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Retrospective Results of Hacettepe University Faculty of Medicine Parasitology Laboratory Between 2014-2019

Hacettepe Üniversitesi Tıp Fakültesi Parazitoloji Laboratuvarı'nın 2014-2019 Yılları Arası Sonuçlarının Retrospektif Olarak Değerlendirilmesi

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ABSTRACT

Objective: Parasitic infections emerge as a significant health problem, especially in underdeveloped and developing countries. Epidemiological data play an important role in taking effective measures against parasitic diseases.

Methods: Clinical samples (stool, blood, bone marrow and tissue samples, etc.) that were sent to Hacettepe University Hospitals Parasitology Laboratory between 2014 and 2019 were analyzed retrospectively.

Results: The positivity rates of the parasites detected in this study are as follows; *Blastocystis* sp. (71.6%), *Dientamoeba fragilis* (13.3%), *Giardia lamblia* (4.7%), *Echinococcus* spp. (1.9%), *Enterobius vermicularis* (1.8%) and *Taenia* spp. (0.3%). In this study, four of the patients were found to be positive for *Leishmania* spp. and two patients for *Plasmodium falciparum* and four patients for *Plasmodium* spp. *E. histolytica/E. dispar* cysts and/or trophozoites examined by Trichrome staining in our study were not detected within six years.

Conclusion: According to this data and in the light of the results obtained from different regions of our country, it will be possible to properly direct the necessary strategies for the diagnosis, treatment of parasitic infections and the implementation of preventive measures.

Keywords: *Blastocystis* sp., *Dientamoeba fragilis*, parasitology

ÖZ

Amaç: Paraziter enfeksiyonlar, özellikle az gelişmiş ve gelişmekte olan ülkelerde önemli bir sağlık sorunu olarak ortaya çıkmaktadır. Epidemiyolojik veriler, paraziter hastalıklara karşı etkili önlemlerin alınmasında önemli rol oynamaktadır.

Yöntemler: Çalışmamızda 2014-2019 yılları arasında Hacettepe Üniversitesi Hastanesi Parazitoloji Laboratuvarı'na gönderilen klinik örnekler (dışkı, kan, kemik iliği ve doku örnekleri vb.) retrospektif olarak incelenmiştir.

Bulgular: Parazitlerin pozitiflik oranları; *Blastocystis* sp. (%71,6), *Dientamoeba fragilis* (%13,3), *Giardia lamblia* (%4,7), *Echinococcus* spp. (%1,9), *Enterobius vermicularis* (%1,8) ve *Taenia* spp. (%0,3) şeklinde tespit edilmiştir. Hastaların dördünde *Leishmania* spp., iki hastada *Plasmodium falciparum* ve dört hastada *Plasmodium* spp. saptanmıştır. Çalışmamızda Trichrome boyama ile incelenen *E. histolytica/E. dispar* kistleri ve/veya trofozoitleri altı yıl içinde tespit edilmemiştir.

Sonuç: Bu veriler doğrultusunda ve ülkemizin farklı bölgelerinden elde edilen sonuçlar ışığında paraziter enfeksiyonların teşhisi, tedavisi ve önleyici tedbirlerin uygulanması için gerekli stratejilerin doğru yönlendirilmesi mümkün olacaktır.

Anahtar Kelimeler: *Blastocystis* sp., *Dientamoeba fragilis*, parazitoloji

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Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Parasitic infections emerge as a significant health problem, especially in underdeveloped and developing countries (1). The World Health Organization (WHO) stated that 3.5 billion people on earth are infected with intestinal parasites and 450 million people have the disease symptomatically (2). Intestinal parasitic infections cause malnutrition, malabsorption, anemia, growth retardation, learning difficulties, diarrhea and other gastrointestinal system complaints, especially in children (3).

In this global era, the importance of travel health is understood better and blood and tissue parasites are not only tropical countries problems anymore. *Plasmodium* and *Leishmania* species are important mortality and morbidity causes worldwide. In 2019, approximately 87 million cases of malaria were diagnosed in 87 endemic countries, according to WHO reports (4). Every year, it is estimated that approximately thirty thousand new visceral leishmaniasis cases and one million cutaneous leishmaniasis cases are diagnosed (5).

Epidemiological data play an important role in taking effective measures against parasitic diseases. Regional epidemiological data should be evaluated to take preventive measures and determine treatment strategies. In this study, we aimed to retrospectively evaluate the data of samples processed in the parasitology laboratory of our hospital between 2014-2019.

METHODS

Clinical samples (stool, blood, bone marrow and tissue samples, etc.) that were sent to Hacettepe University Hospitals Parasitology Laboratory between 2014 and 2019 were analyzed retrospectively. Stool samples were collected in containers containing formol. Stool samples were processed by the modified formol ethyl acetate precipitation method and examined with saline and Lugol's iodine. Modified kinyoun's acid-fast staining was performed to detect *Cryptosporidium, Cyclospora or Cystoisospora*. Trichrome staining was performed for other intestinal protozoa. To detect *Enterobius vermicularis* infection, the cellophane tape method was used. Cyst fluids were examined by the direct microscopic examination for the diagnosis of *Echinococcus* spp. Blood, bone marrow and tissue samples were stained with Giemsa stain. Bone marrow samples were inoculated to NNN medium to detect *Leishmania* species and incubated at 23-25 °C for three weeks and the presence of promastigotes was investigated microscopically every week.

Statistical Analysis

Because of the low number of patients, statistical analyses could not be made.

RESULTS

A total of 67,069 clinical samples were sent to the parasitology laboratory between 2014-2019. Of these samples, 99.1% were stool samples. The incidence of intestinal parasites was found as 7.5% in this study. The distribution of samples by year as follows; 2014: 13.3% (n=8.935), 2015: 16.7% (n=11,226), 2016: 19.0% (n=12,785), 2017: 17.0% (n=11,451), 2018: 17.2% (n=11,553), 2019: 16.5% (n=11,119). The rate of parasite positive samples tends to decrease after 2015 (Table 1). The highest parasite rate was found in 2015.

A total of 5.082 clinical samples from 4.793 patients were detected as parasite positive. The distribution of detected parasites is as follows; *Blastocystis* sp. (71.6%), *Dientamoeba fragilis* (13.3%), *Giardia lamblia* (4.7%), *Echinococcus* spp. (1.9%), *Enterobius vermicularis* (1.8%) and *Taenia* spp. (0.3%). The distribution of intestinal parasites by year and species are given in Table 2. In this study, four of the patients were found to be positive for *Leishmania* spp. and two patients for *Plasmodium falciparum* and four patients for *Plasmodium* spp. *Leishmania* spp. was detected only in 2014, 2016, 2018, 2019. *Plasmodium* spp. was detected only in 2018, 2019.

DISCUSSION

The source of intestinal parasites is humans with parasitosis and it can occur by the spread of cysts, oocysts, eggs and larvae to the environment, directly, after they have developed in the soil or by using another living thing as a mediator (6,7). It is reported that the most common mode of transmission in intestinal parasites is oral ingestion of infective forms (8-11). The incidence of intestinal parasites varies according to the socio-economic and cultural level of societies, hygiene conditions, eating habits, demographic characteristics and geographical conditions (12). Today, the prevalence of intestinal parasites is accepted as an indicator of the development level of societies. Globally reported results to vary by year and region. In studies conducted in different regions of our country; the incidence of intestinal parasites varies between 4.1 and 75%, depending on age groups, the laboratory method applied, the experience of the laboratory staff performing the stool examination, and whether apathogenic ones are included in the study (13). Although it is believed that the spread of loses its significance with time, it is obvious that the problem of parasitosis continues. The study which included the distribution of parasites detected by Çaycı et al. (12) between 2014 and 2016 years, also found an incidence of intestinal parasites at 1.89%. The most common parasites also were found as *Giardia intestinalis* and *Blastocystis* sp. In the study of the distribution of pathogenic intestinal parasites in Sivas Cumhuriyet University Faculty of Medicine Research and Application Hospital between 2006-2018, the parasite positivity rate was detected as 10.8% (14). In another study, intestinal parasites were detected in 9.79% of patients who were admitted during five years period. In the study the most frequently identified intestinal parasites were detected *Blastocystis* sp., *Giardia intestinalis* and *Dientamoeba fragilis* in

Table 1. The number of clinical samples sent to the laboratory between 2014-2019 years and the rate of samples with parasites

Years	Number of samples containing parasite % (n)	Total number of samples % (n)
2014	1.3% (881)	13.3% (8.935)
2015	1.7% (1.202)	16.7% (11,226)
2016	1.4% (1.001)	19.0% (12,785)
2017	1.1% (799)	17.0% (11,451)
2018	0.9% (649)	17.2% (11,553)
2019	0.8% (550)	16.5% (11,119)
Total	7.5% (5.082)	100% (67,069)

Table 2. Distribution of the number of patients with intestinal parasites by year and species

Parasite	2014	2015	2016	2017	2018	2019	Total
Blastocystis sp.	10% (527)	16% (806)	14% (715)	11.8% (568)	9.3% (449)	7.6% (365)	71% (3.430)
Dientamoeba fragilis	2.7% (134)	2.7% (132)	2.9% (139)	1.6% (77)	1.7% (84)	1.5% (72)	13.3% (638)
Giardia lamblia	0.8% (42)	1.11% (54)	0.8% (39)	0.83% (40)	0.54% (26)	0.52% (25)	4.6% (226)
Echinococcus	0.1% (8)	0.5% (27)	0.12% (6)	0.25% (12)	0.4% (20)	0.3% (19)	1.9% (92)
Enterobius vermicularis	0.3% (16)	0.05% (24)	0.16% (8)	0.29% (14)	0.25% (12)	0.2 (14)	1.8% (88)
Taenia spp.	0.04% (2)	0.04% (2)	0.06% (3)	0	0.04% (2)	0.06% (3)	0.24% (12)
Ascaris lumbricoides	0	0	0	0	0.02% (1)	0	0.02% (1)
Hymenolepis nana	0.02% (1)	0.04% (2)	0	0	0	0	0.06% (3)
Cystoisospora belli	0	0	0	0	0.02% (1)	0	0.02% (1)
Cryptosporidium parvum	0	0	0	0	0.02% (1)	0	0.02% (1)

stool samples by Baştemir et al. (15). This distribution of parasites is compatible with our study. The incidence of intestinal parasites was found as 7.5% in this our study. The rate of parasite positive samples tends to decrease after 2015 in our study.

Blastocystis sp. is a common intestinal parasite worldwide. The prevalence is higher in developing countries and is usually associated with poor standards of hygiene, exposure to animals and the consumption of contaminated food or water. Its pathogenicity is debated but it is argued to be a causative agent of gastrointestinal and dermatological disorders (16). Accumulating data suggest a correlation between *Blastocystis* sp. with cutaneous lesions, particularly urticarial (17). In our study, the most common intestinal parasite was found to be *Blastocystis* sp. each year. In our study, the positivity rate of *Blastocystis* sp. was found to be %71 (n=3.430) (Figure 1A). The high positivity rate of *Blastocystis* sp. is noteworthy. *Blastocystis* sp. is also found to be the most prevalent parasite in other studies from Turkey (10,18-20). In a study conducted by Uyar et al. (19), the most common parasite was detected as *Blastocystis* sp. (13.1%) between 2011 and 2013. In a study from Dokuz Eylül University Medical Faculty Hospital, *Blastocystis* sp. was the most common parasite and detected positive in 689 patients (4.83%) between 2005-2008. In a study from from Dokuz Eylül University Medical Faculty, the most frequently detected parasite was also *Blastocystis* sp. between 2008-2017 (39.8%) (20). Furthermore, Kaya et al. (21) determined that *Blastocystis* sp. was the most frequently detected parasite in immunosuppressive patients.

Giardia lamblia (69.5%), *Enterobius vermicularis* (9.7%) and *Taenia saginata* (6.8%) were found to be the most common parasites respectively between 1991 and 2001 in our laboratory. Between 2003 and 2010, the most common parasites were *Giardia lamblia* (40%), *Blastocystis* sp. (22%), *Dientamoeba fragilis* (9%), *Enterobius vermicularis* (5%), *Echinococcus* spp. (4%) and *Taenia* spp. (3%) respectively. *Giardia lamblia*, the most common parasite, tended to decrease after 2004 whereas cases with *Blastocystis* sp. showed a clear increase in 2011 and 2012 (1). This tendency can be explained by an increasing in awareness for *Blastocystis* sp. and *Dientamoeba fragilis*. In recent years, there has been significant debate regarding whether *Blastocystis* sp. are intestinal commensals, markers of dysbiosis, or true pathogens and more and more studies are conducted on this parasite (22).

E. histolytica/E. dispar cysts and/or trophozoites, which are examined by trichrome staining in our study were not detected within five years. It has been observed that trichrome staining is the method recommended method for the detection of *E. histolytica/E. dispar* cysts and/or trophozoites (23). Additionally,

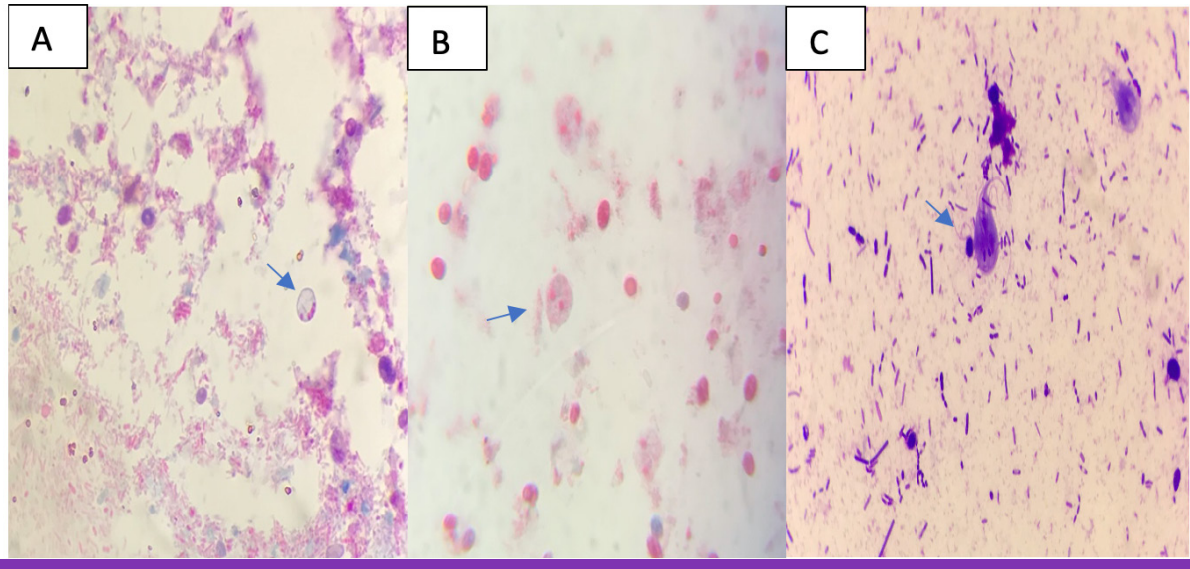

Figure 1. A) Microscopic examination of the vacuolar form of *Blastocystis* sp. in the trichrome stain preparation B) Trophozoite form of *Dientamoeba fragilis* in the trichrome stain preparation (Trichrome stain-1.000x); C) Trophozoite form of *Giardia lamblia* in the Giemsa stain preparation (Giemsa stain-1.000x)

an improvement in hygiene conditions and access to clean water may be the reason for the decreased numbers of positivity for *Giardia lamblia* and *E. histolytica/dispar* in our laboratory.

Between 2003-2012 four *Leishmania* spp. and four *Plasmodium* spp. patients were reported in our hospital in ten years (1). In our study, between 2014-2019 four *Leishmania* spp. and four *Plasmodium* spp. and two *Plasmodium falciparum* positive patients were found. Our region is not endemic for cutaneous and visceral leishmaniasis. It is mostly reported in Southeastern Anatolia, Mediterranean and Aegean Regions in our country. One of the patients diagnosed with *Leishmania* spp. had a travel history abroad, and the other three patients were admitted from endemic regions of our country. Four *Plasmodium* spp. and two *Plasmodium falciparum* positive patients had a travel history abroad. *Plasmodium vivax* is the most common malaria agent in Turkey, but cases of malaria originating abroad have been reported in our study. This shows that there is a tendency to increase in travel related to this infection compared to past years. This underlines the necessity of more awareness studies and effective measures in travel medicine.

In this study, we included the retrospective results of Hacettepe University Faculty of Medicine Parasitology Laboratory between 2014-2019. We did not include the 2020-2021 data because we predicted that the occurring severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) pandemic could affect the parasite data. In the future, results will be shared with the literature about data of parasite infections in the case of the SARS-CoV-2 pandemic will also be shared.

CONCLUSION

The prevention and control of intestinal parasitic infections are now more possible than ever before, owing to the discovery of safe and effective drugs, the improvement and simplification of some diagnostic procedures. In recent years, general health care strategies have emphasized preventive medicine and community cooperation in the control of endemic disease and have created a favourable environment for the design and implementation of control measures against intestinal parasitic infections. As a result, this study shows a decrease in parasite rates since 2015.

In our study, we have found that the rate of positivity of *Blastocystis* has increased and *Giardia lamblia* has decreased. Improvement in hygiene conditions can be a reason for the decrease in *Giardia lamblia*. The pathogenicity of *Blastocystis* sp. has been under debate and more studies are needed to understand its pathogenic ability. Therefore, the clinicians give their treatment according to the patient's clinical situation. The low numbers of patients were detected with *Leishmania* and *Plasmodium* but an increase in annual rate has been noticed. The evaluation of the results of our laboratory, which is one of the centers that can screen many patients in its region, will contribute to the epidemiological data of our country. In the light of the results obtained from different regions of our country, it will be possible to properly direct the necessary strategies for the diagnosis, treatment of parasitic infections and the implementation of preventive measures. This highlights the fact that parasitic infections are still an important public health problem.

*Ethics

Ethics Committee Approval: Not necessary.

Informed Consent: Not necessary.

Peer-review: Externally peer-reviewed.

*Authorship Contributions

Concept: N.İ., T.Ü.A., Design: N.İ., T.Ü.A., Data Collection or Processing: N.İ., Analysis or Interpretation: N.İ., S.E., Y.A.Y., Literature Search: N.İ., T.Ü.A., S.E., Y.A.Y., Writing: N.İ., T.Ü.A., S.E., Y.A.Y.

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