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Prevalence of Microsporidiosis in Different Hosts in Turkey: A Meta-analysis

Türkiye'de Farklı Konaklarda Microsporidiosis Prevalansı: Bir Meta-analiz

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ABSTRACT

Objective: Microsporidia are opportunistic obligate intracellular pathogens which infect many vertebrate and invertebrate hosts. This study aimed at investigating all evidence about microsporidia infection in human and other vertebrate hosts in Turkey.
Methods: This study covered all prevalence studies, related to microsporidiosis in Turkey until April 2020, that were found in Web of Science, PubMed, Scopus, and ULAKBIM databases were considered in this meta-analysis. A total of 168 studies were identified in the systematic literature research. After the initial assessment, only 15 articles (12 humans and three other vertebrates) were included for meta-analysis. Data analysis was carried out using the Revman 5.3 (Review Manage 5.3) software.
Results: With the evaluation of these studies, it was found that the prevalence of microsporidia in humans (n=6.707) and other vertebrate hosts (n=506) was 13.4% and 15.2%, respectively. The risk ratio in the patient groups was 2.87 compared to the control group [95% confidence interval (CI): 1.20-6.87, I^2=87%, p<0.00001]. There was no difference between genders and parasite prevalence (95% CI: 1.00-1.39, I^2=18%, p=0.29). The prevalence of microsporidia was also found to be high in patients with diarrhea (95% CI: 1.09-1.58, I^2=86%, p=0.0001) and in immunosuppressed individuals (95% CI: 1.86-3.70, I^2=16%, p=0.31).
Conclusion: Although there are few studies on the prevalence of these parasites, the results of this meta-analysis provides extensive information about the current situation in Turkey.
Keywords: Microsporidia, Turkey, prevalence, meta-analysis

ÖZ

Amaç: Microsporidia türleri, omurgalı ve omurgasız konakçıların çoğunu enfekte eden, zorunlu hücre içi fırsatçı patojenlerdir. Bu çalışmada, Türkiye'de insan ve diğer omurgalı konaklardan bildirilen microsporidia enfeksiyonlarıyla ilgili tüm kanıtların incelenmesi ve değerlendirilmesi amaçlanmıştır.
Yöntemler: Bu meta-analizde, Web of Science, PubMed, Scopus ve ULAKBIM veri tabanlarında, Nisan 2020'ye kadar Türkiye'de microsporidiosis prevalansı ile ilgili yapılmış çalışmalar dikkate alınmıştır. Sistematik literatür araştırmasında 168 çalışma tespit edilmiştir. İlk değerlendirmenin ardından sadece 15 makale (12'si insan ve 3'ü diğer omurgalılar) meta-analize dahil edilmiştir. Verilerin analizinde Revman 5.3 (Review Manage 5.3) yazılımı kullanılmıştır.
Bulgular: Bu çalışmaların değerlendirilmesi ile insanlarda (n=6,707) ve diğer omurgalı konaklarda (n=506) microsporidia prevalansının sırasıyla %13,4 ve %15,2 olduğu görülmüştür. Hasta grubu/kontrol grubu risk oranı 2,87 idi [%95 güven aralığı (GA): 1,20-6,87, I^2=%87, p<0,00001]. Cinsiyetler ve parazit prevalansı arasında istatistiksel anlamlı fark yoktu (%95 GA: 1,00-1,39, I^2=%18, p=0,29). İshali olan hastalarda (%95 GA: 1,09-1,58, I^2=%86, p=0,0001) ve bağışıklığı baskılanmış bireylerde (%95 GA: 1,86-3,70, I^2=%16, p=0,31) microsporidia prevalansının yüksek olduğu görüldü.
Sonuç: Bu parazitlerin prevalansı hakkında çok az çalışma olmasına rağmen, bu meta-analiz Türkiye'deki mevcut durum hakkında genel bir bilgi vermektedir.
Anahtar Kelimeler: Microsporidia, Türkiye, prevalans, meta-analiz

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Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Microsporidia are obligate intracellular pathogens infecting eukaryotic cells of many vertebrates and invertebrates (1,2). More than 1.200 species have been reported in 144 genera and 14 out of these species in seven genera have been identified as human pathogens. *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* are the most common species and are often associated with infections of the gastrointestinal tract. *Encephalitozoon* species are not limited to intestinal system enterocytes, but they also parasitize in other organs and tissues such as nervous system and respiratory system, causing different clinical situations (1,2).

Although microsporidia species generally cause self-limiting diarrhea in immunocompetent individuals, it is a serious cause of morbidity and mortality in immunocompromised patients. While the number of microsporidiosis cases was quite limited due to the insufficient interest in this issue before the AIDS pandemic, the role of microsporidia species in human pathology has become well known since the outbreak of AIDS and they are considered to be opportunistic pathogens that cause life-threatening infections in many immune-compromised diseases (3,4). Besides humans, microsporidia are known as pathogens of various animals such as fishes, fur-bearing animals, pets, honey bees, silkworms and grasshoppers. Especially, *E. bieneusi* infections are reported from primates, cats, cattle, dogs, horses, pigs, birds and various wild mammals from various parts of the world emphasizing that they are potential reservoirs for human infections (5-7).

In our country, the number of studies on the frequency of microsporidian pathogens in humans or other vertebrates is very limited. In this study, it was aimed to determine the prevalence of microsporidiosis in humans and other vertebrate hosts through a systematic review and meta-analysis in Turkey, and attract attention to this situation. In addition, the relationship of microsporidia prevalence with gender and various clinical conditions was tried to be revealed.

METHODS

The current study was conducted according to the Meta-analysis of observational studies in epidemiology (PRISMA) guidelines (8).

Search Method

Web of Science, PubMed, Scopus, and ULAKBIM databases were used for searching articles. Articles in both English and Turkish language have been included in this study. After the search of the above databases, manual searches were conducted. All published articles until April 2020 were chosen. Keywords used for searches are as following: *Microsporidium*, Microsporidiosis, Microsporidia, *Encephalitozoon*, *E. bieneusi*, Prevalence, Epidemiology, Turkey.

Inclusion and Exclusion Criteria

All studies were included in this review by the following criteria: 1-All chosen articles should be published before April 2020; 2-The studies reporting the prevalence of microsporidiosis in any age group in Turkey; 3-The studies in humans and other vertebrate; 4-Original research articles; 5-Full text studies.

Studies were excluded from the review by the following criteria: 1-The review articles; 2-The studies in invertebrates; 3-The articles that used other diagnostic methods, except staining, molecular techniques and IFA-Mabs; 4-The articles written in another language than English and Turkish; 5-The studies without raw data; 6-The studies with sample size less than 20.

The suitability of all studies was considered by two different authors. After selecting articles, the authors recorded the following information in a standard data extraction form. A flow diagram of the study design process is shown in Figure 1.

Data Extraction

After the searches were completed, each study was transferred to the pre-designed excel form as main titles. Information recorded was first author's name, publication year, gender, host, patient group, total participants, type of sample, positive cases, diagnostic methods, genus or species of the organism and type of study. Two different groups were established from the studies with control group to investigate the relationship between immune status/ diarrhea and microsporidiosis.

Statistical Analysis

Random effects model and fixed effect model were used in meta-analysis of the data. However, the results of the random effects model were taken as basis in the interpretation. In the random effects model, the Mantel-Haenszel method was applied to estimate the variance (tau-square) among the studies. 95% confidence interval (CI) was chosen. Heterogeneity among the studies was evaluated with I^2 values. I^2 value that is above 50% was evaluated as high heterogeneity and p-value <0.05 was interpreted as meaning heterogeneity was significant.

RESULTS

Selection of Studies

The systematic literature search resulted in with 168 studies. After removing duplicates, 62 studies remained. 47 studies were

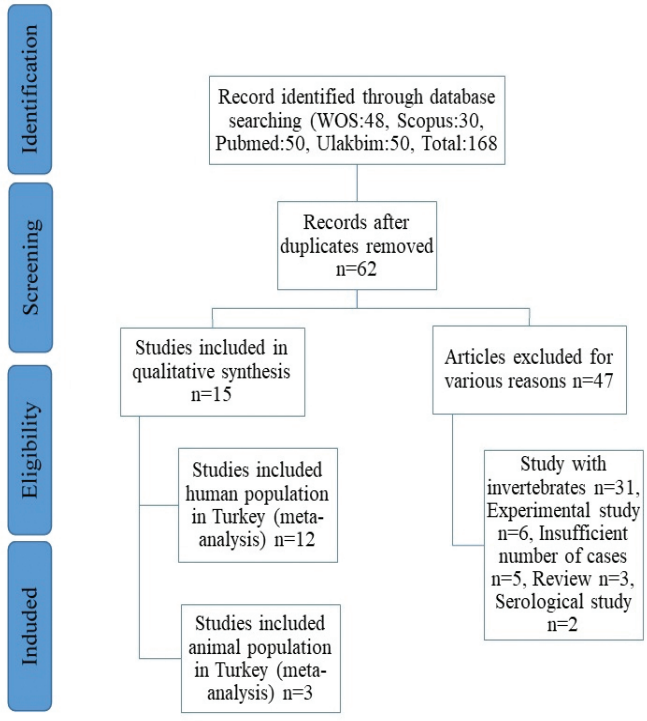

Figure 1. Flow diagram of the search and selection of studies on the prevalence of microsporidiosis in Turkey

excluded for various reason (Thirty-one studies were performed on invertebrate hosts; six studies were experimental; the number of cases in five studies was insufficient; three studies were review articles; different methods were used in two studies). Only 15 articles were included for entry in the meta-analysis (Figure 1).

Prevalence of Microsporidia in Human and Other Vertebrates

Prevalence information of human microsporidiosis (n=6.707) in Turkey are reported in 12 studies (Table 1). Overall prevalence of human microsporidiosis was 13.4% (9-20).

Five studies without control group were excluded from the analysis. Seven studies with control group were evaluated as patient and control group. For the prevalence of microsporidia infection in the patient group, due to the heterogeneity of the studies, meta-analysis calculations were performed with selecting random effect model. The risk rate of microsporidia in the total patient group is 2.87 and 95% CI 1.20, 6.87. Considering the meta-analysis data of the patient group, heterogeneity between studies was I^2 =87% (p<0.00001) and statistically significant. There is a statistically significant difference in microsporidia positivity between the control group and the patient group (Figure 2). In other vertebrate hosts, three publications investigating the prevalence of microsporidia (n=504) were found (Table 2). Forest graphics were not drawn because these studies are not controlled. Highest prevalence was found in cattle (19.3%) followed by cat (14.5%) and dog (9.7%). Overall prevalence of microsporidiosis for all animal species was 15.2% (21-23).

Microsporidiosis in Humans Based on Subgroups

Prevalence of microsporidiosis in humans according to different genders, stool appearance and immune status is summarized in Table 3.

In eight studies reporting genders and microsporidiosis positivity, the number of infected individuals has been found over thousands (Female n=1.141, Male n=1.219). For the prevalence of microsporidiosis by gender, meta-analysis calculations were performed by selecting random effect model according to the heterogeneity of the studies. According to the meta-analysis evaluation, there was no statistically significant difference (p=0.06) between genders. Among studies, heterogeneity is I^2 =18% and is not statistically significant (p<0.29) (Figure 3A).

Six studies evaluating the relationship between diarrhea and microsporidiosis were found (n=1.118). Only four of these studies had control group (n=756) and only these studies were included for further analyses. The prevalence of microsporidia was 41.9% in patients with diarrhea and 32.1% in those without diarrhea. The incidence of microsporidia

Table 1. Characteristics of the included studies reporting human microsporidiosis in different patient group and controls

First author	Year	Ref.	Type of disease	Patients			Control			Sample	Method	Genus-species	Type of study
				Total	Positive	Prevalence (%)	Total	Positive	Prevalence (%)				
Atambay M	2008	9	Gastrointestinal complaints	781	126	6.5	-	-	-	Stool	Staining	-	CS
Karaman Ü	2008	10	Cancer	320	35	10.9	320	18	5.6	Stool	Staining	-	CC
Karaman Ü	2009	11	Gastrointestinal complaints	2.665	226	8.5	-	-	-	Stool	Staining	-	CS
Calik S	2011	12	Gastrointestinal complaints	1.181	92	7.8	-	-	-	Stool	Staining	-	CS
Karaman Ü	2011	13	Urticaria	132	26	19.7	36	1	2.8	Stool	Staining	-	CC
Türk S	2012	14	Gastrointestinal complaints	225	22	9.8	-	-	-	Stool	Staining	-	CS
Cetinkaya Ü	2015	15	Bone Marrow Transplant	200	78	39	80	9	11.3	Stool	IFA-MAbs	*E. intestinalis-E. bieneusi*	CC
Hamamcı B	2015	16	Cancer	93	65	69.9	30	5	16.7	Stool	Staining-IFA-MAbs	*E. intestinalis-E. bieneusi*	CC
Özkoç S	2015	17	Different patients group	63	9	14.2	28	0	0	BAL	PCR	*Microsporidia* spp.	CC
Cetinkaya Ü	2016	18	Different patients group	100	31	31	50	8	16	Stool	PCR	*E.intestinalis*	CC
Mumcuoğlu I	2016	19	Gastrointestinal complaints	115	34	29.6	88	40	45.5	Stool	Staining	-	CC
Oğuz Kaya I	2018	20	Gastrointestinal complaints	200	77	38.5	-	-	-	Stool	PCR	*Encephalitozoon* spp.- *E.bieneusi*	CS

CC: Case-control study, CS: Cross-sectional study, PCR: Polymerase chain reaction

Table 2. Characteristics of the included studies reporting microsporidiosis in other vertebrate hosts

First author	Year	Ref.	Host	Total	Positive	Prevalence (%)	Sample	Method	Genus-species
Duzlu O	2019	21	Dog	282	41	14.5	Stool	PCR	*E. intestinalis- E. hellem*
Pekmezci D	2019	22	Domestic Cat	72	7	9.7	Stool	PCR	*Encephalitozoon* spp.- *E. bieneusi*
Bilgin T	2020	23	Cattle	150	29	19.3	Stool	PCR	*E. bieneusi*

PCR: Polymerase chain reaction

Table 3. Prevalence of microsporidiosis in humans according to different gender, stool appearance and immune status

	Total	Case	Prevalence (%)	p	I squared	Reference
Gender						
Male	1.219	237	19.4	0.06	18%	12, 13, 15-20
Female	1.141	237	20.7			
Stool appearance						
Diarrhoea	367	154	41.9	0.004	86%	15, 16, 18, 19
Non-diarrhoea	389	125	32.1			
Immune status						
Immunosuppressed	726	203	28.0	0.00001	16%	10, 15-18
Immunocompetent	608	55	9.0			

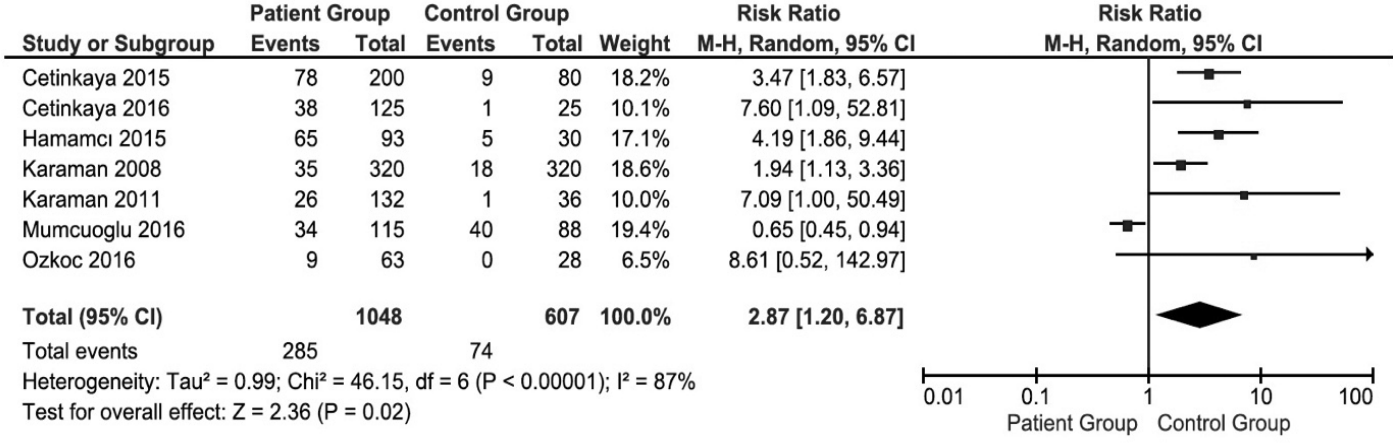

Figure 2. Forest plot diagram showing microsporidiosis in human
CI: Confidence interval

between two groups is statistically significant (p=0.004). The risk ratio of microsporidia was 1.31 in those with diarrhea [95% CI (1.09, 1.58)]. Heterogeneity (I^2 =86%) was found to be high and statistically significant among patients with diarrhea (p<0.00001) (Figure 3B).

Eight studies evaluating the relationship between immune status and microsporidiosis were found (n=3.499). Only five of these studies had control group (n=1.334) and only these studies were included for further analyses. The prevalence of microsporidia was 28% in immunocompromised patients and 9% in immunocompetent patients. The incidence of microsporidia between two groups is statistically significant (p<0.00001). The risk ratio of microsporidia was 2.62 in those with immunocompromised patients [95% CI (1.86, 3.70)]. Heterogeneity is I^2 =16%. It was found to be high and is not statistically significant (p=0.31) (Figure 3C).

Discussion

This systematic review by meta-analysis provides a comprehensive overview of the prevalence of microsporidiosis in both humans and other vertebrates from 2008 to the present day. The data were analyzed by considering healthy individuals and patient groups such as, patients with and without diarrhea symptoms, the immune status of the patients as well as a gender of patients. This study was conducted using 15 articles found in the four databases and documenting the prevalence of microsporidiosis in Turkey until April 2020.

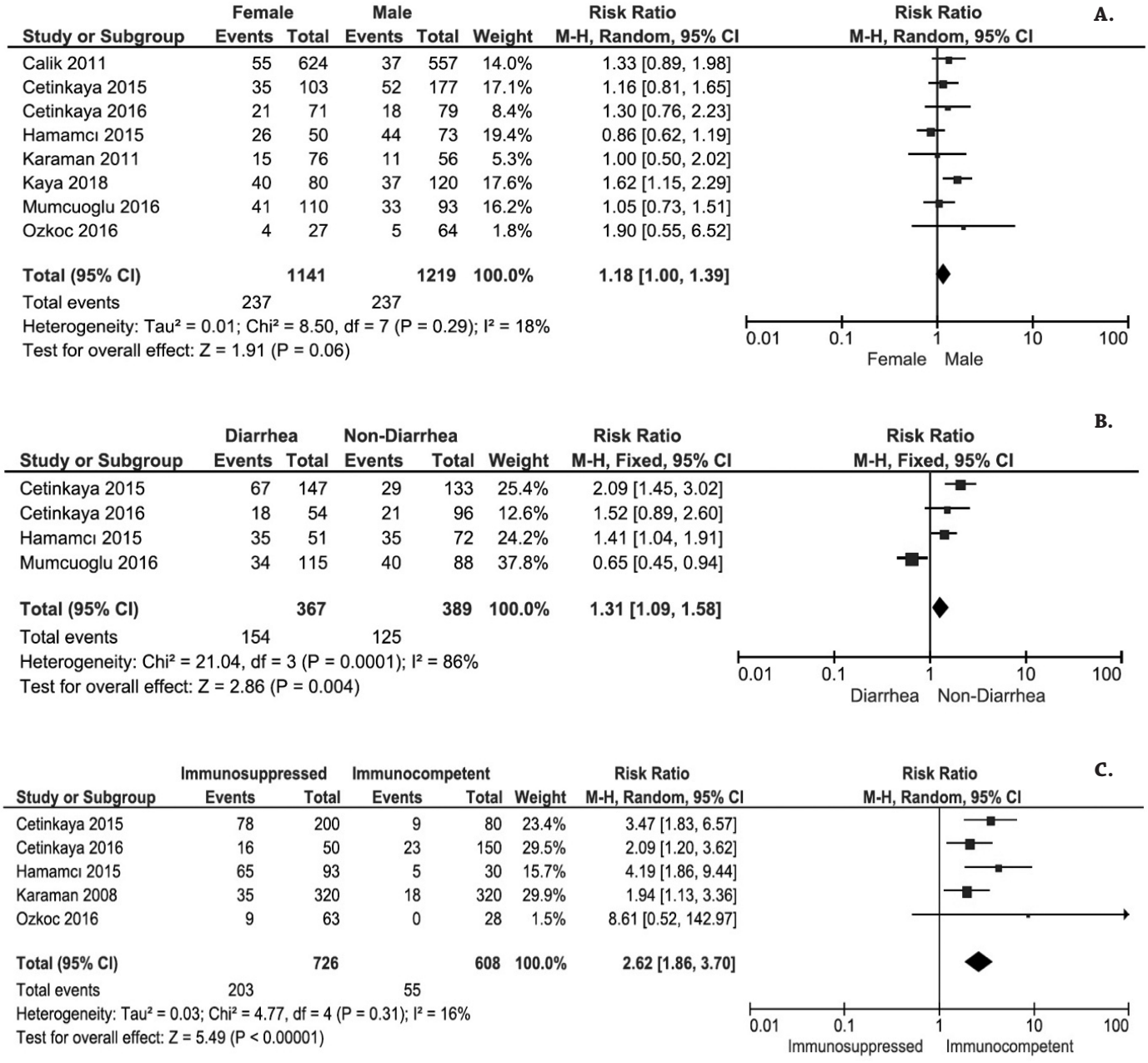

Figure 3. Forest plot diagram showing prevalence of microsporidiosis in different genders (A), patients with diarrhea (B) and according to immune status of individuals (C)

CI: Confidence interval

The highest prevalence reported in humans is in patients diagnosed with cancer (69.9%) (16). The highest prevalence reported in other vertebrate hosts is in healthy-looking cattle (19.3%) (21). This large difference is thought to be due to the all prevalence studies in humans are conducted on patients with clinical complaints or immunosuppressed individuals, while in other vertebrates studies were mainly investigating this parasite in healthy-looking animals. However, when all the cases in the articles were evaluated, it was found that there was a lower prevalence in humans (13.4%) than other vertebrates (15.2%). It was observed that this situation is similar to that of the Chinese population (24). Studies on human microsporidiosis have also been reported that *E. intestinalis* is the dominant species and other species are reported to be a lesser abundant (15,16,20). These findings are similar to the findings of the study on dogs (21), that suggesting zoonotic transmission. In similar studies, some animal species have been reported to be infected by microsporidian species that commonly found in humans and are considered as reservoirs for human infections (25,26).

In our study, microsporidia were observed at a higher rate in female than male, but this difference between gender was not statistically significant. On the other hand, in meta-analysis studies conducted in Iran (27) and in China (24), it was reported that the parasite was more common in male, although it was also not statistically significant. Another subgroup evaluated in terms of microsporidia prevalence in our study was the presence of

diarrhea in patients. In this study, microsporidia prevalence was found higher in patients with diarrhea and this difference was statistically significant. These findings are similar to the study conducted on the Iranian (27) and Chinese population (24).

The microsporidia are opportunistic pathogens. The immune system of the persons plays a key role in the severity and course of the disease (1,2). Microsporidia prevalence in immunocompromised patients is 28% in Turkey (Table 3). This is a very high rate compared to the control group and statistically significant. This rate is highest in patients with cancer (Table 1). These findings are similar to the study conducted on the Iranian (27) and Chinese population (24). The prevalence of this pathogen has been studied in different patients such as cancer or organ transplant (10,15,16,18), but there is no study conducted in HIV-positive individuals in our country, while these studies are concentrated on AIDS patients worldwide. This is a big gap that needs to be filled.

To our knowledge, this study is the first systematic review and meta-analysis of microsporidiosis prevalence in Turkey. However, this meta-analysis has some limitations that can affect the results. The first of these is being made only on people in four cities. There are still regions with no data. Secondly, there are only three studies on other vertebrate hosts and all these studies are on different species of animals. In these studies, it should be expanded both in different provinces and in different hosts. Thirdly, the methods used in diagnosis are very different from each other. In addition, the number of studies performed for species distinction and molecular characterization is very low. Especially, the phylogenetic analysis of the species detected in humans is not well studied and the inadequacy in studies on vertebrate hosts constitute a large gap in terms of zoonotic transition and reservoir hosts. As a result, we think that increasing the use of molecular methods in diagnosis and performing more detailed studies on different hosts will be beneficial for our knowledge about the transmission of these parasites and risk factors.

* Ethics

Ethics Committee Approval: Ethics committee approval was not because it is a meta-analysis study.

Informed Consent: No patients were used in the study. It is a meta-analysis study.

Peer-review: Externally and internally peer-reviewed.

* Authorship Contributions

Surgical and Medical Practices: Ü.Ç., Concept: Ü.Ç., Design: Ü.Ç., Data Collection or Processing: Ü.Ç., Analysis or Interpretation: A.C., Literature Search: Ü.Ç., Writing: Ü.Ç.

Conflict of Interest: The authors confirm that this article content has no conflict of interest.

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