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The Epidemiology of Microsporidias in Humans (Malatya sample)*

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Aim: Microsporidias, first isolated and defined in 1857, are obligate intracellular parasites observed in animal groups and especially invertebrates. Generally homosexual patients and patients who are HIV positive and immune suppressed constitute the participants of international studies about the epidemiology of microsporidias. No previous studies about the prevalence of the parasite in Turkey have been found in the literature. This study aimed to determine the epidemiology of microsporidias in and around Malatya.

Materials and Methods: Feces samples (n: 2665) from patients who presented at İnönü University Medical Faculty policlinics in 2006 with some digestive system complaints and were referred to the Parasitology Department were analyzed. The samples were analyzed using modified trichrome (MTS), Acid-Fast-Trichrome, Calcofluor, and Giemsa staining.

Results: In all 226 samples (8.5%) were positive. There was a statistically significant relationship between lack of appetite, general body pruritus, immune suppression + cancer, dyspnea, and ulcerative colitis.

Conclusions: It is important to check feces samples for *Microsporidium* spp. parasites regularly for cases including unexplained diarrhea, stomachache, lack of appetite, general pruritus, immune suppression + cancer, asthma, and ulcerative colitis, since *Microsporidium* spp. is not a commonly known parasite in Turkey.

Key Words: *Microsporidium* spp., Malatya, epidemiology, Turkey

İnsanlarda Microsporidia'ların Epidemiyolojisi (Malatya ili örneği)

Amaç: İlk kez 1857'de izole edilip tanımlanan ve zorunlu hücre içi paraziti olan microsporidialar farklı hayvan gruplarında ve omurgasızlarda görülmektedir. Microsporidiaların epidemiyolojisi ile ilgili yurt dışı çalışmalarında genellikle HIV pozitif, immün süpresif ve homoseksüel hastalar araştırma grubunu oluşturmuştur. Ülkemizde parazitin görülme yüzdesiyle ilgili araştırmalara, ulaşılan kaynak bilgilerde rastlanılmamıştır. Çalışmada microsporidiaların epidemiyolojisinin belirlenmesi amaçlanmıştır.

Yöntem ve Gereç: Malatya ili ve çevresinden, 2006 yılında sindirim sistemi şikayetleri ile İnönü Üniversitesi Tıp Fakültesi polikliniklerine başvuran ve Parazitoloji Anabilim Dalına gelen hastalar dikkate alınarak sindirim sistemi şikayetler olan bireylere ulaşma hedefi konmuş olup bu doğrultuda 2665 dışkı örneği incelenmiştir.

Bulgular: Dışkı örnekleri modifiye trichrome boyası (MTS), Asit-Fast-Trichrome, Calcofluor white ve Giemsa boyama yöntemi ile incelenmiş ve 226 (% 8,5) pozitiflik saptanmıştır. Çalışmada iştahsızlık, genel vücut kaşıntısı, allerji, immün süpresif+kanserde, nefes darlığı olanlarda ve ülseratif kolitli hastalarda yapılan istatistiki değerlendirmeye göre anlamlı ilişki bulunmuştur.

Sonuç: Sonuç olarak *Microsporidium* spp.'nin ülkemizde tanınan bir parazit olmadığından nedeni belirlenemeyen ishallerde, karın ağrısında, iştahsızlıkta, genel vücut kaşıntısı ve allerjide, immün süpresif+kanserde, nefes darlığında ve ülseratif kolitte rutin olarak dışkıda parazitin aranması gereğinin önemi vurgulanmıştır.

Anahtar Sözcükler: *Microsporidium* spp., epidemiyoloji, Malatya, Turkey

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Introduction

Microsporidium spp. cause sporadic cases characterized by symptoms such as diarrhea, cornea ulcer, and myositis that generally resolve on their own. Moreover, they are thought to be important agents of diarrhea in patients with immune deficiency, and are defined as opportunistic pathogens in Human Immunodeficiency Virus (HIV)

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infections. Some previous studies found that 15%-30% of the chronic diarrhea cases in patients with Acquired Immune Deficiency Syndrome (AIDS) where no other enteric pathogens were found were of microsporidial origin (1-4).

The diagnosis of microsporidias is made upon the detection of the spores or evolution periods in the biopsies of tissue, urine, sinus aspirates, nasal mucus, bronchoalveolar lavage, or stool. The diagnostic practices include staining methods (Modified Trichrome, Gram staining, Giemsa, fluorescent stains, acid-fast, Periodic Acid Schiff (PAS), Hematoxylin-eosin, and Wartin-Stary), serologic methods, polymerase chain reaction (PCR), and electron microscopy (5,6).

HIV positive, immune suppressed, and homosexual patients are generally selected as the participants of studies abroad about the epidemiology of microsporidias. However, no previous studies have been found in the literature regarding the prevalence of the parasite in Turkey. The aim of this study was to determine the epidemiology of the microsporidias in an expanded population covering Malatya and its surroundings (children, patients with digestive system problems etc.). Moreover, the association between the parasite and unexplained diarrhea, stomachache, and digestive system disorders was examined. It is discussed that a significant association implies the necessity of a routine examination of feces for microsporidia.

Materials and Methods

The population of this study comprised the patients in and around Malatya province who presented at İnönü University Medical Faculty policlinics in 2006 with some digestive system complaints.

While selecting the participants, patients with digestive system complaints and with stool specimens sent to the Parasitology Department were targeted. Consequently 2665 stool specimens were examined.

Data collection

A report from the ethical council was granted prior to the study. Then a questionnaire form was developed to determine the epidemiology of the *Microsporidium* spp. among people living in and around Malatya province as the dependent variable of the study. Moreover, each patient was asked to complete and sign a form to certify that they were informed about the study.

Questionnaire Form

A questionnaire form was developed to collect data that can be associated with the presence of microsporidium. The form was planned to be used during the process of stool specimen submission.

Data and Specimen Collection

The instruments for data collection were administered to patients who visited İnönü University Medical Faculty policlinics including Internal Diseases, General Surgery, Hematology, Pediatrics, Infectious Diseases, Radiation Oncology, Dermatology, Emergency Room, and Gastroenterology with some digestive system complaints and who were then referred to the Parasitology Department between 01.01.2006 and 31.12.2006.

The patients were given special containers for stool specimens and instructed to supply 3-4 tablespoons of stool specimen in case of diarrhea or the size of a walnut if there was no diarrhea and to close the cover firmly for return to the parasitology laboratory in no more than 1 h. After the instructions were given the questionnaire form was administered.

The questions in the form were asked and completed by the researcher. Moreover the diagnostic data were provided by the physician and the patient during the patient's anamnesis.

Only complaints concerning microsporidium were covered in the questionnaire.

It was assumed that the participating patients completed the questionnaire form truly and frankly. Moreover the form was assumed to have the adequate level of validity and reliability.

Development of the method used for the diagnosis of the *Microsporidium* spp.

Since no studies concerning the diagnosis and epidemiology of the parasite were found in the literature review in Turkey, 2 foreign experts, Lynne S. Garcia (from Santa Monica, California, USA) and Prof Dr. Rainer Weber (from the Department of Infectious Diseases, Zurich University Medical Faculty, Switzerland) were contacted for a standardized method and they were asked for their recommendations after the possible diagnostic methods were specified in detail. Stains were prepared in line with the recommendations from both experts.

Since staining had to be done in parallel with positive specimens for the accuracy of the stain method to be used, Dr. Weber was asked to send some positive feces

samples and some stained positive preparations for ease in the examination. The specimens obtained from the participating patients were stained and examined in parallel with the positive sample, and a total of 10 specimens which were positive, negative, or suspicious were sent to Dr. Weber for his approval. Then, it was seen that the results from Dr. Weber were in compliance with the findings of this study and we concluded that a diagnosis of microsporidium could be made.

The feces samples were analyzed using modified trichrome (MTS) (5), Aist-Fast-Trichrome (7), Calcofluor (8), and Giemsa staining.

Data Analysis

The data obtained in this study were presented as means, standard errors, or figures/percentages. Independent samples t-test and chi-square test were used for the statistical analysis. Moreover, multivariate logistic regression analysis was used to assess the relationship between the parasite and some variables including age, gender, nausea-vomiting, immunosuppression + cancer, retarded growth and development (RGD), diarrhea, constipation, pruritus ani, salivation, stomachache, lack of appetite, dyspnea, anemia, general pruritus, and ulcerative colitis. In assessing the results, $P < 0.05$ was considered statistically significant. The analysis of the data was done using SPSS 13.0 for Windows (SPSS Inc., Chicago, USA).

Results

The fecal specimens of a total 2665 voluntary patients residing in or around Malatya province who presented at İnönü University Medical Faculty policlinics including Internal Diseases, General Surgery, Hematology, Podiatry, Infectious Diseases, Radiation Oncology, Dermatology, Emergency Room, and Gastroenterology with some digestive system complaints and who were then referred to the Parasitology Department between January 2006 and December 2006 were analyzed.

The methods used for the diagnosis of the 226 cases with detected *Microsporidium* spp. included MTS (Figure 1), Acid-Fast-Trichrome (Figure 2), Calcofluor white (Figure 3), and Giemsa staining. Since the spores could not be distinguished clearly in the preparations stained with Giemsa, only those positive results obtained by the remaining 3 methods (MTS, Acid-Fast-Trichrome, and Calcofluor white) were taken into consideration.

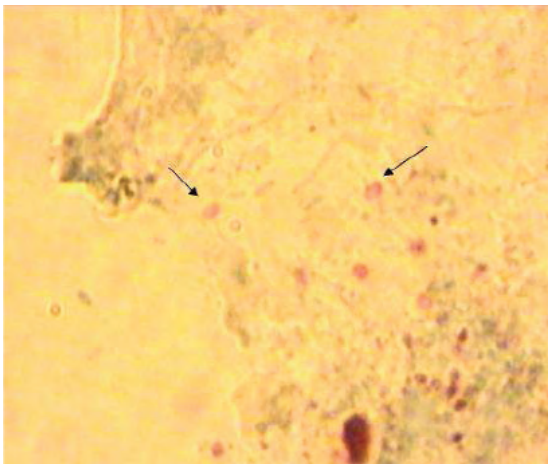

Figure 1. *Microsporidium* spp. spores in positive sample 100 (MTS).

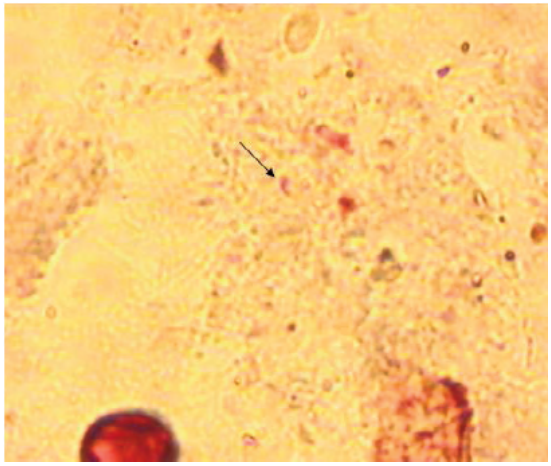

Figure 2. *Microsporidium* spp. spores in positive sample 100 (Acid-fast-trichrome).

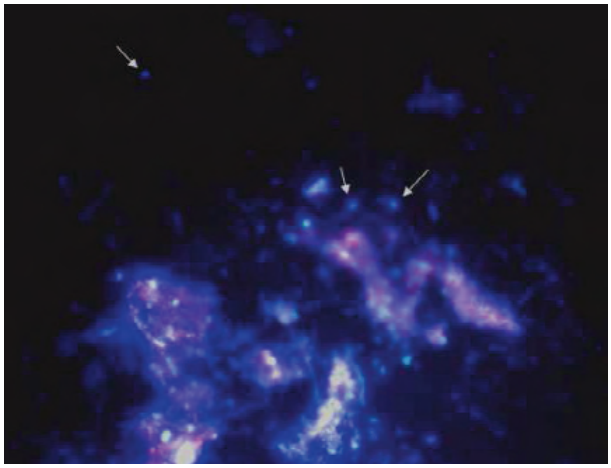

Figure 3. *Microsporidium* spp. spores in positive sample 100 (Calcofluor white).

Microsporidium spp. rates in and around Malatya province according to the study results are shown in Table 1.

Table 1. *Microspordium* spp. in and around Malatya province.

Microsporidium spp. prevalence	Number	%
Negative	2439	91.5
Positive	226	8.5
Total	2665	100.0

The participating patients were aged 24.56 (SEM = 0.38) on average. The distribution of positive cases by month is given in Figure 4.

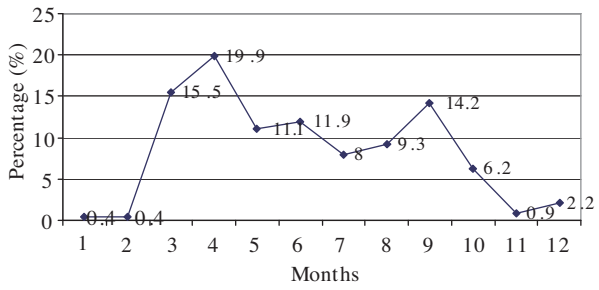

Figure 4. Distribution of positive cases by months.

Based on the results of the analysis, no significant difference was found for the disease variable between different ages (P = 0.60). The distribution of the parasites according to age groups is given in Figure 5.

Distribution of participating patients by the provinces they come from is given in Table 2.

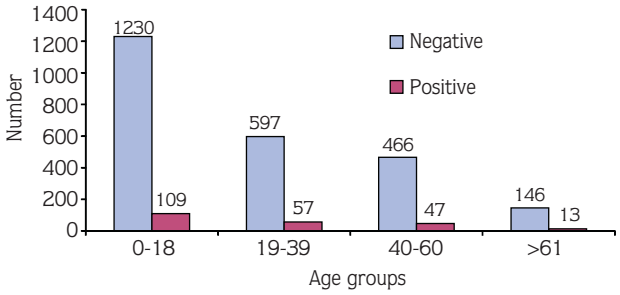

Figure 5. The distribution of the parasites according to age groups.

In this study stool specimens obtained from 2665 patients with digestive system complaints were analyzed using modified trichrome, Calcofluor, and acid fast-trichrome staining, and the *Microsporidium* spp. were found at a rate of 8.5%. The statistical analysis revealed a significant association between both general and positive cases among patients with lack of appetite, general pruritus, allergic, immunosuppression + cancer, dyspnea, and ulcerative colitis ($P < 0.05$). There was no association in the general assessment among cases of diarrhea, stomachache, salivation, constipation, nausea/vomiting, retarded growth and development, and anemia ($P > 0.05$). The analysis of the positive cases revealed a significant difference ($P < 0.05$). The results of multivariate logistic regression analysis are presented in Table 3.

The logistic regression analysis revealed a significant association between the prevalence of the parasite and RGD, stomachache, lack of appetite, dyspnea, general pruritus, and ulcerative colitis ($P < 0.05$). The logistic regression model showed good fit, which was confirmed by the Hosmer-Lemeshow test ($P = 0.96$).

Discussion

In this study, we aimed to determine the epidemiology of microsporidium. First the patients with digestive system complaints were analyzed and the findings were discussed and interpreted in terms of hypotheses.

The review of the relevant literature revealed studies on the epidemiology of the parasite in Argentina, Australia, Brazil, Canada, Czech Republic, France, Germany, China, Italy, Japan, New Zealand, Spain, Sri Lanka, Switzerland, Thailand, Uganda, USA, Zambia, Sweden, Botswana, and Holland (5). No studies about the epidemiology of the parasite in Turkey, however, have been found. On the other hand, Yazar et al. (9) reported *Microsporidium* spp. in a cancer patient and Buget et al. (10) reported it in an AIDS-infected patient, while Ozkirim and Keskin (11) reported Nosema among bees, Eroksuz et al. (12) reported *Encephalitozoon* spp. among a rabbit colony, and Yaman and Radek (13) reported Nosema among coleopteras at 42%.

The first studies about the presence of the parasite were presented as case studies. Then epidemiological studies about microsporidium were generally conducted in immune suppressed patients (14-17). Accordingly, the prevalence of the parasite reported by several researchers

Table 2. Distribution of participating patients by provinces.

Provinces	Negative		Positive		Total	
	Number	%	Number	%	Number	%
Malatya/Center	2173	89.1	192	85.0	2365	88.8
Other provinces*	51	2.1	5	2.2	56	2.1
Adıyaman	131	5.4	18	8.0	149	5.6
Kahramanmaraş	84	3.4	11	4.9	95	3.5
Total	2439	100.0	226	100.0	2665	100

* İzmit, Kars, İstanbul, Ankara, Batman, Bingöl, Bitlis, Kilis, Adana, and Gaziantep

Table 3. The factors related to the parasite.

Variable	Estimate	SEM	Wald	df	P	Odds Ratio	95% Confidence Interval	
							Lower	Upper
RGD	0.485	0.240	4.096	1	0.043	1.625	1.015	2.600
Stomachache	0.252	0.144	3.074	1	0.080	1.286	0.971	1.704
Lack of appetite	0.971	0.467	4.320	1	0.038	2.641	1.057	6.598
Dyspnea	0.936	0.462	4.098	1	0.043	2.549	1.030	6.307
General pruritus	0.686	0.211	10.569	1	0.001	1.986	1.313	3.003
Ulcerative colitis	0.979	0.378	6.710	1	0.010	2.661	1.269	5.579
Constant	-0,499	0.396	1.590	1	0.207	0.607		

df: degree of freedom; SEM: Standard error mean, RGD: Retarded Growth and Development

is as follows: Bretagne et al. (18) at 7%, Field et al. (15) at 33%, Kotler et al. (19) at 39%, Garcia et al. (20) at 42%, Kokoskin et al. (21) at 12%, Brasil et al. (22) at 27.5%, and Kumar et al. (23) at 6.5%. Furthermore, Fournier et al. (16) have reported *Microsporodium* spp. in the urine samples of 12 AIDS-infected patients.

The studies on subjects with healthy immune systems proved that the parasite can cause acute and chronic diarrhea (24,25). Furthermore, some researchers have reported microsporidium in patients at varying rates: Bretagne et al. (18) at 7%, Termmathurapoj et al. (26) at 1.3%, Tumwine et al. (27) at 17.4%, and Abreu-Acosta et al. (28) at 11.5% in 156 stool specimens, at 2.5% in 40 urine specimens, and at 16.2% in 37 saliva specimens. Moreover, Raynaud et al. (29) reported the presence of the parasite in the stool specimens of 4 young negative immune suppressed patients with chronic diarrhea. In the present study *Microsporidium* spp. were found in 226 (8.5%) stool specimens out of 2665 forwarded to the parasitology laboratory (Table 1). The differences in the rates of positive cases obtained in the studies quoted above can be thought to stem from characteristics of the regions worked on, the subjects chosen, the methods used, and the experience of the researchers.

Although the study was performed in the Malatya region, other patients coming from the surrounding provinces were also involved in the study. Patients coming from Malatya province and surroundings constituted 85% of the positive cases. According to the assessment results of the patients coming from other cities, the rate of the *Microsporidium* spp. parasite among patients from the Adıyaman region visiting the hospital with a digestive system complaint was detected as 5.3% and the rate was 11.5% among similar patients coming

from Kahramanmaraş region. This finding indicated that a future epidemiologic study in these 2 regions would likely reveal a higher prevalence of *Microsporidium* spp. cases. Furthermore, 2.2% of the positive cases were found to be those patients who were actually residing in another city but were in Malatya on holiday (Table 2). Muller et al. (30) reported microsporidium among 6.0% of 148 tourists visiting the hospital with diarrhea complaints. This finding can be considered to support the idea that the parasite can cause diarrhea in tourists.

An analysis of the distribution of the positive cases by months (Figure 4) indicates an increase in the prevalence of the parasite during rainy months. In a similar study Tumwine et al. (31) also reported an increase in the prevalence of the parasite in rainy months. It is thought that, coupled with the results of this study, a regular trace of the *Microsporidium* spp. cases in subsequent years can clarify the seasonal distribution pattern of the prevalence of the parasite.

The fecal specimens were stained using Calcofluor, MTS, and acid-fast-Trichrome stains and suspected specimens were re-stained for double-checks, which revealed parallel results.

Since the typical characteristics of the parasite could not been observed as a result of the staining practice with Giemsa stain, no definite diagnosis could be made. This could have been caused by the staining method, researchers, and specimens selected.

The positivity rate (8.5%) obtained in this study was restricted by the staining method. The relevant literature seemed to emphasize the importance of the staining method in routine diagnosis. Kokoskin et al. (21) increased the temperature specified in Weber's method to 50 °C and reduced the time to 10 min, which they reported as an effective modification to the method. DeGirolami et al. (22) reported to have found the parasite at a rate of 18.6% using the MTS and Uvitex 2B methods. Raynaud et al. (29) also reported to have found *E. intestinalis* using MTS and Uvitex 2B staining methods. Carter et al. (31) emphasized the efficacy of the MTS method in diagnosing the parasite. Similarly Ryan et al. (32) reported using aniline blue instead of fast green in the MTS stain and obtained better staining on the parasite in this way (Figure 2). Ignatius et al. (33), on the other hand, recommended using MTS and Uvitex 2B in the routine diagnosis of microsporidia.

DeGirolami et al. (22) recommended both Uvitex 2B staining and MTS for better results.

Chioralia et al. (8) reported that the parallel use of Calcofluor White 2MR and MTS gives effective results in the diagnosis of the parasite. Again Joseph et al. (34) reported to have prepared the Calcofluor stain with KOH, which resulted in a more effective diagnosis. Reisner et al. (7) reported that only the Acid-Fast-Trichrome method is sufficient in the diagnosis of *Crytosporidium parvum* and microspordia.

It has also been reported that other microorganism containing chitin and especially fungus spores are stained by these stains, as well (1,3,35). The specimens in this study were assessed bearing this in mind throughout the study.

It has been reported that Calcofluor M2R and Sytox green stains work in detecting whether the microsporidias are alive or not, with alive spores appearing oval-shaped turquoise-blue at 395-415 nm wavelength in Calcofluor M2R method and dead spores appearing white-yellow (36). White-yellow spores were also found in the positive cases in this study.

It was found that young patients constituted 44.2% of the positive cases. Bretagne et al. (18) reported to have found *Microsporidium* spp. in the feces of 8 out of 980 African children who were clinically healthy. Based on this finding it can be concluded that *Microsporidium* spp. can be found in children and patient groups who are not immune suppressed, and that those patients presenting to the hospital with different complaints should also be examined in terms of microsporidium presence.

Based on the findings of this study following suggestions can be made:

1. Since the study about the epidemiology of the *Microsporidium* spp. was limited to the Malatya region, similar studies regarding the epidemiology of the parasite should be conducted in other regions of the country,

2. Some experimental studies should be done concerning the infection and evolution of *Microsporidium* spp. and steps should be taken to inform the public about protective measures,

3. Given the clinical symptoms apparent in the diagnosis of the *Microsporidium* spp., patients with prolonged digestive system complaints should be referred

to the parasitology laboratory for investigation of the parasite,

4. Specimens should be taken and analyzed for possible *Microsporidium* spp. presence during and after the treatment process of immune suppressed and cancer patients based on their complaints,

5. Staining methods should be used in routine diagnosis,

6. In cases of suspicious diagnosis, staining should be repeated, and if the problem continues TEM and PZR techniques should be employed to the extent that laboratory conditions allow,

7. *Microsporidium* spp. should also be considered as an agent while making a diagnosis in case of complaints including ulcerative colitis, lack of appetite, dyspnea, general pruritus, RGD, and stomachache, and related diseases.

8. The relationship between the prevalence of *Microsporidium* spp. among patients with digestive system complaints and urinary system infection, diabetics, obesity, dyspepsia, enuresis nocturne, chronic liver disease, fever, and eosinophilia was not assessed since the number of participants was not sufficient (below n = 30). Thus it is suggested that future studies investigate the relationship with larger groups.

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