kayseri	Con, KAF	2009	3	3	100.00	[51]	
CR	Н	Eskişehir	MAF	2010	1	1	100.00	[52]	
CR	Н	İzmir	MAF, Tric	2010	1	1	100.00	[53]	
CR	Н	Diyarbakır	KAF	2011	2	2	100.00	[54]	
CR	Н	Malatya	KAF	2012	1	1	100.00	[55]	
CR	Н	Van	EZN	2012	2	2	100.00	[56]	
CR	Н	Van	Con, MAF	2016	7	7	100.00	[57]	

MAF: Modified acid fast, KAF: Kinyoun acid fast, EZN: Erlich Ziehl Neelson, Con: Concentration method, Tric: Trichrome strain, Flor: Fluorescence method, OR: Original research, CR: Case Report, H: Human, W: Water

bias in the reviewed studies is presented in *Fig. 3*. Metaregression of the effects of sample size and year on the prevalence of *C. cayetanensis* in human is presented in *Fig. 4* and *Fig. 5*.

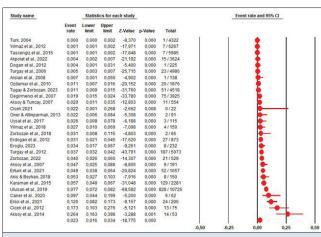
Of the articles, 41 were studied from patient stool samples, while 6 were water samples (*Table 1*). Of the six water samples, five were surface freshwater and one was wastewater sample. In the original studies, the highest positivity rate was observed in the water sample (66.67%) and the lowest positivity rate was observed in a retrospective study on patients (0.02%). The average prevalence of *C. cayetanensis* in water samples was 32.7%.

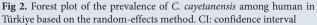
In the studies, at least one of the native Lugol, sedimentation, acid-fast staining, trichrome staining, and molecular methods were used to detect the parasite. The distribution of the methods used is given in *Table 2*. When the studies were examined, it was found that the acid-fast staining method was used in 43 out of 47 studies (Different modifications of the acid-fast staining method were evaluated together. Two acid-fast staining methods were used in one study).

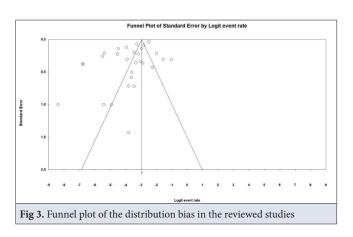
The most common complaints in patients with *C. cayetanensis* are prolonged diarrhea, abdominal pain, and vomitin. Forest plot of the prevalence of *C. cayetanensis* among human in Türkiye based on the random-effects

Table 2. The distribution of methods used for the diagnosis of C. cayetanensis							
Methods	Number of Studies	Percent					
Concentration	18	38.30%					
KAF	22	46.81%					
MAF	21	44.68%					
EZN	1	2.13%					
Tricrome	11	23.40%					
PCR	8	17.02%					
Floresan	3	6.38%					
KAE: Kinyoun acid fast MA	F. Modified acid fast FZN. F	Frlich Ziehl Neelson					

KAF: Kinyoun acid fast, **MAF:** Modified acid fast, **EZN:** Erlich Ziehl Neelson



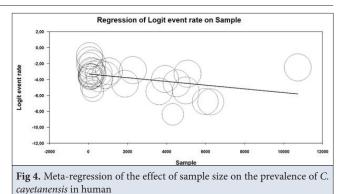


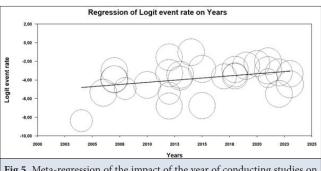


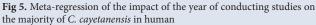
method by years, region and city are presented in *Fig. 6*, *Fig. 7*, and *Fig. 8*. Prevalence rates of *C. cayetanensis* by province in Türkiye is presented in *Fig. 9*. When the incidence of *Cyclospora* was examined over the years, the lowest positivity rate was observed in 2004 (0.05%), while the highest positivity rate was observed in 2011 (15.72%) (Table 1).

In 36 out of 47 studies, no information was found regarding seasonal transmission, while in 11 studies, it was reported

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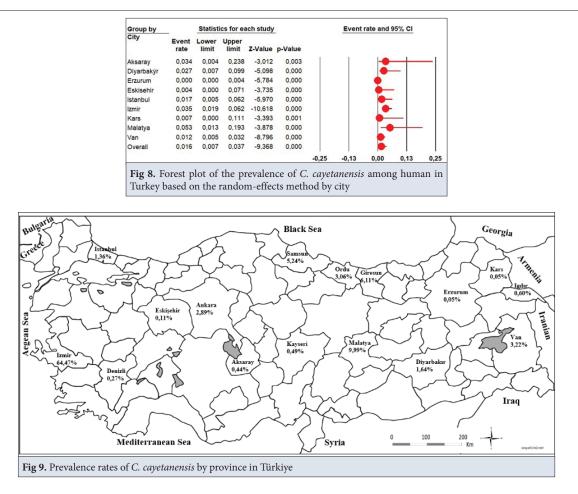
Group by		Statistics for each study					Event rate and 95% CI				
Years	Event	Lower limit	Upper limit	Z-Value	p-Value						
2004	0.000	0.000	0.006	-4,925	0.000		1		1	1	
2006	0.005	0.000	0.066	-3,866	0,000			- -			
2007	0.026	0.005	0.115					- -			
2008	0.007	0.000	0,170	-2.891	0.004			- -			
2010	0.011	0.001	0.142	-3.254	0.001						
2012	0.016	0.004	0.063	-5.650	0.000						
2013	0.027	0.004	0.172	-3,483				- -	1		
2014	0.264	0.022	0.850	-0.727	0.467					_	
2015	0.009	0.001	0.059	-4,760	0.000						
2017	0.026	0.001	0.333	-2.424				- -	_		
2018	0.036	0.007	0,165					- -			
2019	0.077	0.006	0.553	-1.805	0,071			-	_		
2020	0.097	0.006	0.643	-1.551	0,121				_		
2021	0.061	0.011	0.265					-			
2022	0.013	0.002	0.083								
2023	0.019	0.003	0,122	-3,957	0.000			-			
Overall	0,021	0,009	0,046		0,000						
						-1.00	-0,50	0,00	0,50	1,00	

Türkiye based on the random-effects method by years

Group by		Statist	ics for ea	ach study			Event rate and 95% CI			
Region	Event rate	Lower	Upper limit	Z-Value	p-Value					
Aegean	0,035	0,019	0,062	-10,517	0,000	1	- Î			- I
Central_Anatolia	0,016	0,003	0,086	-4,629	0,000			•	-	
Eastern_Anatolia	0,013	0,006	0,028	-11,121	0,000			•		
Marmara	0,017	0,005	0,063	-5,919	0,000			•	-	
Southeastern_Anatolia	0,027	0,007	0,100	-5,055	0,000			-	-	
Overall	0,021	0,012	0,039	-12,022	0,000			•		
						-0,15	-0.08	0,00	0.08	0,15

that diseases are most commonly seen in the spring and summer.

It was determined that studies on *C. cayetanensis* were carried out in six of Türkiye's seven regions and 17 of 81 provinces. In Türkiye, the highest number of studies among regions was conducted in the Aegean region



(13/29), and among provinces Izmir (13/29) (Table 1).

C. cayetanensis was detected in water samples from four regions and six provinces of Türkiye (*Table 1*). Among the studies on *Cyclospora*, the highest positivity rate was observed in water samples (average 32.77%).

When all studies were evaluated together, *C. cayetanensis* was detected most frequently in İzmir (64.47%), Malatya (9.99%), and Giresun (6.11%) (*Fig. 9*).

DISCUSSION

This systematic review provides comprehensive data on the prevalence of *C. cayetanensis* in Türkiye. Of the 47 articles examined in the study, 41 belonged to human samples and 6 to water samples (*Table 1*). The average prevalence of studies conducted in Türkiye has been determined to be 3.21%. The global prevalence of *C. cayetanensis* was reported to be 3.55% in a 2023 study and 3.4% in a 2024 meta-analysis examining *C. cayetanensis* infection worldwide. The results of this study are consistent with the global average ^[58-60]. The prevalence of *C. cayetanensis* varies by country's development status, with rates of 7.6% (83/921) in low-income countries, 4.8% (3.280/48.852) in lower-middle-income countries, and 0.4% (79/17.419)

in high-income countries ^[60] Since cyclosporiasis is an infection transmitted through food and water, it is more common in developing countries with low hygiene standards. The prevalence rates in Türkiye fall between those reported for lower-middle-income and upper-middle-income countries.

The average positivity rate was found to be 2.75% in human samples and 32.77% in water samples, with a significantly higher rate observed in water samples. The high positivity rate in water samples in Türkiye can be attributed to the following factors. Water is an important transmission route for coccidial parasites and many studies have determined that both sporadic and endemic cases are caused by polluted water. Therefore, the detection of coccidian parasites has become the primary goal in studies conducted ^[1,2]. Studies on water samples in Türkiye were conducted by experts in the field, in the seasons when the parasite is most prevalent, using diagnostic methods such as the acid-fast staining technique, which is effective for detecting coccidian parasites. In contrast, studies on human samples were predominantly retrospective, covering the entire year and involving large datasets. Therefore, depending on the seasonality, variations in laboratory conditions, and the expertise of the working personnel, not all cases may have been detected in studies conducted with human samples. Positivity rates in water samples were 27.17% in municipal wastewater and 33.94% in surface freshwater. In a meta-analysis investigating the global prevalence of *C. cayetanensis* in water, the prevalence rate was reported as 6.61% in freshwater and 4.66% in municipal wastewater suggesting that the rates in Türkiye are substantially higher ^[2]. However, only one study on wastewater and five on surface freshwater have been conducted in Türkiye, making the available data insufficient for reliable comparisons with global rates or robust interpretations.

When studies conducted in Türkiye are examined, the most frequently used diagnostic method for detecting *C. cayetanensis* is the acid-fast staining method (43 out of 47 studies) (*Table 2*). Studies have shown that PCR is the most reliable method in the diagnosis of *C. cayetanensis* ^[27,61]. However, when applicability, sensitivity, and cost balance are evaluated together, the most practical method is the acid-fast method.

The most common complaints in people with cyclosporiasis in Türkiye are prolonged diarrhea, abdominal pain and vomiting. Studies indicate that *C. cayetanensis* is more frequently observed in immunosuppressed individuals and causes serious complications ^[1]. In Türkiye, 8 studies were conducted on immunosuppressed individuals with cyclosporiasis. In cases of prolonged diarrhea and abdominal pain where the source cannot be identified, *C. cayetanensis* should be considered as a potential causative agent.

The lowest positivity rate of *Cyclospora* by year was observed in 2004 (0.05%), while the highest positivity rate was observed in 2011 (15.72%). The most important factor affecting the incidence by year is whether the study is original research or retrospective. In the years when research studies were conducted using tests that could identify *Cyclospora*, the positivity rate increased, while in the years when retrospective studies were conducted, it decreased.

Cyclosporiasis is most frequently seen in summer and spring months in Türkiye. *C. cayetanensis* is a waterborne parasite. During these seasons, increased human contact with untreated water sources and soil, along with higher consumption of fruits and vegetables grown in wetlands, such as raspberries and blackberries, contributes to a rise in parasite incidence.

C. cayetanensis has been detected in 6 regions and 17 provinces in Türkiye (*Table 1*) (*Fig. 9*). It has been detected most frequently in the Aegean Region among the regions and in Izmir among the provinces. Cyclosporiasis is more commonly seen in underdeveloped rural areas where hygiene is low ^[5]. However, the Aegean Region and Izmir are among the most developed areas of Türkiye. There

is an inconsistency between the results obtained and this situation. The reason for the apparent contradiction between the studies in Türkiye and the literature data can be explained as follows. *C. cayetanensis* cannot be identified by the native-Lugol method and it is a parasite without a commercial diagnostic kit such as cassette tests. To identify this parasite, specific diagnostic methods such as acid-fast staining methods and expert technical personnel in the field of parasitology are required. Therefore, it is very difficult to detect *C. cayetanensis* in routine microbiology laboratories. When the studies were examined, it was observed that all hospitals in regions where *C. cayetanensis* was diagnosed have either a Parasitology Department or a parasitology specialist available.

C. cayetanensis was detected in water samples from four regions and six provinces of Türkiye, and the highest positivity rate among all studies was observed in water samples (average 32.77%) (*Table 1*). The main reason is that these studies were performed by experts familiar with *C. cayetanensis* and the acid-fast method was used to diagnose coccidian protozoa, an important cause of waterborne diarrhoea ^[62]. There was no correlation between the locations of these studies and the locations of human *Cyclospora* infection. The studies were designed only to identify waterborne parasitic agents, and *Cyclospora* was the most frequently identified parasitic agent after Cryptosporidium. If similar studies are conducted in other regions and provinces in Türkiye, *C. cayetanensis* will likely be detected in these regions as well.

When all studies were evaluated together, *C. cayetanensis* was detected most frequently in İzmir, Malatya, and Giresun (*Fig. 1*). The reason for the high prevalence of *C. cayetanensis* in these provinces is, as mentioned above, the existence of expert parasitologists and laboratory conditions that can detect this agent. The prevalence of *C. cayetanensis* in humans in retrospective studies in Izmir was higher than in the original studies in other provinces ^[31]. The most likely reason for this is that this province, which has the oldest Parasitology Department in Türkiye, has a well-established system of examining all samples received in the laboratory for coccidian parasites.

CONCLUSION

This systematic review is important as it is the first study to reveal the general prevalence of *C. cayetanensis*, an important but neglected public health problem, in Türkiye. *C. cayetanensis* is a parasite that causes prolonged diarrhea and severe symptoms, especially in immunocompromised individuals. Due to the limited number of studies conducted in Türkiye, the available data cannot accurately reveal the prevalence of this parasite in the country. All of the studies were conducted by specialized parasitologists. Studies with human specimens are mostly retrospective or include *Cyclospora* agents seen while investigating intestinal protozoa. Limited research has been conducted with water samples, but these studies have observed high positivity rates. Considering these findings, *Cyclospora* is likely to be detected in studies conducted in other regions and provinces of Türkiye. As a result, more accurate epidemiological data can be obtained if clinicians pay more attention to this parasite and if specialized personnel and adequate laboratory conditions are provided in the field of parasitology.

DECLARATIONS

Availability of Data and Materials: Not applicable.

Acknowledgements: Not applicable.

Competing Interests: The authors declared that there is no conflict of interest.

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