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EDİTÖRDEN

2024 yılının ikinci sayısını üçü yurt dışı kaynaklı 7 özgün araştırma makalesi, 1 olgu sunumu, 2 derleme ve 1 editöre mektup ile çıkarmaktayız. Özgün araştırmalarda anti-*Trichomonas* aktivite gösteren maddelerin denendiği bir araştırma, metilen mavisinin sülükler üzerine etkisinin araştırıldığı bir çalışma ve ülkemizdeki parazitoloji alanında yapılan yüksek lisans ve doktora tezlerinin bibliometrik analizlerinin yapıldığı bir çalışmaya yer verilmiştir. Yurt dışından gelen makalelerde ise İran'da *Blastocystis* varlığı ile ürtikerin ilişkisini araştıran bir makale, kıyı sularında *Cryptosporidium* ve Giardia enfeksiyonlarının risklerini analiz eden bir makale ve Orta Asya'da kistik Ekinokokkozis durumunu ortaya koyan kapsamlı bir makale sunulmaktadır.

Olgu sunumu olarak tavukta ilk kez saptanan *M. pallidulus* olgusu verilmiştir. Derleme makalelerde ise parazitlerin faydalı yönleri olabileceğini yorumlayan bir yazı ile *Toxoplasma*'nın insan ve hayvanlardaki moleküler modellerini derleyen bir makaleye yer verilmiştir.

Dergimizin ESCI için de başvurusu yeniden yapılmış olup sonucu beklenmektedir. Bu sürece büyük katkısı olan ve gönderilen makalelere özveri ile hakemlik yapan, bu sayının sonunda da listesi yayınlanan akademisyenlerimize de teşekkür etmek ve minnetlerimi sunmak isterim.

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Prof. Dr. Yusuf Özbel

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EDİTÖRE MEKTUP / LETTER to the EDITOR

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Investigation of the Antitrichomonal Activity of Cinnamaldehyde, Carvacrol and Thymol and Synergy with Metronidazole

Sinnamaldehit, Karvakrol ve Timolün Antitrichomonal Aktivitesi ve Metronidazol ile Sinerjisinin Araştırılması

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ABSTRACT

Objective: *Trichomonas vaginalis* is a sexually transmitted protozoan parasite that usually causes infections in women. Metronidazole is used as the first choice in the treatment of this parasitic disease, but there is a need for new drugs since 1980's with increasing numbers of reported resistance. In this study, it was aimed to determine the antitrichomonal activity of the major components of *Cinnamomum zeylanicum* (cinnamon) and *Thymus vulgaris* (thyme) essential oils, cinnamaldehyde, carvacrol and thymol against metronidazole resistant and susceptible *T. vaginalis* strains, and to determine their interaction with metronidazole by checkerboard method.

Methods: Cinnamaldehyde, carvacrol, thymol and metronidazole were obtained commercially. Two clinical isolates and one metronidazole resistant *T. vaginalis* reference strain were used in the study. MIC_{50} and MLC values of essential oil components and metronidazole were determined by broth microdilution method. The combinations of essential oil components with metronidazole were determined by the checkerboard method.

Results: According to *in vitro* activity tests, cinnamaldehyde was determined to be most effective essential oil component. Clinical isolates were susceptible to metronidazole. In combination study, metronidazole showed synergy with cinnamaldehyde and carvacrol, and partial synergy with thymol.

Conclusion: It was determined that cinnamaldehyde, carvacrol and thymol, which are known to have high antimicrobial activity, also have strong activity against *T. vaginalis* isolates and show a synergistic interaction with metronidazole. The use of metronidazole at lower doses in the synergistic interaction may contribute to the literature in terms of reducing drug side effects, creating a versatile antimicrobial target, and reducing the rate of resistance development.

Keywords: Checkerboard, cinnamaldehyde, carvacrol, metronidazole, synergy, thymol, *T. vaginalis*

ÖZ

Amaç: *Trichomonas vaginalis* genellikle kadınlarda enfeksiyona neden olan ve cinsel yolla bulaşan bir protozoon parazittir. Parazitin neden olduğu hastalığın tedavisinde ilk tercih olarak metronidazol kullanılmaktadır. Ancak 1980 yılından sonra artan sayılarda direnç gelişiminin rapor edilmesi ile yeni ilaç arayışlarına ihtiyaç duyulmuştur. Bu çalışmada, *Cinnamomum zeylanicum* (tarçın) ve *Thymus vulgaris* (kekik) uçucu yağlarının majör bileşenleri olan sinnamaldehit, karvakrol ve timolün metronidazole dirençli ve duyarlı *T. vaginalis* izolatlarına karşı anti-trichomonal etkinliğinin belirlenmesi ve metronidazol ile etkileşiminin checkerboard (dama tahtası) yöntemi ile gösterilmesi amaçlandı.

Yöntemler: Çalışmada kullanılan sinnamaldehit, karvakrol, timol ve metronidazolün saf formları ticari olarak temin edildi. Çalışmada, iki klinik izolat ve bir adet metronidazole dirençli *T. vaginalis* standart (ATCC 50143) suşu kullanıldı. Uçucu yağ bileşenlerinin ve metronidazolün MIK_{50} ve MLK (minimum letal konsantrasyonu) değerleri sıvı mikrodilüsyon yöntemi, metronidazol ile kombinasyonu ise checkerboard (dama tahtası) yöntemi ile saptandı.

Bulgular: *İn vitro* etkinlik testlerine göre, en etkili uçucu yağ bileşeninin sinnamaldehit olduğu belirlendi. Klinik izolatların metronidazole duyarlı olduğu saptandı. Checkerboard yöntemi ile yapılan kombinasyon çalışması değerlendirildiğinde, sinnamaldehit ve karvakrolün metronidazol ile kombinasyonunda sinerji, timolün metronidazol ile kombinasyonunda ise kısmi sinerji görüldü.

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Türkiye PARAZİTOLOJİ Dergisi

Sonuç: Yüksek antimikrobiyal aktiviteye sahip olduğu bilinen sinnamaldehit, karvakrol ve timol'ün *T. vaginalis* izolatlarına karşı güçlü aktiviteye sahip olduğu ve metronidazol ile sinerjistik etkileşim gösterdiği belirlendi. Sinerjik etkileşimde metronidazolün daha düşük dozlarda kullanılması ilaç yan etkilerinin azaltılması, çok yönlü bir antimikrobiyal hedef oluşturulması ve direnç gelişme hızının düşürülmesi açısından literatüre katkı sağlayabilir.
Anahtar Kelimeler: Checkerboard, sinnamaldehit, karvakrol, metronidazol, sinerji, timol, *T. vaginalis*

INTRODUCTION

Trichomoniasis is a non-viral and sexually transmitted urogenital infection that affects both men and women. According to the World Health Organization, it is estimated that more than 156 million new cases of trichomoniasis will occur in 2020 (1). However, prevalence data vary according to the population studied and the method used for diagnosis. Higher prevalence values have been reported when compared with nucleic acid amplification tests, direct microscopic examination and vaginal pH tests (2,3). *Trichomonas vaginalis* is a protozoan parasite and is usually associated with clinical symptoms including yellow-green vaginal discharge, vulvovaginal irritation and dysuria. Although the infection is usually asymptomatic in men, symptoms such as urethral irritation, discharge, burning after urination or ejaculation and swelling of the prostate gland may be observed (4). The parasite's soluble and membrane-associated enzymes with phospholipase A activity cause microulcerations and microscopic haemorrhages in the vaginal wall and exocervix by causing the breakdown of nucleated cells. Although humoral and cell-mediated immune responses develop against infection, the development of repeated infections indicates non-protective immunity against the parasite (5). Metronidazole is used as the first choice in the treatment of the disease caused by the parasite, yet, after 1980, an increasing number of reports of resistance development led to the need to search for new drugs. In recent years, natural active ingredients derived from plants, called phytochemicals, have become the focus of interest with the discovery of their strong antimicrobial effects (6,7). This study aimed to determine the antitrichomonal activity of cinnamaldehyde, carvacrol and thymol, the major components of *Cinnamomum zeylanicum* (cinnamon) and *Thymus vulgaris* (thyme) essential oils against metronidazole-resistant and susceptible *T. vaginalis* isolates and their synergistic interactions with metronidazole.

METHODS

Ethically Approval

No clinical material or data were used in this study. Therefore, ethics committee approval is not required.

Study Design

Minimum inhibition concentration (MIC_{50}) and (minimum lethal concentration (MLC) values of phytochemicals and metronidazole were determined *in vitro* by broth microdilution method and the combination of each phytochemical with metronidazole was investigated by checkerboard method (Figure 1).

Essential Oil Components and Parasite Strains

Pure forms of cinnamaldehyde, carvacrol, thymol and metronidazole used in this research were obtained commercially (Sigma, the USA). Two clinical isolates and one metronidazole-resistant *T. vaginalis* reference strain (ATCC 50143) were used in this study. Clinical isolates were produced from vaginal swabs sent to our laboratory by sowing them on TYM (trypticase yeast-extract maltose) medium and subcultured until they entered the logarithmic phase (8). Metronidazole-resistant *T. vaginalis* reference strain (ATCC 50143) was obtained from Manisa Celal Bayar University Faculty of Medicine Parasitology Bank.

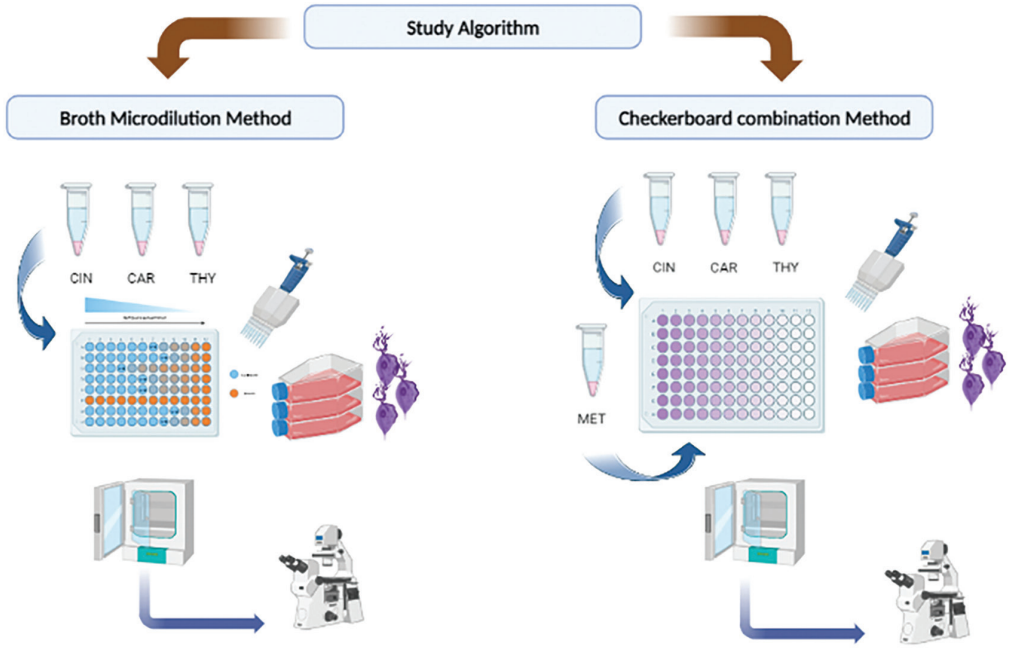

Figure 1. Study workflow

In vitro Drug Screening Test

For all isolates, MIC_{50} and MLC values of phytochemicals and metronidazole were determined *in vitro* by broth microdilution method in 96-well microplates (9). The dilution range for phytochemicals was set to be 1600-0.9 µg/mL and 12500-3 µM for metronidazole. $5x10^3$ parasites/mL *T. vaginalis* trophozoites were added to each well except for negative control. Microplates were incubated at 37 °C for 48 hours. At the end of incubation, the viability and motility of *T. vaginalis* trophozoites were evaluated under a light microscope using a counting chamber. All studies were repeated 3 times on independent days.

Checkerboard Combination Test

The combination of each phytochemical with metronidazole was investigated against a metronidazole-resistant *T. vaginalis* reference (ATCC 50143) strain by checkerboard method (10). Two 96-well microplates were used to determine the combination of phytochemicals with metronidazole. Serial dilutions of phytochemicals were made from top to bottom in the first microplate and metronidazole from right to left in the second microplate. Wells containing all combinations of both substances were obtained by transferring serial dilutions in the second microplate to the corresponding wells in the first microplate. $5x10^3$ parasites/mL *T. vaginalis* trophozoites were added to each well except for negative control. The plates were incubated at 37 °C for 48 hours and the viability and motility of *T. vaginalis* trophozoites were evaluated under a light microscope and FICI (fractional inhibitory concentration index) values were calculated. Interactions were interpreted as synergy if the FICI value was <0.5, partial synergy if between 0.5-0.75, additive if between 0.75-1, indifferent if between 1-4, and antagonism if >4 (11). All studies were repeated 3 times on independent days.

Statistical Analysis

In this study, statistical analysis was not required.

RESULTS

In vitro Drug Screening Tests

MIC_{50} and MLC values of cinnamaldehyde, carvacrol, thymol and metronidazole were respectively 3.9/31.25 µg/mL, 15.6/125 µg/mL, 62.5/500 µg/mL and 6/24 µM for the first clinical isolate; 1.8/7.81 µg/mL, 31.25/250 µg/mL, 15.6/125 µg/mL and 6/24 µM for the second clinical isolate; 3.9/15.6 µg/mL, 62.5/250 µg/mL, 125/500 µg/mL and 48/390 µM for metronidazole resistant isolate (Table 1).

Checkerboard Combination Test

The FICI values for cinnamaldehyde/metronidazole, carvacrol/metronidazole and thymol/metronidazole combinations were 0.374, 0.187 and 0.750, respectively (Table 2). According to these values, the combination of cinnamaldehyde and carvacrol with metronidazole was found to be synergistic and thymol partially synergistic.

DISCUSSION

Although trichomoniasis is thought to be a simple parasitic disease, it is a serious parasitic infection that can cause premature birth and rupture of membranes in pregnant women, and outside of pregnancy, it is associated with the post-partum syndrome with fever and foul-smelling discharge and can predispose to many infections. Many studies have reported that cervical cancer, atypical pelvic inflammatory disease and infertility are more

Table 1. Antitrichomonal activity values of essential oil components and metronidazole

Isolates	Phytochemicals (µg/mL)	MIC_{50}	MLC
T. vaginalis BAUN-TV1	Cinnamaldehyde	3.9	31.25
	Carvacrol	15.62	125
	Thymol	62.5	500
	Metronidazole (µM)	6	24
T. vaginalis BAUN-TV2	Cinnamaldehyde	1.8	7.81
	Carvacrol	31.25	250
	Thymol	15.62	125
	Metronidazole (µM)	6	24
T. vaginalis ATCC 50143	Cinnamaldehyde	3.9	15.62
	Carvacrol	62.5	250
	Thymol	125	500
	Metronidazole (µM)	48	390

MIC_{50}: Minimum inhibition concentration, MLC: Minimum lethal concentration

Table 2. Results of checkerboard combination of phytochemicals and metronidazole

Isolate	Combination	ΣFICI	Interaction
T. vaginalis ATCC 50143	Cinnamaldehyde/metronidazole	0.374	Synergy
	Carvacrol/metronidazole	0.187	Synergy
	Thymol/metronidazole	0.75	Partial synergy

ΣFICI: Fractional inhibition concentration index

common in patients infected with *T. vaginalis*. In addition, in women infected with *T. vaginalis*, the collection of HIV-infected cells such as lymphocytes and macrophages in the vagina and cervix facilitates the transmission of HIV (12,13). In a study conducted by Gram et al. (14) on 43016 Norwegian women, *T. vaginalis* infection was shown to increase the risk of cervical neoplasia caused by human papillomavirus (HPV). Studies conducted in Finland (15) and India (16) also indicated a similar correlation between trichomoniasis and cervical cancer caused by HPV. Another study demonstrated that *T. vaginalis* infection increased HPV infection 6.5 times (17).

The 5-nitroimidazole group of drugs is widely used in the treatment of trichomoniasis. Among these drugs, only metronidazole and tinidazole are authorized by the Food and Drug Administration in the United States for the treatment of trichomoniasis. Metronidazole is a relatively inexpensive, effective, and generally well-tolerated drug as well as generally mild gastrointestinal side effects. Occasionally, hematologic and neurotoxic side effects have also been reported. Recently, however, metronidazole-induced side effects have become a real problem in resistant and recurrent cases of trichomoniasis. Treatment of such infections requires a long-term treatment protocol with high doses of metronidazole. Increasing the drug dose leads to an increase in side effects and treatment fails with discontinuation of prophylaxis (18). New drug alternatives are needed today due to the emergence of resistant strains due to incomplete treatment processes and serious side effects of existing drugs at high doses (19).

In traditional medicine, the use of medicinal plants for the treatment of various diseases dates back thousands of years, according to records from ancient Babylon, Egypt, China and India. Despite the wide variety of chemically synthesized molecules in the modern pharmaceutical industry, natural components play a key role in drug development (20). Currently, around 35% of approved medicines are derived from natural ingredients or semi-synthetic derivatives, while 30% are synthetic molecules inspired by natural products. Remarkably, 65% of the 15 antiparasitic drugs approved by health authorities between January 1981 and June 2006 were natural ingredients or derivatives (21). Therefore, interest in medicinal plants has greatly increased in recent years. Given the need for new alternatives in the treatment of trichomoniasis, research focusing on the efficacy of natural ingredients against *T. vaginalis* has also increased.

Cinnamaldehyde, carvacrol and thymol, whose anti-trichomonal activity we investigated in our research, are the major natural components found in Cinnamomum zeylanicum and Thymus vulgaris essential oil. The antimicrobial activity of these components has been indicated by many researchers (22,23). Nevertheless, there are no studies demonstrating the efficacy of cinnamaldehyde, carvacrol and thymol against *T. vaginalis*. In our study, these components were found to reveal potent antitrichomonal activity. In particular, the fact that cinnamaldehyde is effective at very low concentrations of 0.9 μg/mL and has no cellular cytotoxicity at these doses (24) makes this essential oil component an important drug alternative. There is no clear information in the literature on the antimicrobial mechanisms of action of cinnamaldehyde, carvacrol and thymol, yet the general view is that these compounds bind to cell membranes due to their lipophilic character, increase membrane permeability, cause physical damage by accumulating in the membrane, inhibit the production of various enzymes and kill the microorganism by negatively affecting energy metabolism (25,26). At the MLCs determined for all three essential oil components, it was detected that the cell integrity of *T. vaginalis* trophozoites was preserved but they were immobile and lifeless. This suggests that these components may have an effect on the energy metabolism of the parasite.

In persistent infections caused by drug-resistant strains, increasing the drug dose often leads to treatment failure due to serious side effects. In such cases, combined drug use is often preferred as an alternative method. Phytochemicals offer promising adjuvants for antimicrobial drugs and the synergistic interaction of these metabolites with antimicrobials has been identified by researchers (6). The most common method used to determine the synergistic interaction between antimicrobials is the checkerboard method. In our study, a synergistic interaction was found between cinnamaldehyde, carvacrol and thymol in combination with metronidazole against metronidazole-resistant *T. vaginalis* strains. The observation of synergistic interaction between essential oil components and metronidazole may contribute to the literature in terms of using metronidazole at lower doses, reducing drug side effects, creating a versatile antimicrobial target and reducing the rate of resistance development.

CONCLUSION

Cinnamaldehyde, carvacrol and thymol, which have been shown to have antimicrobial activity against many microorganisms, were also found to have strong activity against *T. vaginalis* isolates. In addition, synergy was detected in combinations of essential oil components with metronidazole against metronidazole-resistant *T. vaginalis* isolate. The synergistic interaction in combinations provides advantages in treatment in terms of using the drug at lower doses, reducing drug side effects and preventing the development of resistance. Investigating both the individual efficacy of phytochemicals and their combinations with existing drugs can contribute to efforts to combat drug resistance.

* Ethics

Ethics Committee Approval: No clinical material or data were used in this study. Therefore, ethics committee approval is not required.

Informed Consent: Not required.

* Authorship Contributions

Concept: Y.Ö., İ.Ç., Design: Y.Ö., İ.Ç., G.Ü., M.Ü., A.Ö., Data Collection or Processing: Y.Ö., İ.Ç., G.Ü., M.Ü., A.Ö., Analysis or Interpretation: Y.Ö., İ.Ç., G.Ü., M.Ü., A.Ö., Literature Search: Y.Ö., İ.Ç., G.Ü., M.Ü., A.Ö., Writing: Y.Ö., İ.Ç.

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Prevalence of *Blastocystis* in Patients Referred to Bushehr Medical Centers and Its Relationship with Urticaria

Buşehr Tıp Merkezlerine Başvuran Hastalarda Blastocystis Prevalansı ve Ürtiker ile İlişkisi

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ABSTRACT

Objective: Recent studies determined that the amoeboid form of *Blastocystis* acts as a factor in stimulating the host's immune responses and ultimately results in urticaria and other skin disorders. The present study was conducted in order to determine the prevalence of *Blastocystis* in people referred to Bushehr city health centers and the relationship of this parasite with urticaria.
Methods: Fecal samples were collected from 180 males and females referred to Bushehr health centers and a questionnaire containing demographic information was completed for each person. Samples were examined by preparing direct smear (wet mount) and then formalin-detergent sedimentation techniques. Data were analyzed using SPSS 22.0 software and chi-square test.
Results: The results showed that 11.1% of cases infected with *Blastocystis* and 55% of patients with *Blastocystis* had various gastrointestinal symptoms. Statistical analysis showed that there was no significant relationship between infection with some demographic factors such as sex, age, literacy level and residence, but this was significant with some clinical symptoms such as itching and urticaria.
Conclusion: Despite the existence of conflicting information and many ambiguities about the *Blastocystis*, this emerging pathogen is very important in terms of causing allergic and skin disorders in sufferers, therefore, it is necessary that patients with urticaria be evaluated for *Blastocystis* along with other diagnostic procedures and physicians should request a test before any medical intervention. Thus, diagnosis and treatment of these people can play an important role in improving the health of society.
Keywords: Prevalence, *Blastocystis*, urticaria, Bushehr, Iran

ÖZ

Amaç: Son araştırmalar *Blastocystis*'in ameboid formunun konağın bağışıklık yanıtlarını uyaran bir faktör olarak hareket ettiğini ve sonuçta ürtiker ve diğer deri bozukluklarına yol açtığını göstermiştir. Bu çalışma Bushehr şehir sağlık merkezlerine başvuran kişilerde *Blastocystis* prevalansını ve bu parazitin ürtiker ile ilişkisini belirlemek amacıyla yapılmıştır.
Yöntemler: Bushehr sağlık merkezlerine başvuran 180 erkek ve kadından dışkı örnekleri toplandı ve her kişiye demografik bilgileri içeren bir anket doldurduldu. Örnekler direkt smear (wet mount) hazırlanarak ve ardından formol eter çöktürme teknikleri kullanılarak incelendi. Veriler SPSS 22.0 programı ve ki-kare testi kullanılarak analiz edildi.
Bulgular: Bulgular olguların %11,1'inin *Blastocystis* ile enfekte olduğunu ve *Blastocystis* ile enfekte olan hastaların %55'inde çeşitli gastrointestinal semptomların bulunduğunu gösterdi. İstatistiksel analiz, enfeksiyon ile cinsiyet, yaş, okuryazarlık düzeyi ve yerleşim yeri gibi bazı demografik faktörler arasında anlamlı ilişki olmadığını, ancak enfeksiyon ile kaşıntı ve ürtiker gibi bazı klinik semptomlar arasında anlamlı ilişki olduğunu gösterdi.
Sonuç: *Blastocystis* hakkında çelişkili bilgiler ve birçok belirsizlik bulunmasına rağmen bu patojen, alerjik deri bozukluklarına neden olması açısından oldukça önemlidir, bu nedenle ürtikerli hastaların diğer tanı işlemleriyle birlikte *Blastocystis* açısından da

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değerlendirilmesi ve hekimlerin herhangi bir tıbbi müdahale öncesinde test istemesi gerekmektedir. Dolayısıyla bu kişilerin tanı ve tedavisi toplum sağlığının iyileştirilmesinde önemli bir rol oynayabilir.
Anahtar Kelimeler: Prevalans, *Blastocystis*, ürtiker, Bushehr, İran

INTRODUCTION

Blastocystis is one of the most common zoonotic protozoan parasites that can be found anaerobically in the digestive system of humans and many vertebrates, including mammals, birds, amphibians, reptiles and fish, and even in invertebrates such as insects (1).

This protozoan was first described by Alexieff in 1912 as a fungus, but years later, Zierdh placed this microorganism in the amoeba order of protozoa according to its phenotypic and morphological characteristics. Finally, in 1996, Siberman named this protozoan as a member of the highly diverse group of Stramenopiles, citing phylogenic studies of ribosomal RNA (2).

For many years, *Blastocystis* was considered a saprophytic and non-pathogenic microorganism, so that even with the diagnosis of positive cases and laboratory reports, no action was taken to treat the infected patients by physicians. But recently, due to new studies and findings about the physiological properties and pathogenesis mechanisms of this parasite, the importance of diagnosing the infection and treating the patients has increased (3-5). In many reports, *Blastocystis* has been mentioned as an emerging pathogen or a mysterious organism (2).

This parasite is a polymorphic protozoan and has different forms such as vacuolar, granular, amoeboid, and cystic transfer form, which is directly transmitted to different hosts through contaminated water and food (6,7).

The prevalence of infection in the world is variable and this rate is different in different countries and even in different societies (0.8-61.8%). In general, countries located in tropical regions have a higher prevalence than other regions. Factors affecting the prevalence of this parasitic infection include poor health in communities, close contact with animals, and seasonal weather changes. Some studies have shown that in some seasons, the prevalence of infection increases and unlike many intestinal parasites, its prevalence is higher in adults than children (3,8,9). In a research conducted for the first time in Lorestan province, Iran, the rate of infection was reported to be about 6.5% (10). In a similar study in Mazandaran province, this amount was found to be about 14.3% (11).

The main method of diagnosing this parasite is stool exam by preparing a wet smear and direct examination under a microscope. Other complementary methods such as permanent staining, culture or molecular polymerase chain reaction test can also be used for this purpose.

The disease caused by this protozoan includes a wide range of non-specific gastrointestinal disorders which is known as *Blastocystis*. Symptoms such as diarrhea, abdominal pain, bloating, nausea, vomiting, constipation, and weight loss can appear in patients with varying severity from acute to chronic. There are even hypotheses about the relationship between this parasite and colorectal cancer (12). On the other hand, it has been found that in people with irritable bowel syndrome, the prevalence of *Blastocystis* is significantly higher than in people without this syndrome (13,14).

Also, the recent studies have introduced the amoeboid form of this protozoan as a factor in stimulating the host's immune responses through binding to the intestinal epithelium and affecting its homeostasis. Correlation between *Blastocystis* infection and urticaria has been repeatedly reported in various studies (15-19). Even some researchers have considered the role of different subtypes and alleles of *Blastocystis* in the development of chronic urticaria symptoms.

In the study conducted by Aykur et al. (20) in Türkiye, stool samples were collected from patients with chronic spontaneous urticaria and healthy individuals as control group and investigated using different conventional and molecular methods. The results revealed a relationship between chronic spontaneous urticaria and *Blastocystis* subtypes as well as a significant difference in total IgE levels between the *Blastocystis* ST2 and ST3 positive group and the negative group (20).

Based on this, and in terms of the high importance of this disease, as well as insufficient information and many uncertainties about this emerging pathogen, the present study was designed to determine the prevalence of *Blastocystis* infection in people referred to health centers in Bushehr city, Iran, in order to take positive steps towards solving the existing ambiguities about pathogenicity and infection control by investigating the relationship between infection with this protozoan and skin urticaria and various epidemiological factors and clinical symptoms.

METHODS

Ethical Approval and Informed Consent

This study was approved in Ethical Committee of Bushehr University of Medical Sciences with ethics code: IR.BPUMS.REC.1400.145. Informed consent was obtained from the patients and in the case of minors, from their parents and confidentiality of the information was guaranteed.

Study Area

This study was carried out in Bushehr city, located in the northwest margin of the Gulf and southwest of Iran. The population of the

Figure 1. Geographical location of Bushehr city on the map of Iran

city is close to three hundred thousand people and this area has hot and humid climate most of the year (Figure 1).

Sample Collection

In this descriptive-analytical study, samples were taken between December 2021 and June 2022, from 180 patients referred to Bushehr medical centers with various gastrointestinal symptoms as well as those suffering from skin disorders. A questionnaire containing demographic information and variables related to *Blastocystis* infection was completed for each patient.

Stool Examination

The collected samples were first examined by direct microscopic examination using the wet mount method, and in the next step, in order to increase the sensitivity of the test, were evaluated using the modified formalin-detergent concentration method. The detergent used in this research was a liquid with the formula of dodecyl benzene, coconut diethanolamide, and antibacterial, viscosifying agent, essential oil, urea and dye.

Commercial kits (Parasite Test, KaraTeb Nur, Iran) were used for this purpose. Briefly; first, 3.5 mL of working solution (containing formalin 10% and detergent) was added to the cylindrical part of the kit, and then, with the spoon of the brain part, about 2 grams of stool sample is taken and entered into the cylindrical tube. After completely closing the two parts, the tubes were vortexed for 30 seconds until the samples were completely suspended. The next step was the incubation of the tests for 24 hours at room temperature. Then, the tubes were inverted and centrifuged at 1000 rpm for 1 minute. Finally, the two parts were slowly separated and the sediment remaining in the conical part was transferred to the slide with Lugol and examined under the light microscope.

Statistical Analysis

The results of the tests along with the questionnaire data were analyzed using the statistical software SPSS (Chicago, IL version 22, SPSS Inc.) and the chi-squared test.

RESULTS

In this study, out of a total of 180 examined patients, 20 samples (11.1%) had blastocystosis. Table 1 shows the prevalence of *Blastocystis* infection by sex and as it can be seen, there is no significant relationship between *Blastocystis* infection and the gender of the examined patients (Table 1).

The results of the present study showed that the highest rate of infection is in the age group of 21-40 years and the lowest rate is in the group under 20 years, which in fact, considering that the most and least examined patients were in these age groups respectively, this difference was not statistically significant (p-value 0.208) (Table 2).

Table 1. Distribution of *Blastocystis* in patients referred to Bushehr medical centers by sex

Sex	Pos		Neg		p-value
	n	%	n	%	
Male	8	4.4	47	26.1	
Female	12	6.7	113	62.8	0.234
Total	20	11.1	160	88.9	

Despite the fact that the majority of cases infected with *Blastocystis* were village residents (10.5%) and only 0.6% of them lived in the city, statistical analysis showed that there is no significant relationship between the place of residence and *Blastocystis* infection (Table 3).

Out of all 20 patients with blastocystosis, 11 patients (55%) had various gastrointestinal symptoms such as anorexia, nausea, vomiting, abdominal pain and diarrhea, which is very significant, but as can be seen from Table 3, in the separate analysis of each of these variables, there was no significant difference between the prevalence rate and clinical symptoms (Table 3).

Other variables analyzed in this study were respiratory symptoms, skin itching and acute or chronic urticaria. Statistical

Table 2. Distribution of *Blastocystis* in patients referred to Bushehr medical centers by age

Age group	Pos		Neg		p-value
	n	%	n	%	
<20 y	0	0	11	6.1	
21-40 y	17	9.4	112	62.2	
41-60 y	3	1.7	32	17.8	0.208
>60 y	0	0	5	2.8	
Total	20	11.1	160	88.9	

Table 3. Distribution of *Blastocystis* in patients referred to Bushehr medical centers by some effective factors

Variables	Results				p-value
	Pos		Neg		
	%	n	%	n	
Residence					
Rural	10.5	19	77.8	140	0.288
Urban	0.6	1	11.1	20	
Abdominal pain					
Yes	2.8	5	28.3	51	0.365
No	8.3	15	60.6	109	
Anorexia					
Yes	3.3	6	32.8	59	0.367
No	7.8	14	56.1	101	
Diarrhea					
Yes	3.3	6	37.2	67	0.220
No	7.8	14	51.7	93	
Nausea and vomiting					
Yes	3.3	6	19.4	35	0.287
No	7.8	14	69.4	125	
Respiratory symptoms					
Yes	3.9	7	28.9	52	0.502
No	7.2	13	60	108	
Skin itching					
Yes	5.6	10	22.8	41	0.025
No	5.6	10	66.1	119	
Acute urticaria					
Yes	3.9	7	10	18	0.010
No	7.2	13	78.9	142	
Chronic urticaria					
Yes	2.2	4	7.8	14	0.121
No	8.9	16	81.1	146	

analysis showed that there is a significant relationship between *Blastocystis* infection and these variables (Table 3).

DISCUSSION

The ability of *Blastocystis* to cause various gastrointestinal disorders as well as skin disorders is still being discussed by researchers, although recent clinical and epidemiological studies emphasize the pathogenicity of this parasite (3-5). Some researchers also believe that eradication of *Blastocystis* is not necessary and it is essential just in some cases where it is the only infectious agent and patients show significant symptoms (21).

A wide range of non-specific gastrointestinal disorders, such as diarrhea, abdominal pain, bloating, nausea, vomiting, constipation, and weight loss can appear in patients with varying severity from acute to chronic, are known as blastocystosis. There are even hypotheses about the relationship between this parasite and colorectal cancer (12).

Despite the fact that limited studies have reported the role of *Blastocystis* in the occurrence of acute urticaria, many other studies have mentioned this important role in the development of chronic urticaria symptoms (16). It is assumed that the amoeboid form of ST1, ST2 and ST3 subtypes of the parasite acts as a factor in stimulating the host's immune responses through binding to the intestinal epithelium and affecting its homeostasis, and finally leads to the secretion of mediators such as histamine which can cause allergic reactions and chronic urticaria in sufferers (15,18,22-26).

In the present study conducted in Bushehr clinical centers in order to determine the rate of *Blastocystis* infection and also to investigate the relationship between *Blastocystis* with various epidemiological factors and various clinical symptoms such as urticaria, a prevalence of 11.1% was obtained, which is significant. Other studies conducted in Iran and other parts of the world have announced different results. In a study in Tabriz, Iran, 558 stool samples were collected from those who referred to medical centers and tested by direct microscopic methods, formal ethyl acetate concentration and trichrome staining. The results indicated a 26.17% prevalence of *Blastocystis* and the most common clinical symptoms were reported as abdominal pain, anorexia and nausea. The *Blastocystis* of the mentioned study have shown that *Blastocystis* is one of the most common intestinal parasite, which if neglected, it can cause many problems for patients (27).

During the years 2010 to 2014, Rahimi et al. (28) analyzed the reports of parasitic infections of 70,978 people who referred to the Baqiyatullah Hospital in Tehran and among the detected parasites, *Blastocystis* with a prevalence of 2.49% had the highest rank. In a similar study by Memar et al. (29), which was conducted on healthy people and AIDS patients, they reported a higher prevalence of *Blastocystis* than other intestinal parasites in both investigated groups.

In analyzing the results of the present study and investigating the correlation of some variables involved, there was no significant relationship with the gender, age, place of residence and education level of the patients. These findings are contrary to the reports of some studies conducted in this field; Shaker et al. (11) found a significant relationship in their study between *Blastocystis* and the variables of age, gender, educational level, place of residence and history of contact with animals, but with some other evaluated factors such as consumable water and symptoms such as diarrhea, bloating and weight loss, this relationship was not significant (11). In the present study, although 55% of patients had various gastrointestinal symptoms such as anorexia, nausea, vomiting, abdominal pain and diarrhea, but in the separate analysis of each of these variables, there was no significant difference between the prevalence rate and clinical symptoms, which is consistent with the results of the study conducted by Shaker et al. (11).

Another valuable finding of this study was the existence of a significant relationship between *Blastocystis* and some allergic symptoms such as skin itching and acute urticaria. Most of the researches conducted in this field have reported the correlation between *Blastocystis* infection and urticaria not completely confirmed, but ambiguously and possibly (18,19,30,31).

In a study that aimed to evaluate the presence of clinical symptoms and skin manifestations in 80 patients with a positive *Blastocystis* test, 11.25% of them showed the relationship between the infection and skin disorders, although these symptoms were mainly reported in women more than men. Also, 73.75% of the examined people had various gastrointestinal disorders. Examination of routine hematological and biochemical tests has shown an increase in C-reactive protein levels in infected patients, but the amount of blood eosinophils was within the normal range. Finally, researchers have recommended that stool exam be done routinely in patients with skin disorders of unknown cause (14).

CONCLUSION

According to the results obtained and the high importance of this emerging pathogen in terms of causing allergic and gastrointestinal disorders, it is necessary that patients with urticaria be evaluated for blastocystosis along with other diagnostic procedures and physicians should request a test before any medical intervention. Thus, diagnosis and treatment of these people can play an important role in improving the health of society.

Also, considering that the severity and type of pathogenicity of this parasite apart from the factors related to the host as well as the number of parasites, has been attributed to different subtypes of the parasite, therefore, in order to respond to many contradictions in this field, it is recommended to carry out additional studies of molecular epidemiology in the region and the dominant subtypes on human and livestock samples be identified relying on advanced molecular techniques.

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* Ethics

Ethics Committee Approval: This study was approved in research committee of Bushehr University of Medical Sciences with Ethics Code: IR.BPUMS.REC.1400.145.

Informed Consent: Informed consent was obtained from the patients and in the case of minors, from their parents and confidentiality of the information was guaranteed.

* Authorship Contributions

Concept: M.F., M.R., A.B., Design: M.F., M.R., A.B., Data Collection or Processing: M.G., H.M., B.A., Analysis or Interpretation: M.G., H.M., B.A., Writing: M.G., M.F., H.M., M.R., B.A., N.S., A.S., N.K.G., A.B.

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Microbial Health Risks of *Cryptosporidium parvum* and *Giardia lamblia* in Tropical Coastal Water in Araromi, Nigeria

Nijerya, Araromi'deki Tropik Kıyı Sularında Cryptosporidium parvum ve Giardia lamblia'nın Mikrobiyal Sağlık Riskleri

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ABSTRACT

Objective: *Giardia* and *Cryptosporidium* are enteric protozoa that can cause a variety of gastrointestinal diseases, especially in vulnerable people like children, the elderly, and those with impaired immune systems. In order to ascertain the microbiological quality of the recreational water from Araromi Beach in Ilaje Local Government Area, Ondo State, Nigeria. This risk assessment is of great significance to human health protection against waterborne diseases. The aim of this study was to determine the microbial quality of recreational water from Araromi Beach in Ilaje Local Government Area, Ondo State, Nigeria.

Methods: Microscopic examination of *Cryptosporidium* and *Giardia* oocysts were done.

Results: Results revealed maximum occurrence of *Cryptosporidium parvum* (20 oocysts/100 mL) of water sample in the month of April and maximum occurrence of *Giardia lamblia* (300 cysts/100 mL) of water sample in the month of June. Additionally, according to Kolmogorov-Smirnov tests for normalcy Ho =0.05, *Giardia lamblia* and *Cryptosporidium parvum* were not regularly distributed in the water samples collected from the beach throughout the study period. The average likelihood of contracting *Giardia lamblia* and *Cryptosporidium parvum* infections after consuming 100 mL of beach water was 0.96 and 0.35, respectively. The risks of infection associated with *Cryptosporidium parvum* was lower than those associated with *Giardia lamblia* in water from the beach, but were both above the acceptable risk limit of 10-4.

Conclusion: The results of this study indicate that *Giardia* and *Cryptosporidium* may represent serious health hazards to people who engage in aquatic activities. Adopting a comprehensive strategy that includes regular inspections, enhanced detection techniques, and the prevention of aquatic environment pollution may provide clean and safe recreational water for all, thereby safeguarding the public's health.

Keywords: Enteric protozoa in coastal water, protozoa, water quality assessment, beach water quality assessment, *Cryptosporidium parvum* and *Giardia lamblia*

ÖZ

Amaç: *Giardia* ve *Cryptosporidium*, özellikle çocuklar, yaşlılar ve bağışıklık sistemi zayıf olanlar gibi hassas kişilerde çeşitli gastrointestinal hastalıklara neden olabilen enterik protozoalardır. Nijerya'nın Ondo Eyaleti, Ilaje Yerel Yönetim Bölgesi'ndeki Araromi Plajı'ndan gelen rekreasyonel suyun mikrobiyolojik kalitesini belirlemek için. Bu risk değerlendirmesi, su kaynaklı hastalıklara karşı insan sağlığının korunması açısından büyük önem taşımaktadır.

Yöntemler: *Cryptosporidium* ve *Giardia* ookistlerinin mikroskobik incelemesi yapıldı.

Bulgular: Sonuçlar, su örneğinde *Cryptosporidium parvum*'un (20 ookist/100 mL) maksimum oluşumunun Nisan ayında, maksimum *Giardia lamblia*'nın (300 kist/100 mL) ise Haziran ayında oluştuğunu ortaya çıkardı. Ayrıca Kolmogorov-Smirnov normallik testlerine göre Ho =0,05, *Giardia lamblia* ve *Cryptosporidium parvum*'un çalışma dönemi boyunca plajdan toplanan su örneklerinde düzenli olarak dağılmadığı görüldü. 100 mL plaj suyu tükettikten sonra *Giardia lamblia* ve *Cryptosporidium parvum* enfeksiyonlarına yakalanma ortalama olasılığı sırasıyla 0,96 ve 0,35 idi. *Cryptosporidium parvum* ile ilişkili enfeksiyon riskleri, plajdaki suda *Giardia lamblia* ile ilişkili olanlardan daha düşüktü, ancak her ikisi de kabul edilebilir risk sınırı olan 10-4'ün üzerindeydi.

Sonuç: Bu çalışmanın sonuçları *Giardia* ve *Cryptosporidium*'un suda yaşayan insanlar için ciddi sağlık tehlikeleri oluşturabileceğini göstermektedir. Düzenli denetimleri, gelişmiş tespit tekniklerini ve su ortamı kirliliğinin önlenmesini içeren kapsamlı bir stratejinin benimsenmesi, herkes için temiz ve güvenli dinlenme suyu sağlayabilir ve böylece halk sağlığının korunmasını sağlayabilir.

Anahtar Kelimeler: Kıyı suyunda enterik protozoa, protozoa, su kalitesi değerlendirmesi, plaj suyu kalitesi değerlendirmesi, *Cryptosporidium parvum* ve *Giardia lamblia*

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Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Water bodies have acquired significant amounts of pollution from a number of sources due to the development of human populations, commercial, and recreational activities. Recreational water refers to rivers, lakes and coastal waters that are used for recreational purposes. Humans use recreational water for all manner of activities, including swimming, surfing, white water sports, diving, boating and fishing (1). Consuming or being exposed to contaminated water can result in the transfer of a number of pathogens, such as enteric bacteria, viruses, and parasites, which can cause serious illnesses and pose a serious threat to global public health (2). Other activities are enhanced by being close to water, such as hiking, nature viewing, and hunting waterfowl. However, recreational waterways can become contaminated with faecal pathogens from human sewage and animal manure. These pathogens can cause gastrointestinal illnesses (causing diarrhoea and vomiting), respiratory diseases, eyes, ear, nose and throat infections.

Protozoa are diverse group of eukaryotic, typically unicellular microorganisms. The majority of protozoa are free-living organisms that can reside in fresh water and pose no risk to human health.

However, some protozoa are pathogenic to humans. These protozoa fall into two functional groups: Enteric protozoa and free-living protozoa. Human infections caused by free-living protozoa are generally the result of contact during recreational bathing (or domestic uses of water other than drinking). Enteric protozoa, on the other hand, have been associated with several drinking water-related outbreaks, and drinking water serves as a significant route of transmission for these organisms. Enteric protozoa are common parasites in the gut of humans and other mammals. The enteric protozoa that are most often associated with waterborne disease in the world are *Giardia* and *Cryptosporidium*. These protozoa are commonly found in surface waters which include recreational waters. Some strains are highly pathogenic and can survive for long periods of time in the environment and are highly resistant to chlorine-based disinfection (3). Protozoan parasites like *Cryptosporidium* spp. and *Giardia* spp. are the most frequently identified cause of diarrheal outbreaks in middle-income and low-income countries (4). The right to access water, which is essential for the survival of living beings, has been acknowledged as a universal human right (5). A sizable portion of epidemics worldwide are caused by waterborne protozoa. According to a recently released comprehensive assessment, from 1953 to 2019 at least one waterborne parasite was documented in 86.7% of eastern African nations (6). Protozoa pollution can lead to waterborne epidemics, which is a serious problem. Despite the application of disinfectants, *Giardia* and *Cryptosporidium* species, which can endure in aquatic environment (7). Unfortunately, many people around the world lack access to clean water that is free of diseases and toxins. Due to its intimate ties to human health, this issue is a substantial public health concern, even for high-income nations (8).

Cryptosporidium spp. is a leading global cause of waterborne disease, with many reported outbreaks related to main water supplies (9). They are known as the main protozoa parasite reported from waterborne outbreaks (10-12). A most recent study reported that, from 251 waterborne outbreaks with parasitic agents *Cryptosporidium* was identified among 198 outbreaks (13). The enteric parasite *Cryptosporidium*, along with *Norovirus*, *Giardia*, *Campylobacter* and *Rotavirus* are among the most frequent causes of waterborne disease (14). Humans can contract *Cryptosporidium* by drinking untreated water from a lake or river that is contaminated, through the faecal-oral route by coming into contact with infected people or animals directly (person-to-person transmission), swallowing recreational water (water in swimming pools, waterparks, fountains, lakes, rivers) contaminated with cryptosporidiosis (15). Oocysts of *Cryptosporidium* and cysts of *Giardia* occur in the aquatic environment throughout the world. They have been found in most surface waters, where their concentration is related to the level of faecal pollution or human use of the water. In fact, it is well known that the prevalence of parasitic protozoan infection is high in low-income countries, this is as a result of their low economic status and poor sanitation. Effects can range from minor illnesses to potentially fatal diseases. Children, the elderly, and people with compromised immune systems are most at risk. *Giardia lamblia* is the second most reported protozoa from watery outbreaks worldwide similar to *Cryptosporidium* spp., (16), the main reason for the high prevalence of *G. lamblia* in waterborne outbreaks is the capability of this protozoan to remain viable during water treatment processes (17,18). However, outbreaks of *G. lamblia* and *Cryptosporidium* spp. are recorded far more frequently in high-income countries than in low-income countries, which may be related to the adoption of more advanced detection methods. According to World Health Organisation, proper monitoring and adjustment of disinfection processes are necessary to ensure adequate control of *Cryptosporidium* and *Giardia* (19).

The aim of this study was to determine the microbial quality of recreational water from Araromi Beach in Ilaje Local Government Area, Ondo State, Nigeria. The objectives were to determine the load of enteric protozoa (*C. parvum* and *G. lamblia*) in water samples from Araromi beach, examine the risk posed by the protozoa on humans and carry out quantitative microbial risk assessment of the pathogen in the water samples.

METHODS

Description of the study area and collection of water samples Araromi Beach is located in Ilaje local government area of Ondo State in the south-western region of Nigeria. There is a popular night market close to the beach where trading starts at night. The beach is situated on the same beach line from Lagos and about 290 km on the shoreline to Lagos. The study area lies between longitudes 2° 24E. 5° 1E of the Greenwich meridian and latitude 5° 51 N. 6° 42 N of the equator (Figure 1). Over the years the beach has been known for its space for relaxation or engagement in activities especially beach ball and picnic etc. It is also used as a major source of fishing activities. It has been recorded over the years that people visit the beach more often during the festive seasons. Water samples were collected from the representative monitoring points on the beach monthly over a period of 12 months (i.e., December 2021 to November 2022). On each sampling occasion, about 10 litres of water was collected using sterile plastic bottles and transported in a cool box with ice packs to the Microbiology Laboratory at the Federal University of Technology, Akure.

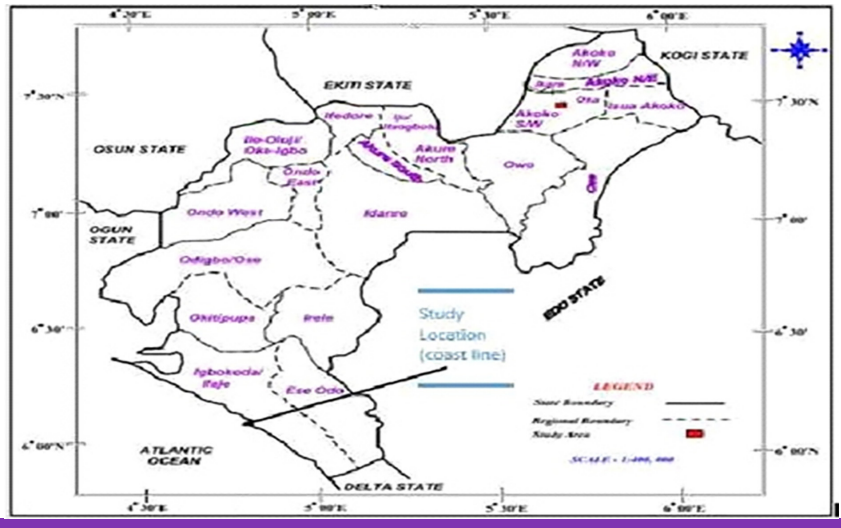

Figure 1. A map showing the location of the beach in Araromi, Ilaje Local Government Area, Ondo State, Nigeria

Enumeration of *Cryptosporidium parvum* in Water Samples from the Beach

Cryptosporidium oocysts were concentrated from the water samples (100 mL) in triplicates by centrifuging at 1000 rpm for 15 minutes. Pellet was suspended in normal saline and placed on a microscopic slide, and then stained using modified Ziehl Neelsen method. The oocysts were identified using shape, size and color, and thereafter counted and expressed as the total number of *Cryptosporidium* per 100 mL.

Enumeration of *Giardia lamblia* in Water Samples from the Beach

Giardia cystic forms were concentrated from the water samples (100 mL) in triplicates, centrifuging at 1000 rpm for 15 min. The centrifugation's pellet product was suspended in sterile saline, put on a microscopic slide and stained using a modified iodine, thereafter counted and expressed as the total number of *Giardia* per 100 mL.

Distribution Pattern of Enteric Protozoa in Water Samples From the Beach

The distribution pattern of *Cryptosporidium parvum* and *Giardia lamblia* in water from the beach were determined using Skewness and kurtosis, Kolmogorov-Smirnov tests for normality.

Microbial Risk Assessment

The risk of infection with *C. parvum* and *G. lamblia* in water from the beach during swimming activities was estimated. *Cryptosporidium parvum* causes watery diarrhea including symptoms such as nausea, vomiting, abdominal cramps and fever. In immune-compromised individuals, infectious dose as low as 1-5 oocysts may result into an infection, whereas about 132 oocysts are capable of causing infection in healthy humans. *Giardia lamblia* causes diarrhea in humans with accompanying symptoms such as weight loss, nausea, vomiting, abdominal pain and fever. Infectious dose a low as 10 cysts may elicit an infection.

The exponential model (Equation 1) was adopted to determine the probability of infection (Table 1) associated with exposure to *Cryptosporidium parvum* and *Giardia lamblia* in water from the beach. The annual probability of infection (Equation 2) was also determined. For exposure assessment, the ingestion of 1 mL, 10 mL and 100 mL was assumed as the volume of water consumed during recreational activities in order to determine the human health risks.

$$Pi = 1 - \exp(-rN) \quad (1)$$

$$P_A = 1 - (1 - Pi)^{365} \quad (2)$$

Where: Pi = Probability of infection; r = Parameters defining the dose-response curve (determined by the infectivity the organism); and N = exposure (colony forming unit); P_A = Annual probability of infection.

Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 20 software and Microsoft Excel.

RESULTS

Detection of *Cryptosporidium parvum* and *Giardia lamblia* in Water Samples from the Beach

The minimum occurrence of *Cryptosporidium parvum* was observed to be 1.1 oocysts/100 mL of water sample in the month of March. On the other hand, the maximum occurrence of *Cryptosporidium parvum* in the water sample was observed to be 20 oocysts/100 mL of water sample in the month of April. Similarly, the minimum occurrence of *Giardia lamblia* was observed to be 73 cysts/100 mL of water sample for the month of July On the other hand, the maximum occurrence of *Giardia lamblia* in the water sample was observed to be 300 cysts/100 mL of water sample in the month of June (Figure 2).

Table 1. Dose-response model adopted to evaluate microbial health risks from exposures to enteric protozoa in water samples from the beach

Pathogens	Model	Parameters	Reference
Cryptosporidium parvum	Exponential	$r=5.72\times10^{-2}$	(19)
Giardia lamblia	Exponential	$r=1.99\times10^{-2}$	

Distribution Pattern of Enteric Protozoa in Water Samples from the Beach

Cryptosporidium parvum and *Giardia lamblia* following Kolmogorov-Smirnov tests for normality H_o =0.05 were not normally distributed in the water samples from the beach over the period of study (Table 2).

Probabilities of Infection

The mean probability of *Cryptosporidium parvum* infection from ingestion of 1 mL of water from the beach was 0.0035, whereas ingestion of 10 mL of water from the beach revealed mean probability of infection of 0.035. The mean probability of *Cryptosporidium parvum* infection from ingestion of 100 mL of water from the beach was 0.35. The mean probability of *Giardia lamblia* infection from ingestion of 1 mL of water from the beach was 0.0096, whereas ingestion of 10 mL of water from the beach revealed mean probability of infection of 0.096. The mean probability of *Giardia lamblia* infection from ingestion of 100 mL of water from the beach was 0.96. The risks of infection associated with *Cryptosporidium parvum* was lower than those associated with *Giardia lamblia* in water from the beach, but were both above the acceptable risk limit of 10^{-4} (Figure 3).

The mean annual probabilities of infection as a result of contact or ingestion of water from the beach were estimated. The mean annual probability of *Cryptosporidium parvum* infection due to ingestion of 1 mL of water from the beach was 0.72; 10 mL of water from the beach was 1.0; and 100 mL of water from the beach was 1.0. The mean annual probability of *Giardia lamblia* infection due to ingestion of 1 mL of water from the beach was 0.97; 10 mL of water from the beach was 1.0; and 100 mL of water from the beach was 1.0. The mean annual probabilities of infection due to *Giardia lamblia* was than those due to *Cryptosporidium parvum* (Figure 4).

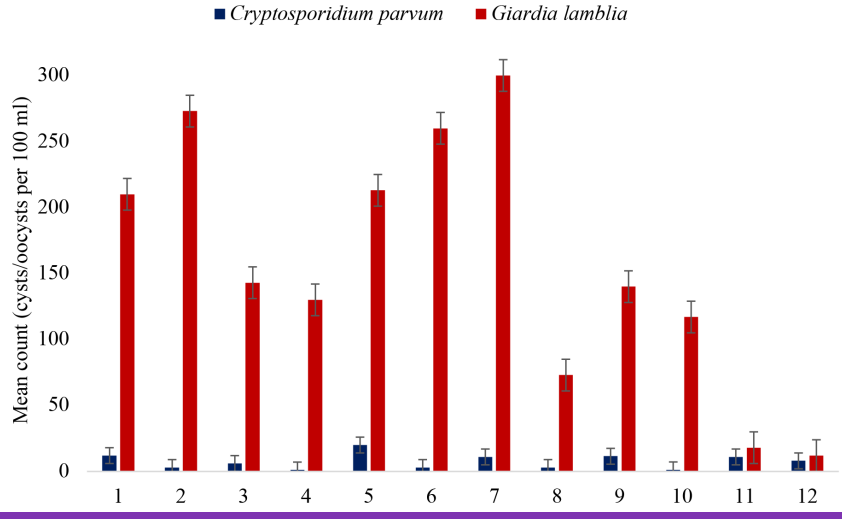

Figure 2. Mean count of *Cryptosporidium parvum* and *Giardia lamblia* in water samples from the beach (1 – Dec'21; 2 – Jan'22; 3 – Feb'22; 4 – Mar'22; 5 – Apr'22; 6 – May'22; 7 – Jun'22; 8 – Jul'22; 9 – Aug'22; 10 – Sep'22; 11 – Oct'22; 12 – Nov'22)

Table 2. Skewness and kurtosis, Kolmogorov-Smirnov tests for normality in water samples from the beach

Microorganisms	Skewness	Kurtosis	Kolmogorov-Smirnov statistic	Normal distribution
Cryptosporidium parvum	-0.502	-0.920	0.776	No
Giardia lamblia	-1.442	1.215	0.985	No

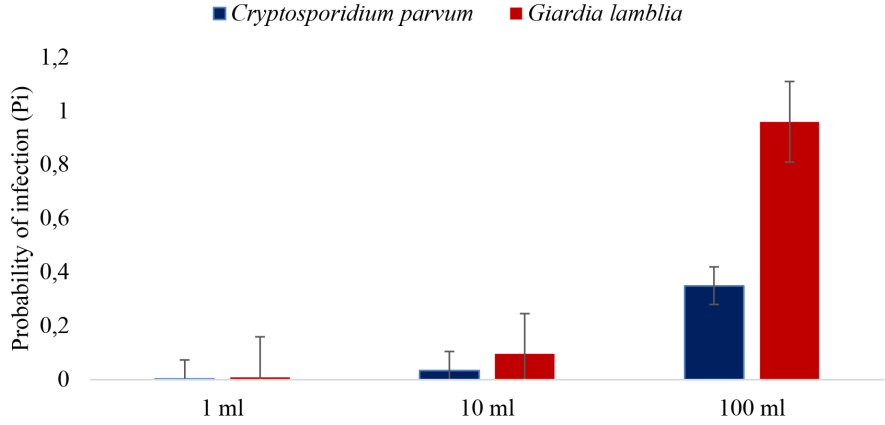

Figure 3. Probability of infection (*Pi*) associated with exposure to pathogens in water from the beach

DISCUSSION

The concentration of enteric protozoa in recreational water from Araromi Beach in Ilaje Local Government Area, Ondo State, Nigeria was determined in this study. The human population in the area depends on the water for fishing, agriculture, and other household and recreational activities in addition to increasing beach activity. *Giardia* and *Cryptosporidium* are important etiological agents of waterborne illnesses primarily because of their high levels of environmental persistence and infectivity. Using conventional water treatment procedures, it is challenging to get rid of them from water supplies. Their small size and resistance to chlorine, commonly used for disinfection, enable them to persist and remain infectious even after standard treatment processes. This persistence has resulted in several waterborne disease outbreaks, underscoring the need for improved detection and control measures. As a result of this it is important to investigate the presence of *Cryptosporidium parvum* and *Giardia lamblia* in recreational waters to estimate the infection risk associated with swimming and other recreational activities.

Cryptosporidium species have been described, infective to a wide range of hosts, including humans, domestic and wild mammals, birds, reptiles, amphibians and fishes (20). These parasites spread between hosts via the faecal-oral pathway, either directly through contact with an infected person or indirectly by transmission in faecally contaminated water or, less frequently, food (21). Water serves as an important channel for the spread of *Cryptosporidium* oocysts throughout the ecosystem. Different water resources, including drinking, recreational, groundwater, and wastewater, are commonly polluted with this parasite (22,23). The distribution of *Cryptosporidium parvum* in the water sample increased regularly with month (April, November), then average increases in the month (December, June, August, October) with little or no increases in the month (January, February, March, May, July, September). This observation might be the effect of more people being near and in the water between the months of April and November.

One of the most frequent global causes of water and food borne illnesses is *Giardia* infection (giardiasis). In this study, the distribution of *Giardia lamblia* was sporadic, as there was an

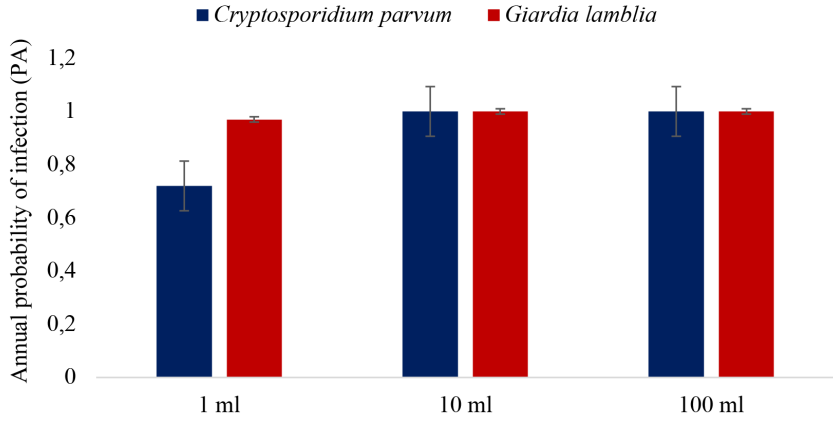

Figure 4. Annual probability of infection (P_A) associated with exposure to pathogens in water from the beach
In children, the mean probability of infection from contact or ingestion of 37 mL of water from the beach due to *Cryptosporidium parvum* was 0.13 and *Giardia lamblia* was 0.36. The risks of infection associated with exposure of children to the enteric protozoa in water from the beach were lower for *Cryptosporidium parvum* than *Giardia lamblia*. In adults, the mean probability of infection from contact or ingestion of 16 mL of water from the beach due to *Cryptosporidium parvum* was 0.056 and *Giardia lamblia* was 0.15. Again, the risks of infection associated with exposure of adults to the enteric protozoa in water from the beach were were lower for *Cryptosporidium parvum* than *Giardia lamblia* (Figure 5).

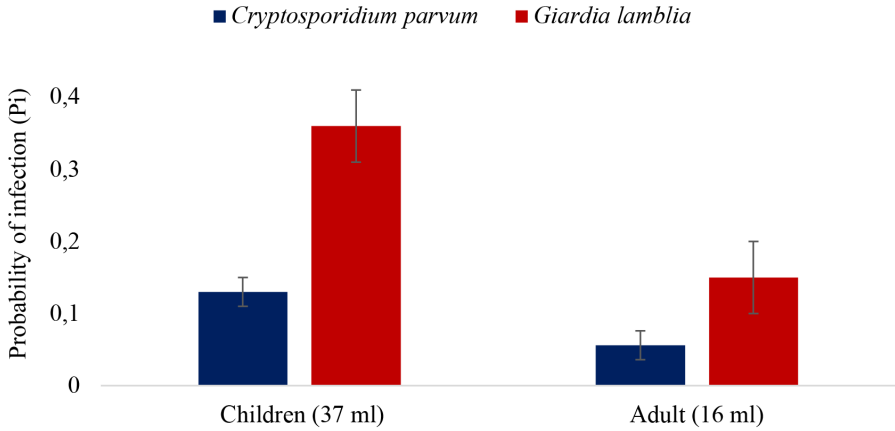

Figure 5. The probability of infection associated with exposure of children and adults to *Crptosporidium parvum* and *Giardia lamblia* in the water from the beach

increase in the counts observed in the months of January, May and June. This could be as a result of the increase in human populations that visited the beach. *Giardia lamblia* was more prevalent in this study than *Cryptosporidium parvum*, and its prevalence exceeded the acceptable risk level of 10^{-4} set by the World Health Organization (24).

Giardia lamblia was irregular in its spread, with increases in counts seen in the months of January, May, and June. This could be as a result of the increase in human populations that visited the beach. In this study, *Giardia lamblia* prevalence was much greater than *Cryptosporidium parvum* prevalence. The amount of *Cryptosporidium parvum* in the water sample increased consistently in the months April and November, followed by average monthly increases in December, June, August, October and then minimal or no monthly increases in January, February, March, May, July and September.

QMRA was conducted to assess health risks associated with the beach water in the presence of the pathogens. Two pathogenic microorganisms were chosen, *Cryptosporidium parvum* and *Giardia lamblia*. These organisms are known to cause the most common water-related gastrointestinal illnesses caused by protozoa. Cryptosporidiosis is caused by the intestinal parasite *Cryptosporidium*. Cryptosporidiosis is an acute illness characterised by diarrhoea and abdominal pain, whereas Giardiasis is caused by the protozoan parasite *Giardia lambia*. The main symptoms are diarrhoea and cramps. These gastrointestinal illnesses can be contracted in various ways, including contact with contaminated water, farm animals, sick animals, faecal matter, other symptomatic people, and eating contaminated food. Therefore, the presence of *Cryptosporidium parvum* and *Giardia lamblia* in water at Araromi Beach may pose significant risk to public health. The estimated risks of infection with *Cryptosporidium parvum* were highest in the month of April and lowest in the month of March and September. The estimated risks of infection with *Giardia lamblia* were highest in the month of June and lowest in the month of November.

CONCLUSION

The findings of this study demonstrated the presence of *Cryptosporidium parvum* and *Giardia lamblia* in water at Araromi beach. The presence of these enteric protozoa in the water samples suggest faecal pollution of the water and may pose significant health risks to humans using the water for recreational activities. The estimated risks of *Cryptosporidium parvum* and *Giardia lamblia* infections which could be as a result of human exposure to the beach were highest in April and June respectively. Adopting a comprehensive approach that involves routine monitoring with improved detection methods and prevention of pollution of aquatic environment may offer clean and safe recreational water for all, thereby protecting public health.

* Ethics

Ethics Committee Approval: N/A.

Informed Consent: N/A.

* Authorship Contributions

Surgical and Medical Practices: O.T.O., A.O., E-O.T., Concept: O.T.O., A.O., E-O.T., Design: O.T.O., A.O., E-O.T., Data Collection or Processing: O.T.O., A.O., E-O.T., Analysis or Interpretation: O.T.O., A.O., E-O.T., Literature Search: O.T.O., A.O., E-O.T., Writing: O.T.O., A.O., E-O.T.

Conflict of Interest: No conflict of interest was declared by the authors.

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The Impact of Human Cystic Echinococcosis in the Central Asian Region, 1990-2019

İnsan Kistik Ekinokokkozisinin Orta Asya Bölgesi'ndeki Etkisi, 1990-2019

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ABSTRACT

Objective: This research aims to update knowledge on the regional and national sickness burden attributable to cystic echinococcosis (CE) from 1990 to 2019, as well as epidemiology and disease control, with a particular emphasis on the People's Central Asian Regions.

Methods: We calculated the morbidity, mortality, and disability-adjusted life years at the global, regional, and national levels for CE in all central Asian countries from 1990 to 2019, and we analyzed the association between GDP per capita and the disease burden of CE.

Results: In 2019, the three greatest numbers of CE cases were recorded in Kazakhstan [23986; 95% uncertainty interval (UI); 19796; 28908]; Uzbekistan (41079; 18351; 76048); and Tajikistan (10887; 4891; 20170) among all 9 countries. The three countries with the greatest ASIR of CE were estimated to be Kazakhstan (127.56; 95% UI: 105.34-153.8), Uzbekistan (123.53; 95% UI: 58.65-219.16), and Tajikistan (121.88; 58.57-213.93). Kyrgyzstan, Tajikistan, and Uzbekistan had the biggest increases (125%, 97%, and 83%, respectively) in the number of incident cases of CE, whereas Georgia, Kazakhstan, and Armenia saw the largest decreases (45%, 8%, and 3%, respectively).

Conclusion: To reduce the illness burden caused by CE, our findings may help public health professionals and policymakers design cost-benefit initiatives. To lessen the impact of CE on society, it is suggested that more money be given to the region's most endemic nations. Echinococcosis, cystic, negative health effects, life-years lost due to disability, rate of occurrence as a function of age, rate of death as a function of age.

Keywords: Burden of disease, cystic echinococcosis (CE), age-standardized mortality rate, disability-adjusted life year (DALY), age-standardized incidence rate, Central Asia

ÖZ

Amaç: Bu araştırma, 1990'dan 2019'a kadar kistik ekinokokkoza (CE) atfedilebilecek bölgesel ve ulusal hastalık yükünün yanı sıra epidemiyoloji ve hastalık kontrolüne ilişkin bilgileri, özellikle Halkın Orta Asya Bölgeleri'ne vurgu yaparak güncellemeyi amaçlamaktadır.

Yöntemler: 1990'dan 2019'a kadar tüm Orta Asya ülkelerinde CE için küresel, bölgesel ve ulusal düzeyde morbidite, mortalite ve engelliliğe göre düzeltilmiş yaşam yıllarını hesapladık ve kişi başına GSYİH ile CE'nin hastalık yükü arasındaki ilişkiyi analiz ettik.

Bulgular: 2019 yılında en fazla sayıda CE olgusu Kazakistan'da kaydedildi [23986; %95 belirsizlik aralığı (Üİ); 19796; 28908]; Özbekistan (41079; 18351; 76048); ve Tacikistan (10887; 4891; 20170) 9 ülke arasında yer alıyor. CE'nin en büyük ASIR'sine sahip üç ülkenin Kazakistan (127,56; %95 UI: 105,34-153,8), Özbekistan (123,53; %95 UI: 58,65-219,16) ve Tacikistan (121,88; 58,57-213,93) olduğu tahmin edilmektedir. Kırgızistan, Tacikistan ve Özbekistan CE olgularının sayısında en büyük artışları yaşarken (sırasıyla %125, %97 ve %83), Gürcistan, Kazakistan ve Ermenistan ise en büyük düşüşleri (%45, %8, %8 gördü ve sırasıyla %3).

Sonuç: CE'nin neden olduğu hastalık yükünü azaltmak için bulgularımız halk sağlığı profesyonellerinin ve politika yapıcıların maliyet-fayda girişimlerini tasarlamasına yardımcı olabilir. CE'nin toplum üzerindeki etkisini azaltmak için bölgenin endemik ülkelerine daha fazla para verilmesi öneriliyor. Ekinokokkoz, kistik, olumsuz sağlık etkileri, engellilik nedeniyle kaybedilen yaşam yılı, yaşın bir fonksiyonu olarak ortaya çıkma oranı, yaşın bir fonksiyonu olarak ölüm oranıdır.

Anahtar Kelimeler: Hastalık yükü, kistik ekinokokkoz (CE), yaşa standardize edilmiş ölüm oranı, engelliliğe göre düzeltilmiş yaşam yılı (DALY), yaşa standardize edilmiş insidans hızı, Orta Asya

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Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

The microscopic tapeworm, Taenia echinococcus, of which there are four species, is the causative agent of echinococcosis, a parasitic zoonotic illness that mostly affects dogs (1). Nine species of Echinococcus have been described so far; four pose a threat to humans. The most common of these four are *E. granulosus* and *E. multilocularis*, which are responsible for cystic echinococcosis (CE) and alveolar echinococcosis, respectively (2). The parasitic infection known as echinococcosis has already spread to all five continents, causing widespread illness in people and animals (3). It is likely one of the most significant parasitic illnesses in the world today. CE patients may have no signs of the disease until the hydatid cysts have progressed to a later stage (4). With a death rate of 2.2% after surgery and a recurrence rate of 6.5%, the outlook for untreated or poorly treated patients is bleak (5). The human CE incidence estimates range from 50 to 100 per 100,000 person-years in endemic foci, with frequencies of 5 to 10% in certain areas of Argentina, Peru, eastern Africa, Central Asia, and China (6,7). There is an annual loss of $760,000,000 owing to compounding interest since CE is a worldwide health concern that shortens people's lives by an estimated 184,000 disability-adjusted life years (DALYs) (8). The worldwide public health burdens and economic costs of this zoonotic parasitic disease are substantial (9). Recent research has shown that the illness is rapidly expanding in several regions throughout the globe (3).

Five of the ten former Soviet Republics in Central Asia -Kazakhstan, Uzbekistan, Kyrgyzstan, Tajikistan, and Turkmenistan- have seen a resurgence of CE after the fall of the Soviet Union in 1991 (10,11). The cattle industry has seen radical shifts due to this process. The decline in the value of meat has led to the closure of several large mechanical slaughterhouses. Veterinarian oversight of the slaughtering process has been compromised by the lack of government money to pay veterinarians' salaries and the closing of these massive slaughterhouses. The cumulative effect of these changes is a general tendency toward smaller farms and an increase in illegal home slaughter or slaughter at markets without veterinary monitoring. As a result of lax veterinary public health precautions and the careless slaughter of animals, echinococcosis has become widespread in the region (12). In Central Asia, the number of recorded cases of human CE has increased dramatically and persistently, with certain localities seeing surges of four- or five-fold. Human echinococcosis is now endemic in the livestock-rearing regions of southern and western Kazakhstan and the other four republics, with an overall incidence rate of more than 3 cases/100,000 per year and an incidence rate of more than 25 cases/100,000 in some districts (13-16).

The data release includes a comprehensive and detailed analysis of the extent of health loss in various local areas within the Central Asian region. On the other hand, the global burden of disease (GBD) data offers a global perspective on mortality and disability, including countries, periods, age groups, and genders. It is achieved by an examination of how the adverse health conditions and premature mortality resulting from various ailments and injuries contribute to the overall burden of disease in the Central Asian region, as well as within local communities. These deliverables aim to enhance the local population health monitoring activities in the Central Asian region, both now and in the future. Despite being a parasitic tropical illness, CE receives little attention despite its high disease, social, and economic effects globally. While significant progress has been made toward the World Health Organizations (WHO) ambitious goal of eradicating CE by 2030, several obstacles remain. Estimates of disease burden help organize eradication efforts, but data on the worldwide burden of CE in terms of death, morbidity, and DALYs was lacking until recently. This research aims to provide the most recent data on the regional and national disease burden attributable to CE from 1990 to 2019, together with information on epidemiology and disease management, emphasizing the people's central Asian locations.

METHODS

Design

The GBD research annually evaluates and analyzes the global burden of illness. GBD 2019 presents the most recent analysis of descriptive epidemiology for 204 nations and territories from 1990 to 2019. This list of illnesses and injuries is thorough individually and individually (17).

Study Area

The GBD super regions classify the Republic of Armenia as part of Central Asia, which consists of 8 other independent states. Included on this map are the countries of Azerbaijan, Kyrgyzstan, Mongolia, Tajikistan, Turkmenistan, Georgia, Kazakhstan, and Uzbekistan. Natural disasters, like earthquakes and floods, as well as constant water shortages, threaten the lives of the people there. Furthermore, water disputes are a common cause of climate-related conflicts. Many lives are lost, property is destroyed, and resources are depleted when these calamities strike. The situation in Afghanistan and the aftereffects of Russia's conflict with Ukraine have contributed to the influx of new refugees into Central Asia in the spring of 2021. As of Friday, July 7, 2023, the United Nations estimates that the population of Central Asia is 77,706,704. The people who live in Central Asia make up less than one percent of the global population. Regarding population density, Central Asia is the fifth most populous area in all of Asia. Central Asia has a population density of 19 per km^2 (49 per mi^2). The entire land area is 3,926,790 km^2 (1,516,141 sq. miles); in 2019, 48.0% (or 35,681,394) of the population will live in cities. Central Asians, on average, are 27.6 years old (Figure 1) (18).

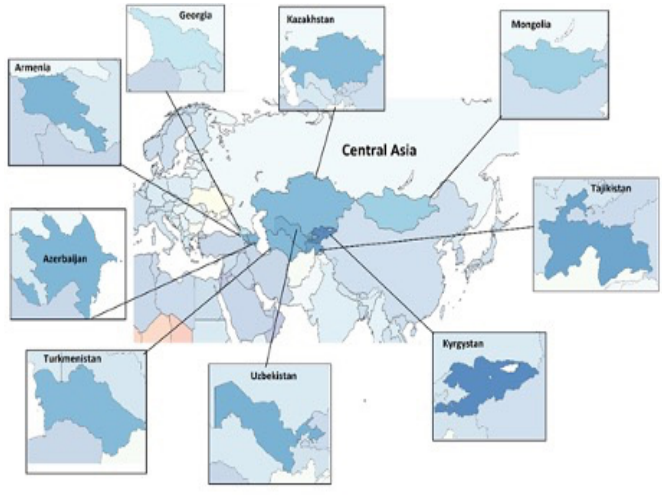

Figure 1. Study area

Data Source

Using data from the GBD 2019 retrieved from the Global Health Data Exchange tool (http://ghdx.healthdata.org/gbd-results-tool), we calculated the incidence, ASIR, mortality, ASMR, DALYs, and age-standardized DALY rate of CE in 9 countries from 1990 to 2019. From the GBD database, we retrieved information for each Central Asian nation from 1990 to 2019 on the incidence, prevalence, mortality, and DALYs of CE.

Death Rate Estimation

Previous studies (19,20) have examined the sources and management of cause-of-death (COD) data for the GBD project. The current dataset for COD has information from a cumulative count of 2809 sources across 21 nations, owing to the inclusion of 89 supplementary sources for the GBD 2021. In essence, COD data refer to documented information on the reasons behind mortality within a certain population or a specific subgroup of that population, often sourced through vital registration systems and similar repositories. The data pertaining to deaths in COD (cause of death) were subjected to a mapping process, whereby each death entry in the dataset was assigned a cause of death according to the GBD classification system, or alternatively, a garbage code. Additionally, before to the modeling phase, the age and sex categories were normalized via the use of aggregation or disaggregation techniques. The inclusion of HCE in the GBD list of valid underlying causes of death was undertaken to address the issue of fatalities that were initially assigned to causes of death that are either inappropriate or unsuitable as the underlying cause of death (referred to as "garbage code" deaths). In order to address the potential influence of stochastic temporal or geographical patterns, a Bayesian noise-reduction approach was used to the data pertaining to unusual causes of death or cases with small sample sizes. The age-, sex-, year-, and location-specific death rates of inflammatory bowel disease (IBD), Crohn's disease (CD), and ulcerative colitis (UC) were computed using the cause of death ensemble modelling (CODEm) approach, as described in detail in another source (8). The CODEm algorithm is a systematic Bayesian approach that employs ensemble modeling to analyze geospatial and temporal data. It generates and evaluates sub-models of two distinct types: Linear mixed-effects models and spatiotemporal Gaussian process regression models. These sub-models are designed to predict two response variables, namely the logarithm of mortality rate and the logarithm of cause of death fraction (expressed as a logit). Additionally, CODEm incorporates predetermined combinations of potential predictive covariates in its modeling process. The primary linear mixed-effects model consisted of sub-models that included covariate and age as fixed effects, and super-region, region, country, and age as nested random effects. The selection and assessment of variables by IBD modelers were determined by the robustness of the existing scientific data supporting their association with IBD. If the predictive variables of a sub-model exhibited a statistically significant link ($p<0.05$) and were epidemiologically valid in terms of the predicted direction of association with the response variable, the algorithm assessed the sub-model and included it into the ensemble. The evaluation of the prediction performance for each sub model included randomly deleting 15% of the whole data set. The selection criteria for exclusion were based on the cause-specific missingness patterns observed for ages and years across different geographic locations. The evaluation of the model's predictive validity outside of the training set involved the utilization of root-mean-squared error sum, trend error, which refers to the proportion of predictions accurately predicting the direction of the time trend between adjacent points, and coverage of the predicted 95% uncertainty interval. The performance of each sub-model was assessed by 20 measurements. The sub-models that exhibited the highest performance were assigned weights and then included into ensemble models. These ensemble models were then subjected to identical testing protocols. To get the CODEm estimates of mortality across the whole spectrum of IBD, the estimates of death specifically attributed to CD and UC were adjusted using the cause of death correct approach (CoDCorrect). The CoDCorrect tool is a core processing tool for the GBD study. Its purpose is to ensure the accuracy of mortality data by verifying that the sum of fatalities for each age group, sex, year, and geographic location aligns with the overall number of deaths attributed to all causes (20).

Secondary Analyses

Two more analyses were conducted. Initially, a decomposition analysis was conducted to ascertain the proportion of the observed change in prevalence of IBD between 1990 and 2021 that could be attributable to demographic factors such as population aging, as well as epidemiological factors such as alterations in sickness rates based on age and sex. In this research, we used the demographic methodology developed by Das Gupta (8). In order to ascertain the general incidence of HCE, we took into account two hypothetical scenarios. In the "population growth scenario", we made predictions regarding the expected number of prevalent cases by maintaining the age-sex distribution of the population and the age-sex-specific prevalence of HCE at their 1990 values while allowing for changes in population growth observed between 1990 and 2021. The second scenario, referred to as the "population growth and population aging scenario", closely resembled the first scenario. However, it assumed that the age-sex-specific prevalence of IBD would remain constant in 2021, as in 1990. This scenario accounted for both population growth and changes in population age-sex structure between 1990 and 2021. The disparity between the GBDs estimate of prevalent cases in 1990 and the projected 2021 under the first counterfactual scenario was used to determine the population growth that may be ascribed to causative causes. Through a comparative analysis of the forecasts for 1990 and 2021, we have successfully ascertained the extent to which population aging may be attributable to changes in demographic patterns. The attribution of any changes in age- and sex-specific prevalence, which could not be explained by demographic shifts such as population growth and aging, was attributed to epidemiological factors.

Next, we compared the national socio-economic development, quantified by the socio-demographic index (SDI) estimates provided by GBD, and the changes in prevalence of inflammatory bowel disease (IBD) across different regions and time periods. The SDI is a comprehensive measure incorporating many indicators, including the total fertility rate among women under 25, the average years of education for those aged 15 and above, and the per capita income distribution throughout a country. The classification process included categorizing 21 countries and territories into distinct quintiles based on their SDI scores. These quintiles were high, high-middle, medium, low-middle, and low. Further details on constructing and evaluating a SDI may be found in other resources.

Standardization

We calculated age-standardized incidence and death rates using the WHO's world standard population to facilitate comparisons across populations with varying ages.

Statistical Analysis

Thirty years of data were analyzed statistically to reveal patterns. We assessed the pace of change over time by calculating the annual percentage increase or decrease in the age-adjusted incidence and death rates. We also did a sub-regional study to examine the prevalence of CE in various Central Asian nations.

Ethical Considerations

The data utilized in this research was secondary. Hence, permission from any relevant ethics bodies was unnecessary. Each participant's information is private yet accessible to the public in the GBD research data. In order to fully grasp the impact of CE on Central Asia, this technique offers a rigorous and systematic approach.

RESULTS

Global Scenario

The global and Central Asian epidemiological picture of CE illness is shown in Table 1 from 1990 to 2019, broken down by gender. The estimated annual percentage change (EAPC) is 0.54% [95% uncertainty interval (UI): 0.42 to 0.7], while the total number of cases worldwide has climbed from 134980 (95% UI: 93141-195144) in 1990 to 207368 (95% UI: 137807-303233) in 2019. Deaths from CE have decreased worldwide from 2.839 (95% UI: 2.218 to 3.497) in 1990 to 1.349 (95% UI: 987 to 1.762) in 2019, with an EAPC of 0.52% (95% UI: 0.66% to 0.34%). In addition, the number of DALYs lost due to CE worldwide decreased from 210,044 (95% UI: 166,434 to 261,084) in 1990 to 122,457 (95% UI: 89,244 to 168,556) in 2019, with an EAPC of 0.42% (95% UI: 0.57% to 0.23%) (Table 1). Table 1 shows that between 1990 and 2019, females made up a larger proportion of CE sufferers than men. For 2019, the rates of new cases, deaths, and DALYs due to CE were all trending downward (Table 1). From 1990 to 2019, the EAPC for the number of incident cases of CE was +0.18% (95% UI: +0.24% to 0.12%), +4.64% (95% UI: -4.85% to -4.43%), and -3.38% (95% UI: -3.54% to 3.26%) (Table 1).

Central Asia

Table 2 shows that out of the nine countries studied in 2019, Kazakhstan had the largest number of CE cases with 23986 (95% UI: 19796-28908), followed by Uzbekistan with 41079 (18351-76048), and finally Tajikistan with 10887 (4891-20170). As shown in Table 2, the three countries with the highest ASIR of CE were Kazakhstan (127.56; 95% UI: 105.34-153.8), Uzbekistan (123.53; 95% UI: 58.65-219.16), and Tajikistan (121.88; 58.57-213.93). Also, Table 2 shows that although the number of incident instances of CE increased by the greatest percentage in Kyrgyzstan (125%), Tajikistan (97%), and Uzbekistan (83%), it decreased by the greatest percentage in Georgia (45%), Kazakhstan (8%), and Armenia (3%). As for the ASIR of CE, the highest gain was recorded in Kyrgyzstan [EAPC =1.3%, 95% UI: (0.94% to 1.67%)],

Table 1. Comparison of number of global CE, age-standardized rates (ASR), Annual rate of change (ARC), and EAPC of incidence, mortality, and DALYs between 1990 to 2019

Item	Incidence, number (95% UI)		Mortality, number (95% UI)		DALYs, number (95% UI)	
	1990	2019	1990	2019	1990	2019
CE incidence, mortality, and DALYs						
Global						
Overall	134980 (93141-195144)	207368 (137807-303233)	2839 (2218-3497)	1349 (987-1762)	210044 (166434-261084)	122457 (89244-168556)
Male	55004 (36334-84605)	83318 (52851-127144)	1480 (1105-1879)	726 (455-1030)	103864 (78388-133209)	58532 (40465-81788)
Female	79977 (56335-113027)	124050 (84455-175220)	1359 (995-1776)	623 (385-879)	103864 (78388-133209)	63925 (43062-87764)
Central Asia	65909 (43716-102773)	95102 (56969-152588)	27 (17-38)	15 (9-21)	21147 (12039-36064)	30110 (15271-54362)
Age-standardized rates (ASR), incidence, mortality, and DALYs						
Global						
Overall	2.65 (1.87-3.7)	2.6 (1.72-3.79)	0.06 (0.04-0.07)	0.02 (0.01-0.02)	3.82 (3.05-4.7)	1.56 (1.14-2.15)
Male	2.12 (1.43-3.1)	2.09 (1.34-3.21)	0.06 (0.04-0.08)	0.02 (0.01-0.03)	3.74 (2.86-4.75)	1.49 (1.04-2.1)
Female	3.17 (2.28-4.31)	3.1 (2.1-4.38)	0.05 (0.04-0.07)	0.02 (0.01-0.02)	3.9 (2.99-4.94)	1.62 (1.08-2.25)
Central Asia	103.28 (68.82-152.04)	100.32 (61.06-159.16)	0.05 (0.03-0.07)	0.02 (0.01-0.03)	33.74 (19.48-56.19)	31.68 (16.44-56)
Annual rate of change (ARC), and estimated annual percent change (EAPC)						
	ARC (95% UI)	**EAPC (95% UI)**	**ARC (95% UI)**	**EAPC (95% UI)**	**ARC (95% UI)**	**EAPC (95% UI)**
Global						
Overall	0.54% (0.42-0.7)	-0.18% (-0.24, -0.12)	-0.52% (-0.66, -0.34)	-4.64% (-4.85, -4.43)	-0.42% (-0.57, -0.23)	-3.38% (-3.5, -3.26)
Male	0.51% (0.39-0.7)	-0.08% (-0.13, -0.02)	-0.51% (-0.7, -0.24)	-4.54% (-4.75, -4.32)	-0.44% (-0.62, -0.21)	-3.47% (-3.6, -3.35)
Female	0.55% (0.43-0.71)	-0.24% (-0.31, -0.18)	-0.54% (-0.73, -0.27)	-4.78% (-4.99, -4.57)	-0.40% (-0.58, -0.15)	-3.3% (-3.44, -3.17)
Central Asia	0.45% (0.28 - 0.67)	-0.2% (-0.28, -0.12)	-0.45% (-0.07, -0.01)	-3.6% (-4.1, -3.1)	0.42% (0.21-0.67)	-0.35% (-0.42, -0.27)

CE: Cystic echinococcosis, DALY: Disability-adjusted life year, UI: Uncertainty interval

Table 2. Country-wise comparison of the number of global CE, age-standardized rates (ASR), Annual rate of change (ARC), and EAPC of incidence, mortality, and DALYs between 1990 to 2019

Item	Incidence, number (95% UI)		Mortality, number (95% UI)		DALYs, number (95% UI)	
	1990	2019	1990	2019	1990	2019
CE incidence, mortality, and DALYs						
GBD regions						
Armenia	1902 (873-3500)	1849 (862-3383)	0.55 (0.29-0.83)	0.22 (0.12-0.35)	639 (267-1300)	621 (255-1261)
Azerbaijan	3958 (1726-7618)	55004 (36334-84605)	1.46 (0.8-2.23)	0.71 (0.38-1.1)	1335 (556-2640)	2066 (815-4215)
Georgia	381 (313-468)	210 (176-251)	1.01 (0.52-1.58)	0.33 (0.17-0.52)	188 (124-268)	98 (63-143)
Kazakhstan	26055 (21264-31968)	23986 (19796-28908)	3.66 (2.37-5.34)	1.44 (0.78-2.22)	7572 (4751-11273)	6931 (4467-10420)
Kyrgyzstan	2757 (2259-3389)	6211 (5183-7653)	3.11 (1.79-4.54)	1.43 (0.8-2.16)	949 (629-1334)	1870 (1176-2745)
Mongolia	1036 (433-2102)	1858 (815-3484)	0.93 (0.48-1.47)	0.46 (0.23-0.77)	375 (164-772)	635 (256-1338)
Tajikistan	5535 (2492-10858)	10887 (4891-20170)	3.89 (2.17-5.76)	2.37 (1.39-3.5)	1950 (874-3732)	3579 (1496-7224)
Turkmenistan	1825 (775-3600)	2822 (1231-5170)	0.88 (0.43-1.44)	0.40 (0.22-0.63)	624 (260-1252)	934 (354-1880)
Uzbekistan	22460 (9914-43740)	41079 (18351-76048)	11.13 (7.41-14.84)	7.32 (4.53-10.55)	7517 (3214-15371)	13377 (5362-27592)
Age-standardized incidence rates (ASIRs), and estimated annual percent change (EAPC)						
GBD regions	**ASIR 1990 (95% UI)**	**ASIR 2019 (95% UI)**	**Num change (95% UI)**		**EAPC (95% UI)**	
Armenia	56.48 (26.55-101.85)	56.11 (25.8-100.3)	-0.03% (-0.21-0.21)		-0.01% (-0.03-0)	
Azerbaijan	56.26 (25.42-99.52)	55.83 (25.38-102.43)	0.57% (0.25-0.9)		-0.02% (-0.04--0.01)	
Georgia	6.52 (5.35-8.01)	5.26 (4.42-6.3)	-0.45% (-0.51--0.39)		-1.04% (-1.23, -0.85)	
Kazakhstan	164.09 (135.28-197.75)	127.56 (105.34-153.8)	-0.08% (-0.14--0.01)		-1.22% (-1.44, -0.99)	
Kyrgyzstan	68.56 (56.57-82.4)	95.61 (80.36-116.11)	1.25% (1.08-1.45)		1.3% (0.94-1.67)	
Mongolia	55.98 (25.19-100.22)	55.52 (25.61-100.56)	0.79% (0.4-1.29)		0% (-0.02-0.01)	
Tajikistan	123.34 (60.01-214.46)	121.88 (58.57-213.93)	0.97% (0.71-1.33)		-0.03% (-0.04--0.02)	
Turkmenistan	56.57 (26.03-101.9)	55.86 (25.25-99.31)	0.55% (0.21-0.91)		-0.04% (-0.07--0.01)	
Uzbekistan	124.35 (57.81-220.68)	123.53 (58.65-219.16)	0.83% (0.57-1.23)		-0.03% (-0.05--0.01)	

CE: Cystic echinococcosis, DALY: Disability-adjusted life year, GBD: Global burden of disease, UI: Uncertainty interval

while the largest decreases were recorded in Kazakhstan [EAPC =1.22%, 95% UI: (1.44% to 0.99%)] and Georgia [EAPC =-1.04%, 95% UI: (1.23% to -0.85%)] (Table 2).

The trends of the number of cases, age-standardized mortality, and DALY rates have shown mixed patterns throughout Central Asian countries (Figure 2).

Between 1990 and 2019, neglected tropical disease and malaria (NTD & M) jumped from the 22nd to the 21st spot on Central Asia's list of leading risk factors (Figure 3A). This was due to a decrease in the DALY rate from 164.11 (95% UI: 98.36 to 272.99 age-standardized DALY rate per 100,000 population for both sexes) to 77.78 (95% UI: 52.3 to 111.05). Change in DALY rate from baseline: 7.67 (95% UI: 5 to 11.75 age-standardized DALY rate per 100,000 population for both sexes) to baseline: 8.94 (95% UI: 4.97 to 15.55) (Figure 3B) Central Asia jumped from third to first place among GBD super regions.

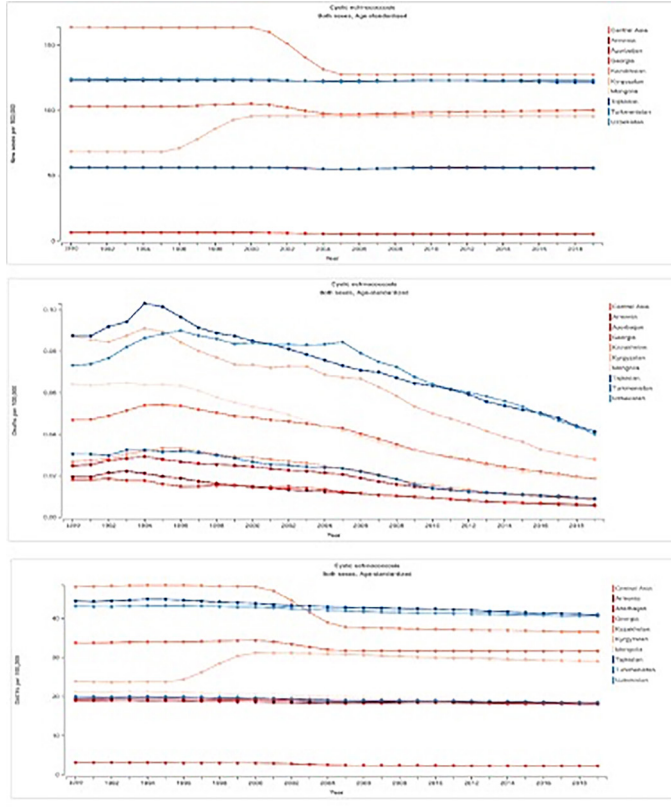

Figure 2. The trends of number of CE cases, age-standardized mortality and DALY rates cases in different Central Asian nations

CE: Cystic echinococcosis, DALY: Disability-adjusted life year

DISCUSSION

In order to devote appropriate healthcare resources, public health professionals and policymakers require a solid knowledge of the evolving epidemiology and sickness burden of CE. This research looked at the prevalence, mortality, and DALY rate of CE in 9 different Central Asian nations between 1990 and 2019. The global disease burden from CE may then be estimated. There were 95,102 cases of CE recorded in nine countries in 2019, a significant rise from the 65,909 cases reported in 1990 (45.18 percent higher: 95,102/207,368). Several countries in central Asia, including Kyrgyzstan, Uzbekistan, and Tajikistan, have reported declines in CE incidence, death, and DALY rate during the previous three decades, from 1990 to 2019. Nonetheless, Georgia, Kazakhstan,

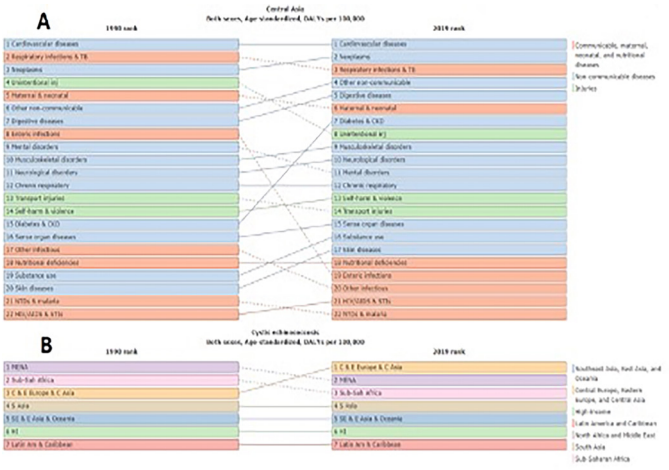

Figure 3. Comparison of CE with other leading risks factors in Central Asia by causes (A) and location (B) in 1990 and 2019, for both sexes, age-standardized DALY rate per 100,000 population

CE: Cystic echinococcosis, DALY: Disability-adjusted life year

and Armenia had the highest drop. In Kazakhstan, the estimated 2016 CE incidence rate was higher than the 1990 rate reported rate (1.4 cases per 100,000 population vs. 4.7 cases per 100,000 people), suggesting a shift in the CE pattern in these nations (21). A high humidity index, moderate temperatures, and a prolonged vegetative phase make the soil in southern and central Asia optimal for *E. granulosus* egg survival (22). Therefore, environmental variables could play a role. Two, it is common practice in pastoral areas to feed dogs the raw offal of sick calves as a diet component (3-6). In addition, the coasts of the Mediterranean, China, Europe, the Middle East, and South America have some of the highest infection rates in the world (8,23). Much of Central Asia's borders are with countries where CE is widespread (24). The expansion of businesses related to livestock raising has helped enhance regional trade and cattle transportation thanks to recent policy changes. This may have increased the circulation of infected intermediate animals, contributing to the illness's active spread (25,26). Increased mobility and better physician awareness of CE diagnosis, treatment, and control are needed since an increased number of immigrants from high-endemic countries and areas may be responsible for the rise in CE cases in low-endemic locations (27). The area is especially susceptible to the effects of natural disasters. This is because of the varied topography of Central Asia, which includes mountains, steppes, deserts, and significant river systems, all of which are affected by global warming. While some nations are more prepared than others, lowering the likelihood of natural disasters has become an important goal for all of the countries in the area. Central Asia has made great strides in the past few years but still needs to improve its disaster management infrastructure. The current situation in Afghanistan may cause an increase in the number of Afghans seeking refuge in Central Asian countries, with Tajikistan being the country most affected. The key to managing freshly arriving migrants demands to have regional contingency plans, stockpile, and replenish necessary assets. In addition, we found a decline in the regional age-standardized mortality and DALY rate of CE, which may be attributable to WHO's publication of the updated roadmap for neglected tropical diseases 2020 (28), and the signing of the London Declaration on Neglected Tropical Diseases in 2012 (29).

Study Limitations

There are several caveats to this research. While several changes were made to the GBD 2019 data's source, processing, and evaluation to increase data accuracy comparability, some degree of bias remains in the data's integrity and correctness. Second, the analysis may have been biased since disability weights for liver cancer were used instead of CE weights because they were the only ones accessible in the GBD 2019 data. Lastly, alveolar echinococcosis data was not provided at GBD 2019, whereas data on CE was. Validation of this study's conclusions requires more research that addresses these limitations.

CONCLUSION

Overall, our results show that the prevalence of CE diseases increased throughout central Asia between 1990 and 2019. Our findings may help public health authorities and policymakers design cost-benefit initiatives to reduce the illness burden caused by CE. It is suggested that most endemic nations in the area get more health funding in order to lessen the impact of CE. The improved diagnostic tools and more public and medical knowledge of CE may contribute to the growing incidence rate. This notion is bolstered by the fact that just around two instances in the sample were relapses, even though the majority were new cases. The burden of CE nationwide may be reduced with early diagnosis through ultrasound screening and preventative measures among high-risk populations.

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* Ethics

Ethics Committee Approval: The data utilized in this research was secondary. Hence, permission from any relevant ethics bodies was unnecessary.

Informed Consent: Not necessary.

* Authorship Contributions

Concept: F.R., K.T., K.O.D., Design: F.R., K.T., Data Collection or Processing: F.R., K.T., K.O.D., Analysis or Interpretation: F.R., K.T., K.O.D., Literature Search: F.R., K.T., K.O.D., Writing: F.R., K.O.D.

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Evaluation of Methylene Blue as An Effective Antiseptic for Medicinal Leeches (*Hirudo verbana*)

Metilen Mavisinin Tıbbi Sülükler (Hirudo verbana) için Etkili Bir Antiseptik Olarak Değerlendirilmesi

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ABSTRACT

Objective: Medicinal leeches (*Hirudo* spp.) have been used for therapeutic purposes in humans since ancient times. Because of their growth conditions, leeches carry certain bacteria and endosymbionts (e.g., *Aeromonas* spp). In both leech farms and hirudotherapy clinics, there are no reliable antiseptics that can be used with leeches. This study aimed to determine whether methylene blue (MB) is a safe antiseptic for medicinal leeches and assess its safe usage.

Methods: This study evaluated the efficacy of MB by determining lethal concentrations (LC), effective concentrations (EC), and lethal times (LT) for the medicinal leech *Hirudo verbena* Carena, 1820. A total of 570 *H. verbana* specimens obtained from a local farm were used in this study. Eighteen different concentrations of MB (between 1 ppm and 512 ppm) were tested.

Results: The LC_{50} and EC_{50} values for *H. verbana* were determined to be 60.381 (53.674-66.636) ppm and 2.013 (1.789-2.221) ppm, respectively. The LT_{50} durations for MB concentrations of 32 and 512 ppm were calculated as 212.92 h (138.43 h-1485.78 h) and 17.82 h (8.08 h-23.90 h), respectively.

Conclusion: The results show that MB concentrations between 2 and 19 ppm can be safely used as antiseptics in hirudotherapy clinics and leech farms to address bacterial concerns caused by medicinal leeches.

Keywords: Medicinal leech, hirudotherapy, methylene blue, antiseptics, lethal concentrations, lethal times

ÖZ

Amaç: Tıbbi sülükler (*Hirudo* spp.) eski çağlardan beri insanlarda tedavi amaçlı kullanılmaktadır. Sülükler, büyüme koşulları nedeniyle bazı bakterileri ve endosimbiontları (örneğin; *Aeromonas* spp.) taşırlar. Hem sülük çiftliklerinde hem de hirudoterapi kliniklerinde sülüklerle birlikte kullanılabilecek güvenilir antiseptikler bulunmamaktadır. Bu çalışmanın amacı, metilen mavisinin (MB) tıbbi sülükler için güvenli bir antiseptik olup olmadığını belirlemek ve güvenli kullanımını değerlendirmektir.

Yöntemler: Bu çalışmada, tıbbi sülük Hirudo verbana Carena, 1820 için ölümcül konsantrasyonlar (LC), etkili konsantrasyonlar (EC) ve ölümcül süreler (LT) belirlenerek MB'nin etkinliği değerlendirilmiştir. Bu çalışmada yerel bir çiftlikten elde edilen toplam 570 *H. verbana* örneği kullanılmıştır. On sekiz farklı MB konsantrasyonu (1 ppm ile 512 ppm arasında) test edilmiştir.

Bulgular: *H. verbana* için LC_{50} ve EC_{50} değerleri sırasıyla 60.381 (53.674-66.636) ppm ve 2.013 (1.789-2.221) ppm olarak belirlenmiştir. 32 ve 512 ppm MB konsantrasyonları için LT_{50} süreleri sırasıyla 212.92 saat (138.43 saat-1485.78 saat) ve 17.82 saat (8.08 saat-23.90 saat) olarak hesaplanmıştır.

Sonuç: Sonuçlar, 2 ila 19 ppm arasındaki MB konsantrasyonlarının, tıbbi sülüklerin neden olduğu bakteriyel endişeleri gidermek için hirudoterapi kliniklerinde ve sülük çiftliklerinde antiseptik olarak güvenle kullanılabileceğini göstermektedir.

Anahtar Kelimeler: Tıbbi sülük, hirudoterapi, metilen mavisi, antiseptikler, öldürücü konsantrasyonlar, öldürücü süreler

INTRODUCTION

The therapeutic potential of medicinal leeches has long been recognised, and their saliva contains a potent anticoagulant compound. These natural healing creatures have a rich history of use in various cultures, including Central Asian countries, Egypt, Arabian regions, Anglo-Saxon communities, and ancient Greek and Roman civilisations (1-3). The application of hirudotherapy, which includes the use of medicinal leeches, is among the most popular methods in clinics in France, Russia, Canada, and Germany. These extraordinary creatures can even be found in European pharmacies, and more than 300 hirudotherapy clinics offer medicinal leech treatment

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in Germany alone (4). In particular, in 2004, the US Food and Drug Administration authorised medicinal leeches in plastic and reconstructive surgery. As a result, their adoption in traditional and complementary medicine practices (TCMP) has gained global momentum and led many countries to establish legal frameworks to regulate their use (5-9).

As confirmed by many studies, medicinal leeches require endosymbiont bacteria, especially *Aeromonas* species, to digest the blood they consume (10-12). Unfortunately, medicinal leeches have also been associated with *Aeromonas* infections in patients requiring antibiotic treatment (10,13-17). To address this problem, researchers found that immersion of *Hirudo medicinalis* Linnaeus, 1758 in a 12.5 ppm hypochloric acid solution for 10 min effectively disinfected the handling material and suppressed oral bacterial flora without harming the leeches or compromising their sucking function (18). Furthermore, the antibiotics ciprofloxacin and cefotaxime added to the feeding blood of medicinal leeches significantly reduced the number of *Aeromonas* species (19).

MB is a heterocyclic aromatic compound characterized by the chemical formula $C_{16}H_{18}ClN_3S$ H_2O. This dark greenish-blue dye has a wide range of applications in medicine, biology, and chemistry. It is classified as a thiazine dye because of the presence of nitrogen and sulphur atoms in the aromatic ring structure. It has a molecular weight of 319.85 g/mol, making it highly soluble in water. Its synthesis in 1876 marked the beginning of its widespread use in various fields (20). In 1928, Schultz (21) elucidated the ability of MB to photoinactivate bacteria, leading to its widespread adoption. Later, Perdrau and Todd (22) revealed the antiviral mechanism of action. Notable applications of MB include surgical staining, treatment of malaria, and treatment of methaemoglobinemia. MB effectively treats methaemoglobinemia by reducing methaemoglobin to haemoglobin, which is crucial for oxygen transport in the bloodstream. Furthermore, MB serves as a remedy for cyanide poisoning by detoxifying the body by converting cyanide into less toxic substances (23). Beyond these critical applications, MB also plays a role in photodynamic therapy for lung, breast, and prostate cancers. Remarkably, no cases of severe acute respiratory syndrome-coronavirus-2 infection associated with MB use in chemotherapy patients have been reported in France (24). In addition, low doses of MB have been shown to stimulate mitochondrial respiration *in vivo*, and its safety has been well established in animal and human studies (25). In aquaculture, MB prevents bacterial, parasitic, and fungal diseases in fish. It is widely used as a disinfectant in aquarium fisheries, where it has proven to be particularly effective in preventing fungal diseases (26-29). MB has recently expanded so much that its potential as an anti-aging drug has emerged (30).

Despite sterile farm conditions, leeches still carry bacteria as endosymbionts or are contaminated by soil or water. Consequently, it is important to develop antiseptic solutions that can safely eliminate, prevent, or minimize these bacteria before TCMP. However, the mechanisms of action and LC of many antibiotics and antiseptics used in leeches have not yet been determined. Although medicinal leeches are hardy, they are highly sensitive to water quality criteria and chemicals, emphasizing the importance of establishing safe use ranges for chemical antiseptics to protect leech health, prevent contamination on farms, and avoid harm to leeches. Despite the widespread use of MB as an antiseptic in various medical fields, its toxicity in medicinal leeches, which are widely used in clinical applications, requires further investigation. This study aimed to fill this gap by investigating the acute toxicity of MB on medicinal leeches (*H. verbana*) and determining the safe dose range for its use in both clinics and leech farms. The results of this study will contribute to a broader understanding of the effects of this important compound on different organisms and ensure the safety and efficacy of MB in leech treatment.

METHODS

Medicinal Leeches Used in This Study

Medicinal leeches were obtained from a private medical leech production farm in Kayseri (Türkiye) and transported live to the laboratory for research. The average weight, length, and width of *H. verbana* used in the MB test were 1.49±0.16 0.16 g (1.24-187 g), 89.95±6.42 6.42 mm (79.80-99.30 mm) and 10.30±1.02 mm (9.2-12.1 mm), respectively. Considering that leeches feed on blood, they were given three months to digest this blood and acclimatize to the laboratory environment. During this adaptation period, the aquariums established in the laboratory provided water exchange and oxygen support to the leeches. After the completion of the 3-month adaptation period, toxicity tests (LC_{50}, EC_{50} and LT_{50}) were performed to determine the efficacy of MB on *H. verbana*.

Experimental Design

This study utilized a static acute toxicity assay, as prescribed by the APHA (31) standard method, to determine the $LC_{10\text{-}90}$, $EC_{10\text{-}90}$ and $LT_{10\text{-}90}$ values of MB ($C_{16}H_{18}ClN_3S$ x H_2O; Sigma-Aldrich) for medicinal leeches (*H. verbana*) throughout 24, 48, 72 and 96 h. In this experiment, nineteen aquariums were used to study the effects of MB on leeches. One aquarium was designated as the control group, while the remaining eighteen were test groups, each containing 20 L of spring water. The water quality characteristics in each aquarium were carefully monitored, with the temperature maintained at 20 °C and dissolved oxygen and pH values falling within the range of 5.00-5.37 ppm and 7.21-7.26, respectively, in accordance with APHA (31) guidelines. A stock solution of MB was created to prepare for the experiment at a daily concentration of 10 g/L. The solution was used to achieve concentrations of 1, 2, 4, 8, 16, 25, 32, 50, 64, 75, 100, 125, 128, 150, 175, 200, 256, and 512 ppm in the eighteen test group aquariums. Aeration was applied to each aquarium for 2 h to ensure that the MB concentration was evenly distributed throughout the water. In contrast, the control group aquarium contained only spring water without any MB solution. Ten medicinal leeches were placed in each aquarium, resulting in 190 leeches per test replication, including the control group. The experiments were performed in three replicates, and 570 medicinal leeches were used. The mortality of leeches in each replicate was monitored 24, 48, 72, and 96 h after the start of the experiment and recorded for evaluation. Dead leeches were immediately removed from the experimental media. The toxicity parameters in the study were obtained on the basis of the data of medicinal leech mortality at each concentration.

Statistical Analysis

The mean, standard deviation, and range values with 95% confidence limits were used to express the results. All replicates were included in the calculation of the mean values. Statistical analysis was performed using the computer program SPSS 25 (SPSS Inc.). Statistical probit analysis was employed to determine

the LC_{10-90}, EC_{10-90}, and LT_{10-90} values based on the MB acute toxicity test data. The chi-square test was conducted to compare mean mortality values using a significance level of 0.05. The relationship between the LT data was determined by correlation analysis. The Kruskal-Wallis test was used to statistically analyse the LC, EC, and LT between the concentrations tested with MB for 96 h. ANOVA Dunnett's T3 analysis was performed to determine differences between groups.

RESULTS

Behavioural Changes in Leeches

The present study investigated the effects of exposure to varying concentrations of MB on the mortality and behaviour of medicinal leeches, depending on the exposure of adult *H. verbana*. The number of dead medicinal leeches was examined across a range of MB concentrations (1-512 ppm), and the results were recorded for 96 h. Notably, no deaths were observed in the test groups exposed to MB concentrations between 1 and 25 ppm. No significant behavioural abnormalities were detected in repeated trials conducted with these leeches. However, behavioural responses were observed on the first day of exposure to higher concentrations of MB. The findings indicate that the mortality of medicinal leeches is dose-dependent on MB concentration and exposure duration, whereas behavioural changes were primarily observed at higher MB concentrations. The survival rates of leeches according to MB concentrations within 24-96 h are shown in Figure 1.

Observations revealed that movements such as excessive mucus secretion, acceleration in their actions, and attempts to move away from the test environment were observed in leeches exposed to 32 ppm of MB after 24 h. Additionally, blue staining in the colors of medicinal leeches, loss of adhesion ability on the posterior sucker of leeches and inactivity of some leeches on the water floor were detected at the same concentration. These behaviours and changes were also detected in other MB concentrations, but the severity was higher and occurred in a shorter time. Similar behavioural changes occurred at 50-75 ppm in 24 h, while at 100-512 ppm, it was observed to take shape in the first 3-6 h. Excess mucus secretion in leeches started at 12 h at 50-75 ppm doses and was detected within the first three h at 100-512 ppm concentrations.

Lethal (LC) and Effective (EC) Concentrations

H. verbana, when exposed to increasing concentrations of MB for 96 h, demonstrated a significant increase in the number of deaths ($p<0.05$ per case). The first death in medicinal leeches occurred at the end of 24 h at MB concentrations ranging from 100-512 ppm, and subsequent deaths were observed at concentrations of 50-75 ppm in 48 h and 32 ppm in 96 h. Furthermore, a significant difference in the number of deaths was observed over the four designated time intervals (24, 48, 72, and 96 hours) in leeches exposed to MB concentrations ($p<0.05$; chi-square test). Additionally, significant differences in the number of dead leeches were observed between the 24-96-h period for each concentration ($p<0.05$), with the highest concentration of MB (512 ppm) resulting in the highest leech mortality. The 24, 48, 72, and 96 h LC_{50} values (95% confidence limits) of MB, determined using a static bioassay system for *H. verbana*, were calculated as 223.429 (182.520-301.140) ppm, 129.192 (116.232-143.853) ppm, 84.430 (77.028-91.752) ppm, and 60.381 (53.674-66.636) ppm, respectively. Moreover, a significant difference in LC_{10-90} values was obtained at different times of exposure ($p<0.05$) (Figure 2, see Supplementary Data Table S1). Our results indicate that high concentrations of MB are toxic to *H. verbana*. However, EC_{10-90} used for sterilization and disinfection in leeches were calculated from the LC values obtained in this study, given that MB is used as an antibacterial agent. The EC_{90} (95% confidence limits), including the factor of safety or *H. verbana*, was determined in the range of 24, 48, 72, and 96 h as 18.983 (13.046-40.410) ppm, 10.409 (8.732-13.313) ppm, 5.147 (4.624-5.924) ppm, and 3.816 (3.411-4.417) ppm, respectively (Figure 3, see Supplementary Data Table S2). Our findings demonstrate that MB concentrations within the range of 2-19 ppm can be safely used to sterilize medicinal leeches and disinfect items used in medicinal leech farms and hirudotherapy clinics. Furthermore, the difference between the LC_{10-90} and EC_{10-90} concentrations of MB occurring after 24 h and the concentrations obtained at the end of 72 and 96 h was determined to be significant ($p<0.05$).

Lethal Times (LT)

The LT50, which represents the time for 50% mortality, was determined as 17.82 h (8.08-23.90 h) for the highest MB concentration of 512 ppm. For the lowest concentration of 32 ppm, the LT50 was calculated as 212.92 h (138.43-1485.78 h) (see Supplementary Data Table S3). The LT_{10-90} for *H. verbana* were analysed at various concentrations of MB using correlation analysis, Kruskal-Wallis analysis, and ANOVA-Dunnett T3 analysis. The results are summarised in Figure 4, which presents a statistical difference matrix indicating the significance of differences between the various concentrations of MB. It was determined that there was a strong positive correlation between the LT obtained according to the concentrations of MB (Figure 4A). Overall, the matrix shows that there were significant differences in LT between the different concentrations of MB. However, specifically, concentrations of 32-64 ppm, 75-125 ppm, and 128-512 ppm showed no significant differences in LT among themselves ($p>0.05$), whereas all other concentrations (75 ppm-512 ppm) showed significant differences in LT compared with each other ($p<0.05$). Moreover, the matrix reveals that the LT at

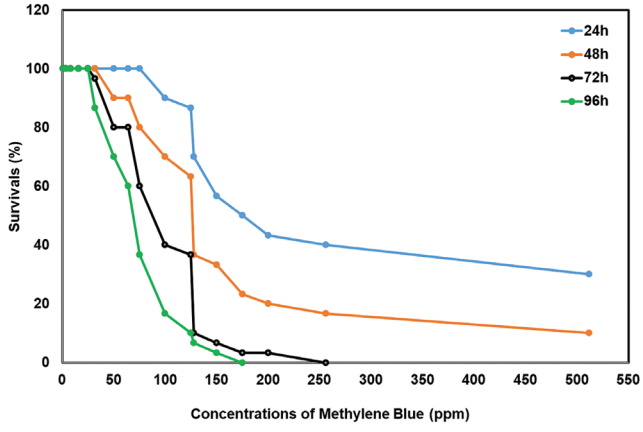

Figure 1. Survival rates of medicinal leeches in the 24-h to 96-h range at MB concentrations

MB: Methylene blue

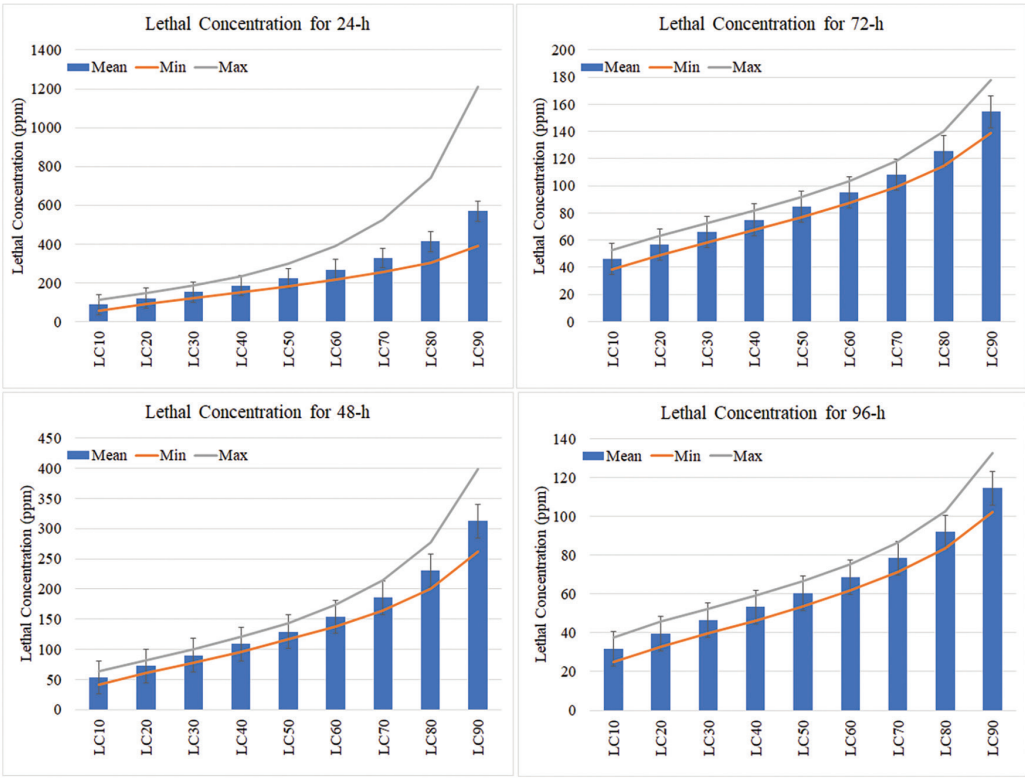

Figure 2. Time-dependent LC_{10-90} of MB for *H. verbana* over 24-96 h (min and max values were obtained within 95% confidence limits)
MB: Methylene blue, LC: Lethal concentrations

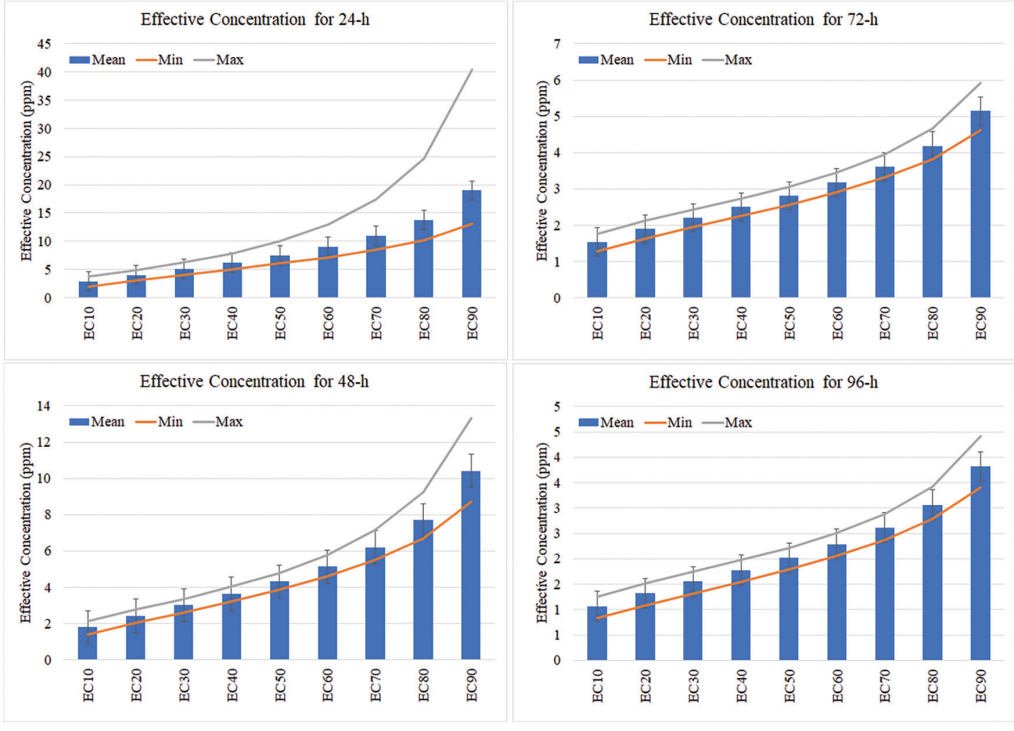

Figure 3. Time-dependent EC_{10-90} of MB for *H. verbana* over 24-96 h (min and max values were obtained within 95% confidence limits)
MB: Methylene blue, EC: Effective concentrations

lower concentrations of MB was generally longer, whereas the LT at higher concentrations was generally shorter. For example, concentrations of 32, 50 and 64 ppm had longer LT, whereas concentrations of 128, 150, 175, 200, 256 and 512 ppm had shorter LT (Figure 4B).

DISCUSSION

This study investigated the toxicity of MB on *H. verbana* and its effect on the survival rate at different concentrations and exposure times. These findings demonstrate that the toxicity of MB on *H. verbana* increased in proportion to both the concentration and exposure time. The medicinal leeches exposed to 32 ppm MB exhibited a mortality rate of only 20% at 96 h, whereas exposure to 512 ppm MB resulted in 100% mortality at 72 h. Moreover, the LC_{50} values of MB for *H. verbana* were determined to be 215.285 ppm after 24 h and 55.271 ppm after 96 h. These results indicate that high concentrations of water-soluble MB can be highly toxic to *H. verbana* if appropriate concentrations are not used.

A study on the toxicity of MB in Nil tilapia [*Oreochromis niloticus* (Linnaeus, 1758)] reported abnormal behaviour, irregular swimming, and premature death in fish exposed to high concentrations of MB. In addition, they determined that the toxicity observed when MB is used in high doses can occur by transferring in the same way (32). These results are consistent with our findings, demonstrating similar effects of MB in leeches exposed to high concentrations. These results agree with our findings showing similar effects of MB in leeches exposed to high concentrations. Again, this reveals that MB can have significant toxic effects on all aquatic living organisms when used in high concentrations.

The LC_{50} values of MB were calculated in three different groups of the marine medaka [*Oryzias dancena* (Hamilton, 1822)], including adults, juveniles, and eggs, after 96 h of exposure as 185.26, 103.84 and 127.15 ppm, respectively (33). In larval fathead minnows [*Pimephales promelas* (Rafinesque, 1820)], the 96-h LC_{50} of MB was found to be 45 ppm at 20 °C (34). Our study determined the LC_{50} value of MB in the medicinal leech *H. verbana* to be 60,381 ppm after 96 h of exposure. These results indicate that *H. verbana* is more sensitive to MB than marine medaka, but more resistant than larval fathead minnows.

In a recent study aimed at determining the lethal dose of zinc in *H. verbana*, it was reported that increasing the concentration of zinc led to behavioural disorders, mucus increase, bleeding, and faster death in the leeches (35). Our study also observed similar anomalous behaviours in leeches exposed to MB. Specifically, the LC_{50} value of MB in *H. verbana* was determined to be 215,285 ppm after 24 h of exposure, whereas the LC_{50} value of zinc in the same species was 48.30 ppm over the same period. This finding indicate that zinc is significantly more harmful to leeches because of its heavy metal nature. At the same time, MB is relatively safe for leeches when used carefully, especially for disinfectant purposes.

Studies have determined the LD_{50} value of copper sulphate on medicinal leeches at low concentrations, specifically at 0.0044 ppm (36). Meanwhile, the LC_{50} value of Cu on *H. verbana* was found to be 0.84 ppm and 15.83 ppm for Zn, indicating a higher sensitivity of *H. verbana* to Cu than to Zn. However, the effect of the mixture of Cu and Zn was higher than that of both elements individually (37). Furthermore, copper has been observed to cause deformations on the body wall of *H. verbana* (38). The mixing of these substances, particularly in the surface waters of natural wetlands, can limit the survival of leeches. Notably, *H. verbana* displays greater resistance to MB solutions than to chemicals such as Cu, Zn, and copper sulphate.

Water-borne heavy metal accumulation in tissues and salivary secretions of *Hirudinaria manillensis* (Lesson, 1842) has been reported in natural environments. However, a study has shown that heavy metal accumulation can decrease by 92.4-99.7% after three weeks of continuous exchange with distilled water (39). This indicates that heavy metal accumulation in *H. verbana* after using MB as a bath can be easily removed when the leeches are transferred to clean water. Moreover, short-term antiseptic applications of MB (e.g., 30 minutes) are unlikely to accumulate in leeches.

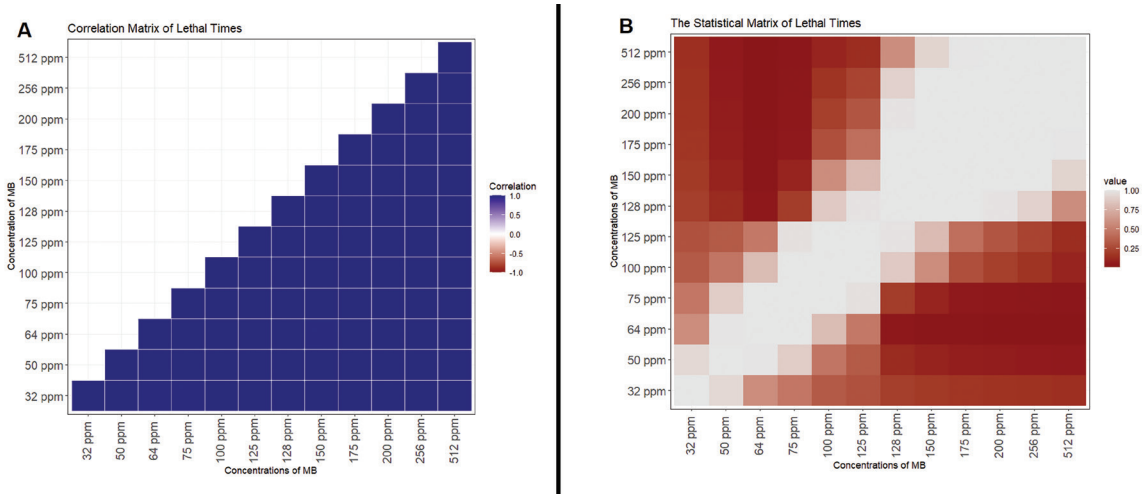

Figure 4. A) Correlation of lethal times obtained with respect to concentrations of MB. B) ANOVA-Dunnett T3 analysis showing the statistical matrix of $LT_{10\text{-}90}$ at varying concentrations of MB used for *H. verbana*. (The change of the p-value from grey to dark red from $p<0.05$ to $p>0.05$ indicates)

MB: Methylene blue, LT: Lethal times

A study has suggested that hypochlorous acid, when used at a concentration of 12.5 ppm, can serve as a safe and effective external decontamination and bacterial suppression method for medicinal leeches without causing any adverse effects on their life or sucking function (18). Nonetheless, chlorinated compounds, including hypochlorous acid, are highly toxic to leeches and should be avoided in leech farming practices (40). Additionally, the presence of chlorine compounds can cause leeches to vomit. Therefore, based on our toxicological findings and effective doses, we recommend using MB as a safe and effective alternative to prevent external contamination and suppress pathogenic bacteria in leeches, without causing harm to the animals.

Previous studies have demonstrated that ciprofloxacin and cefotaxime added to food can suppress *Aeromonas* endosymbionts in the intestine of leeches and control bacterial infections (10,19). Similarly, using MB as a bath at appropriate concentrations can also play a crucial role in reducing the prevalence of *Aeromonas* species both on the surfaces and in the digestive tract of medicinal leeches.

Soft tissue infections and complications resulting from *Aeromonas* infections have been observed in some patients after hirudotherapy (14-16). However, the incidence of such infections can be decreased by taking a bath with MB or other antiseptics and disinfectants one week before using medicinal leeches.

CONCLUSION

MB has a long history of use in medicine and is effective and safe in various applications. This study investigated the toxic effects of different concentrations of MB on medicinal leeches and sought to determine its safe and effective use as an antiseptic. The results showed that longer exposure times to MB concentrations significantly increased mortality and behavioural changes in medicinal leeches. It reveals that MB concentrations between 2 and 19 ppm can be safely used in sterilization of medicinal leeches and can be a valuable tool in reducing the risk of bacterial infection in patients receiving leech therapy. This shows that MB can be safely used as an antiseptic in medicinal leech farms and traditional and complementary medicine clinics, which is becoming increasingly widespread worldwide.

* Ethics

Ethics Committee Approval: Animal Experiments Ethics Committees As stated in the Regulation on Procedures and Principles, an Ethics Committee Approval Certificate is required for vertebrate animals. Because medicinal leeches are invertebrates, they are not included in the definition of "experimental animal". Therefore, an Ethics Committee approval certificate was not obtained for this study.

Informed Consent: This study was conducted in accordance with the EU Directive 2010/63/EU for animal experiments, the National Research Council's Guide for the Care and Use of Laboratory Animals, and the National Ethic Rules of Türkiye.

* Authorship Contributions

Surgical and Medical Practices: S.D., S.F., N.S., Concept: N.S., Design: S.F., N.S., Data Collection or Processing: S.F., B.K., N.S., Analysis or Interpretation: S.D., N.S., Literature Search: S.D., S.F. B.K., Writing: S.D., N.S.

Conflict of Interest: No conflict of interest was declared by the authors.

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Table S1. Time-dependent LC_{10-90} of MB for *H. verbana* over 24-96 h

Point	Lethal concentration (ppm) (95% confidence limits)			
	24-h	48-h	72-h	96-h
LC_{10}	87.658 (57.090-111.529)	53.448 (42.196-63.323)	46.165 (38.303-52.906)	31.851 (25.007-37.727)
LC_{20}	120.860 (90.308-147.765)	72.362 (60.677-82.641)	56.796 (48.999-63.502)	39.671 (32.683-45.610)
LC_{30}	152.358 (121.755-186.867)	90.030 (78.308-100.821)	65.950 (58.344-72.656)	46.477 (39.541-52.432)
LC_{40}	185.699 (152.125-235.947)	108.508 (96.559-120.506)	74.932 (67.500-81.792)	53.209 (46.396-59.234)
LC_{50}	223.429 (182.520-301.140)	129.192 (116.232-143.853)	84.430 (77.028-91.752)	60.381 (53.674-66.636)
LC_{60}	268.826 (215.221-391.072)	153.819 (138.352-173.661)	95.132 (87.431-103.478)	68.519 (61.773-75.350)
LC_{70}	327.654 (253.816-523.140)	185.388 (164.984-214.630)	108.089 (99.465-118.466)	78.445 (71.274-86.570)
LC_{80}	413.046 (305.309-741.511)	230.654 (200.955-277.441)	125.510 (114.782-139.856)	91.901 (83.418-102.871)
LC_{90}	569.495 (391.380-1212.311)	312.279 (261.974-399.398)	154.412 (138.706-177.705)	114.466 (102.318-132.519)

MB: Methylene blue, LC: Lethal concentrations

Table S2. Time-dependent EC_{10-90} of MB for *H. verbana* over 24-96 h

	Effective concentration (ppm) (95% confidence limits)			
Point	24-h	48-h	72-h	96-h
EC_{10}	2.922 (1.903-3.718)	1.782 (1.407-2.111)	1.539 (1.277-1.764)	1.062 (0.834-1.258)
EC_{20}	4.029 (3.010-4.926)	2.412 (2.023-2.755)	1.893 (1.633-2.117)	1.322 (1.089-1.520)
EC_{30}	5.079 (4.059-6.229)	3.001 (2.610-3.361)	2.198 (1.945-2.422)	1.549 (1.318-1.748)
EC_{40}	6.190 (5.071-7.865)	3.617 (3.219-4.017)	2.498 (2.250-2.726)	1.774 (1.547-1.974)
EC_{50}	7.448 (6.084-10.038)	4.306 (3.874-4.795)	2.814 (2.568-3.058)	2.013 (1.789-2.221)
EC_{60}	8.961 (7.174-13.036)	5.127 (4.612-5.789)	3.171 (2.914-3.449)	2.284 (2.059-2.512)
EC_{70}	10.922 (8.461-17.438)	6.180 (5.499-7.154)	3.603 (3.316-3.949)	2.615 (2.376-2.886)
EC_{80}	13.768 (10.177-24.717)	7.688 (6.699-9.248)	4.184 (3.826-4.662)	3.063 (2.781-3.429)
EC_{90}	18.983 (13.046-40.410)	10.409 (8.732-13.313)	5.147 (4.624-5.924)	3.816 (3.411-4.417)

MB: Methylene blue, EC: Effective concentrations

Table S3. LT_{10-90} for *H. verbana* at different concentrations of MB

Point	Lethal time (95% confidence limits)			
	32 ppm	**50 ppm**	**64 ppm**	**75 ppm**
LT_{10}	67.33h (48.00-80.27h)	53.43h (34.30-65.38h)	55.30h (41.63-63.98h)	41.15h (27.40-49.92h)
LT_{20}	99.97h (83.27-181.27h)	71.62h (55.72-84.52h)	66.52h (54.87-74.97h)	51.68h (39.48-60.78h)
LT_{30}	132.93h (102.28-394.95h)	88.47h (74.23-108.25h)	76.28h (66.97-85.93h)	60.92h (50.35-70.62h)
LT_{40}	169.58h (119.88-781.53h)	105.95h (89.57-141.68h)	85.73h (76.27-96.37h)	70.88h (60.33-82.82h)
LT_{50}	212.92h (138.43-1485.78h)	125.42h (103.60-187.73h)	95.63h (85.73-110.83h)	79.92h (69.48-97.87h)
LT_{60}	267.33h (159.53-2830.47h)	148.45h (118.23-252.70h)	106.67h (95.60-127.48h)	91.12h (78.42-119.58h)
LT_{70}	341.05h (185.43-5647.57h)	177.82h (135.27-347.95h)	119.90h (105.17-150.57h)	104.85h (88.13-148.70h)
LT_{80}	453.52h (220.93-12687.65h)	219.62h (157.60-509.75h)	137.48h (117.57-184.22h)	123.58h (100.18-194.28h)
LT_{90}	673.33h (294.42-39022.12h)	294.35h (193.97-869.30h)	166.20h (136.40-245.12h)	155.18h (118.83-284.48h)
	100 ppm	**125 ppm**	**128 ppm**	**150 ppm**
LT_{10}	27.10h (17.28-34.32h)	24.27h (15.37-31.08h)	15.97h (8.13-20.93h)	11.77h (5.02-32.00h)
LT_{20}	35.37h (25.62-42.68h)	31.78h (22.68-38.68h)	20.15h (12.32-26.23h)	16.87h (9.85-22.58h)
LT_{30}	42.97h (33.67-50.53h)	38.62h (29.78-45.73h)	24.82h (16.72-31.02h)	20.78h (13.33-26.72h)
LT_{40}	50.73h (41.87-59.25h)	45.62h (37.15-53.32h)	29.63h (21.55-36.03h)	24.33h (15.72-30.80h)
LT_{50}	59.27h (50.35-70.83h)	53.28h (44.93-62.58h)	34.98h (27.08-41.82h)	29.12h (20.55-35.83h)
LT_{60}	69.22h (59.27-84.72h)	62.27h (53.27-74.93h)	41.32h (33.57-49.23h)	34.83h (26.53-42.28h)
LT_{70}	81.72h (69.22-105.77h)	73.55h (62.63-92.75h)	49.35h (41.25-60.07h)	42.18h (33.98-51.75h)
LT_{80}	99.27h (81.73-139.27h)	89.37h (74.35-121.17h)	60.77h (50.90-78.02h)	52.82h (43.52-68.42h)
LT_{90}	130.02h (101.57-206.72h)	117.88h (92.82-178.35h)	81.10h (65.65-116.55h)	72.90h (57.78-106.90h)
	175 ppm	**200 ppm**	**256 ppm**	**512 ppm**
LT_{10}	11.45h (4.98-16.77h)	9.57h (3.22-15.07h)	9.92h (3.43-15.23h)	7.87h (1.47-13.47h)
LT_{20}	15.07h (7.78-20.63h)	12.87h (5.38-18.72h)	12.98h (5.53-18.50h)	10.42h (2.67-16.30h)
LT_{30}	18.38h (10.68-24.03h)	15.95h (7.77-21.95h)	15.75h (7.67-21.35h)	12.75h (4.07-18.77h)
LT_{40}	21.78h (13.93-27.52h)	19.17h (10.58-25.27h)	18.58h (10.35-24.23h)	15.17h (5.82-21.22h)
LT_{50}	25.53h (17.77-31.40h)	22.73h (14.05-28.97h)	21.72h (13.47-27.40h)	17.82h (8.08-23.90h)
LT_{60}	29.92h (22.42-36.22h)	26.98h (18.48-33.52h)	25.33h (17.37-31.27h)	20.95h (11.18-27.12h)
LT_{70}	35.43h (28.22-42.97h)	32.42h (24.32-39.92h)	29.92h (22.42-36.62h)	24.92h (15.60-31.47h)
LT_{80}	43.22h (35.67-54.37h)	40.17h (32.18-51.07h)	36.30h (29.12-45.72h)	30.52h (22.17-38.93h)
LT_{90}	56.92h (46.55-79.87h)	54.08h (43.65-78.07h)	47.50h (38.68-67.27h)	40.40h (32.02-58.90h)

MB: Methylene blue, LT: Lethal times

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The Bibliometric Analysis of the Postgraduate Theses Written on Medical Parasitology in Türkiye

Türkiye'de Tıbbi Parazitoloji Alanında Hazırlanan Tezlerin Bibliyometrik Analizi

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ABSTRACT

Objective: The aim of this study was to analyze the theses prepared in the field of medical parasitology in Türkiye and to reveal the importance given to the science of parasitology in the groves of academe and to raise awareness in this field.
Methods: Council of Higher Education's National Thesis Center database has been analyzed postgraduate these documents conducted in the field of medical parasitology from January 1985 to September 2022.
Results: As a result of the examining, 393 theses made in the field of medical parasitology were detected. It was determined that 52.9% of the theses prepared were master, 28% of PhD and 19.1% were the thesis of medical specialty thesis and 61.3% of the theses prepared were related to protozoa, 16.5% of helminths, 8.6% arthropods and 12.2% of intestinal parasites (helminth and protozoa). The top five types of parasites in the theses were *Toxoplasma gondii, Leishmania* spp., *Echinococcus* spp., *Giardia intestinalis* and *Cryptosporidium* spp. respectively.
Conclusion: In conclusion, it was of the opinion that more importance should be given to the field of medical parasitology in Türkiye.
Keywords: Bibliometric analysis, postgraduate theses, medical parasitology, Türkiye

ÖZ

Amaç: Bu çalışmanın amacı, Türkiye'de tıbbi parazitoloji alanında hazırlanan tezlerin analizini yaparak, akademik camiada parazitoloji bilimine verilen önemi ortaya koymak ve bu alanda bir farkındalık oluşturmaktır.
Yöntemler: Çalışmaya, Yükseköğretim Kurulu Ulusal Tez Merkezi'nin online uygulamasından arama yapılarak, Ocak 1985-Eylül 2022 tarihleri arasında tıbbi parazitoloji alanında yapılmış tezler dahil edildi.
Bulgular: Arama sonucunda tıbbi parazitoloji alanında yapılmış 393 tez saptandı. Tezlerin %52,9'unun yüksek lisans, %28'inin doktora ve %19,1'inin tıpta uzmanlık tezi olduğu belirlendi. Tezlerin yapıldığı alanlarının dağılımı incelendiğinde, tezlerin %61,3'ünün protozoonlar, %16,5'inin helmintler, %8,6'sının artropodlar ve %12,2'sinin bağırsak parazitleri (helmint ve protozoonlar) ile ilgili olduğu saptandı. Tezlerde en çok çalışılan ilk beş parazit türünün sırasıyla *Toxoplasma gondii, Leishmania* spp., *Echinococcus* spp., *Giardia intestinalis* ve *Cryptosporidium* spp. olduğu belirlendi.
Sonuç: Sonuç olarak, Türkiye'de tıbbi parazitoloji alanına daha çok önem verilmesi gerektiği kanaatine varıldı.
Anahtar Kelimeler: Bibliometrik analiz, lisansüstü tezler, tıbbi parazitoloji, Türkiye

INTRODUCTION

Parasitic diseases are an important public health problem worldwide. These diseases are particularly a serious public health problem in developing countries, and it is estimated that there may be an increase in the prevalence of parasitic diseases in developed countries for many reasons such as climate change and mass migration (1). Thus, parasitosis outbreaks seen in countries that make up the European Union have once again emphasized the importance of struggle with the parasitic diseases (2). It should not be forgotten that

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Türkiye PARAZİTOLOJİ Dergisi

combating these diseases that threaten human health is possible with health workers specialized in field of parasitology (3).

Postgraduate education is a planned/programmed educational process aimed at educating qualified individuals who can contribute to knowledge through scientific research processes and meet the social needs (4). The theses prepared at the end of postgraduate education contribute to the scientific literature and give the researcher analytical thinking skills. The theses prepared at the end of the postgraduate programs are classified as "Master Thesis", "PhD Thesis" or "Specialization Thesis in Medicine" according to the stage in which it is written (5,6).

The aim of this study was to analyze the theses prepared in the field of medical parasitology in Türkiye and to reveal the importance given to the science of parasitology in the groves of academe and to raise awareness in this field.

METHODS

In this study, document analysis as a qualitative research method was used. Council of Higher Education's National Thesis Center database (https://tez.yok.gov.tr/UlusalTezMerkezi) has been analyzed postgraduate theses documents conducted in the field of medical parasitology from January 1985 to September 2022. In this database, which can be accessed online, the words of "parasitology" and "microbiology" were separately searched by entering the "division" line of "detailed search" page. The theses prepared in the fields of veterinary parasitology and veterinary microbiology were not included in the study. The no, title, type and publication date of the theses included in the study were recorded. In the summary of the theses with open access, if the field work was stated, the province(s) and parasite(s) where the study carried out were recorded. The theses that were not open access were not taken into consideration.

Statistical Analysis

Descriptive statistics for categorical variables in our study are expressed as number (n) and percentage (%). Microsoft Excel 2016 program was used for calculations and graphs. The map of distribution by provinces of the theses in the group of intestinal parasites was drawn on the PaintMaps.com website.

RESULTS

A total of 393 theses, 150 (3.6%) of 4119 theses prepared in microbiology departments, were found to be related to field of medical parasitology. Two hundred fourty-three (61.8%) of these theses were prepared in "Parasitology" and "Medical parasitology" departments and 150 (38.2%) were prepared in six different departments of microbiology (Table 1). In addition, 208 (52.9%) of 393 theses were a master thesis, 110 (28%) PhD thesis and 75 (19.1%) were specialization thesis in medicine (Table 2).

Two hundred forty-one (61.3%) of the theses prepared were related to protozoa, 65 (16.5%) helminths, 34 (8.6%) arthropods and 48 (12.2%) intestinal parasites (helminths and protozoa) (Table 3). The distribution of prepared theses subject (protozoa, helminths and arthropods) is as shown in Figure 1. Even though the number of theses prepared in the field of medical parasitology between 2000 and 2022 were small fluctuations between these years, it was found that there was no gradual increase in the number of theses to the until today.

The top 10 parasites in the prepared theses were found to be *Toxoplasma gondii* (68), *Leishmania* spp. (50), *Echinococcus* spp. (37), *Giardia intestinalis* (27), *Cryptosporidium* spp. (21), *Trichomonas vaginalis* (16), *Entamoeba histolytica* (16), *Plasmodium* spp. (15), *Blastocystis hominis* (15) and *Naegleria fowleri* + *Acanthamoeba* spp. (9), respectively (Figure 2).

Forty-four (11.2%) theses were prepared for intestinal parasites. Fifteen (34.1%) of the theses were prepared in Van, Türkiye and six (13.6%) were prepared in different patient groups in Ankara, Türkiye. The distribution of the theses prepared in the field of medical parasitology according to the provinces is as shown in Figure 3. In the theses, the parasites of the investigated frequency and patient groups where the research performed are shown in Table 4.

In the distribution by universities of the theses prepared in the field of medical parasitology, the universities, which took place in the top three, were Ege University, Van Yüzüncü Yıl University and Erciyes University, respectively. In addition, the list of the first ten universities was shown in Table 5.

DISCUSSION

Bibliometric analysis of theses is an important way to determine scientific field trends (7). The upload of the theses prepared in

Table 1. Distributions of theses by departments

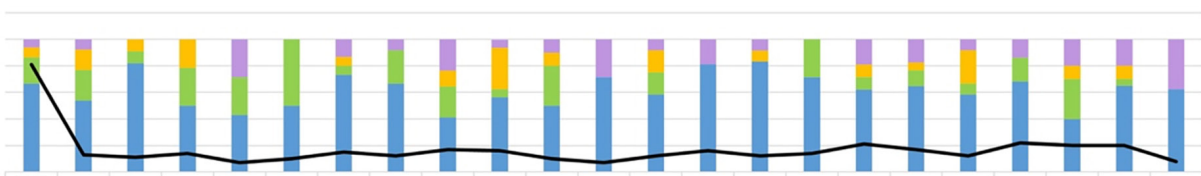
Departments	Number and rate (%) of the theses
Parasitology	209 (53.2)
Medical parasitology	34 (8.6)
Microbiology	40 (10.2)
Medical microbiology	54 (13.7)
Microbiology and clinical microbiology	49 (12.5)
Infectious diseases and clinical microbiology	4 (1.0)
Pharmaceutics microbiology	2 (0.5)
Clinical microbiology and infectious diseases	1 (0.2)

Table 2. Distributions of theses by program degrees

Program degree	Number and rate (%) of the theses
Master	208 (52.9)
PhD	110 (28.0)
Specialization in medicine	75 (19.1)
Total	393

Table 3. The distribution of prepared theses subject

Prepared theses subject	Number and rate (%) of the theses
Protozoan	241 (61.3)
Helminths	65 (16.5)
Arthropod	34 (8.6)
Intestinal parasites	48 (12.2)
Others	5 (1.3)



	2000 and before	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Intestinal parasites (%)	6,1	7,7	0	0	28,5	0	13,3	8,3	23,5	6,2	10	28,6	8,3	18,8	8,3	0	19	17,6	8,3	13,7	20	20	37,5
Arthropod (%)	7,4	15,3	9	21,4	0	0	6,7	0	11,8	31,2	10	0	16,7	0	8,3	0	9,5	5,8	25	0	10	10	0
Helminth (%)	19,8	23	9	28,5	28,5	50	6,7	25	23,5	6,2	30	0	16,7	0	0	28,6	9,5	11,8	8,3	18,1	30	5	0
Protozoan (%)	66,6	53,9	82	50	43	50	73,3	66,7	41,1	56,2	50	71,4	58,3	81,2	83,3	71,4	62	64,8	58,4	68,1	40	65	62,5
Total number of theses	81	13	11	14	7	10	15	12	17	16	10	7	12	16	12	14	21	17	12	22	20	20	8

Figure 1. Distribution of total number of theses and theses subjects by years

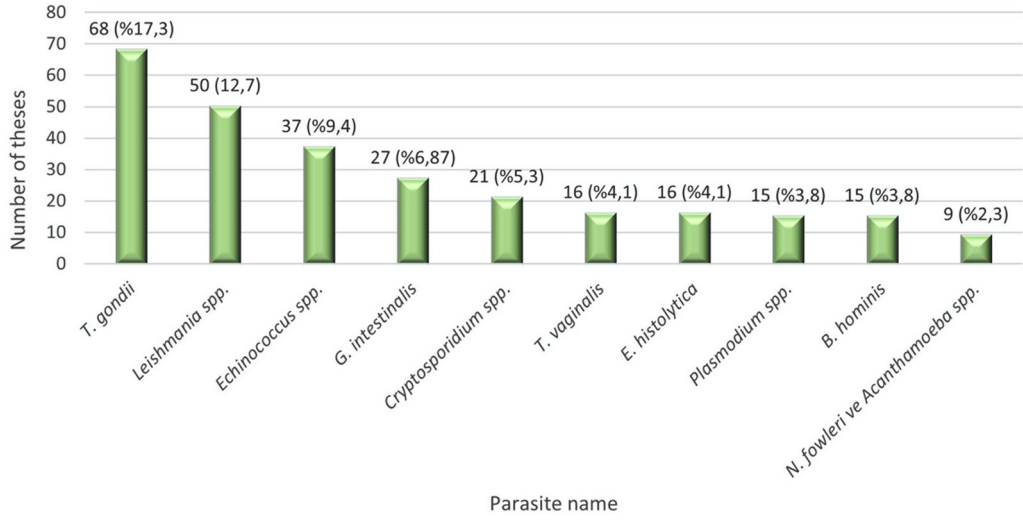


Figure 2. The top 10 parasite species

Türkiye to the Council of Higher Education's National Thesis Center database enables that bibliometric analyzes are made easily according to the thesis subjects.

According to World Malaria Report 2017, it is estimated that there are about 216 million malaria cases annually and approximately 445,000 of these cases result in death. It has been reported that more than 12 million people were influenced by leishmaniasis and about 2 million new leishmaniasis cases. In the world, especially in Central and South America, approximately 6-8 million people were affected by Chagas disease and about 12,000 of these patients died. It has been reported that amebiasis caused the most deaths in children after pneumonia (8). A study conducted by worldwide, it has been reported that 819 million people are estimated to be infected with *Ascaris lumbricoides*, 464.6 million people *Trichuris trichiura* and 438.9 million people with hookworms (9). It has been stated that approximately 187 million people were influenced by schistosomiasis in sub-Saharan Africa, India, China, East Asia and America. In 83 countries of the Africa, Asia, Southern and Central America, approximately 120 million lymphatic filariasis cases have been seen and approximately 90% of these cases were reported to be caused by *Wuchereria bancrofti* (10).

In the data of the T.C. Ministry of Health 2022, the rate of deaths due to some infectious and parasitic diseases among total deaths was reported as 3.66%. In the same data, the sales value of Antiparasitic Products, Insecticides and Repellents in 2018 was 26.6 million TL (5.1 million boxes), while this value was reported as 174.8 million TL (7.5 million boxes) in 2022 (11). In summary, parasitic diseases continue to be important public health problems in Türkiye and worldwide (12). For this reason, it is important to increase the number of scientists specialized in the field of medical parasitology. The number of PhD and specialization theses is an indicator of the number of scientists specialized in their field. It was determined that of the 393 theses written in the field of parasitology in Türkiye, 208 (52.9%) were master's theses, 110 (52.9%) were PhD theses and 75 (19.1%) were medical specialization thesis. We believe that the number of medical speciality theses written in the field of parasitology is insufficient and there should be more specialisation in the field of parasitology due to reasons such as the fact that parasitology laboratories are within the microbiology laboratories in hospitals and parasitology specialists who are not medical doctors are not authorised to evaluate patient results and consult medical specialization students in Türkiye.

Medical parasitology has included the study of parasitic protozoa, parasitic helminths and arthropods that directly cause disease or act as vectors of various pathogens (13). Parasitic diseases cause low productivity, morbidity and mortality in animals, and life-threatening morbidity and mortality in humans by affecting both humans and animals worldwide. Despite recent advances in epidemiology, molecular biology, and treatment of protozoan

Table 4. Species of parasites and patient groups studied in the theses

Species of parasites	Studied patient groups
Intestinal parasites	Applying to the hospital, primary school student, 0-6 years old children, immigrant children, diarrheic, diarrheic children, gastrointestinal complaints, dialysis patients, skin disease, chronic urticaria, allergy complaint, oncologic/cancer patients, stomach cancer, pediatric intensive care unit, substance addicted patients, cardiac disease, schizophrenic patients, HIV-positive
T. gondii	Healthy woman, miscarriage and death–birth, suspected pregnant, 8-25 age group women who have never given birth, women of reproductive age group, hospital staff members, hospital staff members, diabetic, cancerous, dialysis, multiple sclerosis, preeclampsia, ocular toxoplasmosis pre-diagnosis, blood donors, applying to neurology polyclinic, newborns
E. histolytica	Applying to hospital, gastrointestinal complaints, diarrheic, endoscopy/colonoscopy, ulcerative colitis, primary school student, mental retardation
G. intestinalis	Applying to hospital, school-age children, diarrheic, endoscopy/colonoscopy, immunosuppressed, abdominal pain, gastrointestinal complaints
B. hominis	Applying to hospital, gastrointestinal complaints, diarrheic, immunosuppressed, using proton pump inhibitors, ulcerative colitis and Crohn's disease, diabetic, urticaria, coeliac disease
Cryptosporidium spp.	Applying to hospital, gastrointestinal complaints, diarrheic, immunosuppressed, dialysis, endoscopy/colonoscopy, HIV-positive, children in the child welfare agency
C. cayetanensis	Diarrheic, HIV-positive
Microsporidia	Cancer patients, diarrheic, children with gastrointestinal complaints, receive a diagnosis of cancer, bone marrow transplantation, retarded development, itching, dermatitis, urticaria and ulcerative colitis complaint
Dientamoeba fragilis	Gastrointestinal complaints, coeliac disease, irritable bowel syndrome, ulcerative colitis complaint
C. belli	HIV-positive
T. vaginalis	Women admitted to hospital, woman with vaginal discharge, vaginitis pre-diagnosis, female patients with vaginal complaints and male patients with urinary system complaints, male patients with urinary tract infection
Acanthamoeba spp. and *Naegleria* spp.	Applied to the ophthalmology
Pneumocystis jirovecii	Lung disease
Plasmodium spp.	Malaria-like symptoms, blood donors, Malaria suspected individuals working as farm workers
Babesia spp.	A history of tick bites
Leishmania spp.	Individuals over 2 years of age living in Mersin/Türkiye, applying to dermatology outpatient clinic
Echinococcus spp.	Diagnosed intrathoracic or intracardiac hydatid cyst, healthy individuals, pre-diagnosed hydatidosis, eosinophilic, applying to hospital, studies involving sheep, dogs and cattle as well as humans
Toxocara spp.	Toxocariasis suspected, primary school students, healthy individuals of different ages and occupations, eosinophilic, veterions
Fasciola hepatica	Applying to hospital, healthy individuals, eosinophilic
Enterobius vermicularis	Requested cellophane band, primary school students
Trichinella spiralis	Healthy individuals, suspected trichinellosis
Demodex spp.	Applying to dermatology outpatient clinic, applied to the ophthalmology, diabetic, acne complaints, healthy individuals
Pediculus humanus capitis	Primary school students, seven occupational groups (preschoolers primary school students, college students, healthcare personnel, officeholder, workers and peasantry)
Tick species	A history of tick bites

HIV: Human immunodeficiency virus

diseases, research on protozoa is not sufficient. In addition, an increase in protozoan infections is expected due to displacement of human and animal populations, increases in atmospheric temperature, flooding and the need for alternative water sources (14). In this study, it was determined that 61.3% of the theses prepared in our country in the field of medical parasitology were related to protozoa.

Since helminths tend to cause chronic infections, their impact on people's quality of life is enormous. Helminths reduce the quality of life in people with low socio-economic levels and weak immune system. These diseases affect the lives of future generations negatively by preserving their ability to infection in various environments without disappearing for many years (10,15). In this study, it was determined that 16.5% of the theses prepared in the field of medical parasitology were related to helminths. Considering the high prevalence of helminths in the community and their negative widespread effects on human health, it is seen that number of studies in this area is insufficient.

In addition to causing allergic reactions as a result of sucking blood from people, the importance of arthropods in the field of medical parasitology is increasing because they transmit some fatal cause of disease to people. Many pathogenic agents such as viruses, bacteria, rickettsia, spirochaete, protozoa and helminths are transmitted to humans by arthropods feeding on blood. In

Figure 3. Distribution by provinces of the number of theses investigating the incidence of intestinal parasites (https://paintmaps.com/ was used to create the map)

Table 5. Distribution of theses by universities

Name of university	The number of theses				
	Protozoans	Helminths	Arthropoda	Intestinal parasites	Total
Ege University	32	13	4	5	54
Van Yüzüncü Yıl University	14	4	8	19	45
Erciyes University	29	5	5	0	39
Sivas Cumhuriyet University	24	6	1	1	32
İstanbul University	18	6	3	1	28
Çukurova University	22	2	0	0	24
Manisa Celal Bayar University	14	0	0	1	15
Fırat University	7	3	0	4	14
Ankara University	7	2	3	1	13
Harran University	8	2	0	3	13

order to understand the epidemiology of vector-borne diseases, it should be informed about the diseases carried by arthropods and their complex life cycles. This information is necessary for the prevention, recognition, treatment and control of vector-borne diseases with epidemic potential. The adaptability of arthropods to various environments (such as pesticide resistance) more difficult to control the diseases they transmitted (16). For this reason, scientific studies on arthropods will help us in the fighting against arthropods and the disease agents transported by arthropods. In this study, only 8.6% of the theses examined in the field of medical parasitology are related to arthropods, and this result shows that scientific studies related to arthropods in Türkiye are insufficient.

Intestinal parasitosis is among the most common infections in people living in countries with low and middle-income levels. Soil-transmitted helminths such as A. *lumbricoides* and hookworm, nematodes such as *Strongyloides stercoralis* and *T. trichiura* affect more than 2 billion people all around the world (17). While helminth infections can be asymptomatic in humans, it causes a wide variety of symptoms such as fatigue, ailment, arm and leg pain, insomnia, headache, chest pain, cough, eosinophilia, skin rashes, itchy rashes, stomach ache, nausea and vomiting, diarrhea, ileus and volvulus-invagination due to ileus, fever, itchy nose, drooling, lingual papillitis, asthma exacerbations, tetany, epileptic spasm, myoclonus, ageusia, appetite disorders, vision disorders, mental retardation, nyctophobia, anemia and weakening (18).

Parasitizing on human's intestines pathogenic intestinal protozoans like *E. histolytica, G. intestinalis*, *Cryptosporidium* spp., *Cyclospora cayetanensis* and *Cystoisospora belli* have an important place in the world and in our country. *Cryptosporidium* species are an important parasite that causes high rates of death in children under five years of age and immunocompromised patients (17). Intestinal parasitosis continues to be one of the important health

problems in Türkiye (19). Epidemiological studies have shown that there is an increase in the incidence of intestinal parasites moving from east to west in Türkiye. Studies to determine the prevalence of intestinal parasites in Türkiye are very important in the development of effective protection and control strategies to be created against parasites (12). In this study, it was determined that only 12.2% of the theses prepared in the field of medical parasitology performed to determine the prevalence of intestinal parasites. The theses were prepared in 18 provinces, especially Van (34.1%) and Ankara (13.6%). Similarly, in the remaining 63 provinces, we believe that comprehensive thesis studies on the prevalence of intestinal parasitosis should be done.

CONCLUSION

In conclusion, we believe that more importance should be given to the field of medical parasitology in Türkiye and that parasitology departments should be established in all medical faculties.

* Ethics

Ethics Committee Approval: Ethics committee approval is not required for this study.

Informed Consent: No patients were included in the study.

* Authorship Contributions

Concept: S.A., F.B., A.E., Design: S.A., F.B., A.E., Data Collection or Processing: S.A., F.B., K.K., Analysis or Interpretation: H.Y., S.A., F.B., A.E., Literature Search: F.B., S.A., K.K., Writing: H.Y., S.A., F.B., K.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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Molecular Identification of *Encephalitazoon intestinalis* and the Prevalence of Renal Microsporidiosis in Renal Transplant Recipients in Türkiye

Renal Transplant Alıcılarında Renal Microsporidiosis Prevalansı ve Encephalitazoon intestinalis Moleküler Karakterizasyonu

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ABSTRACT

Objective: In patients with end-stage kidney disease, kidney transplantation is the kidney replacement therapy option that provides the most successful survival. However, immunosuppression agents administered after kidney transplantation can increase the risk of opportunistic infections. Microsporidia are obligate intracellular pathogens that can be fatal in immunosuppressed patients. The present study aimed to determine the prevalence of microsporidia in kidney transplantation recipients and the molecular characterization of the detected species.

Methods: To evaluate the prevalence of renal microsporidiosis in kidney transplant recipients, the urine samples from a total of 325 patients were analyzed by real-time and nested polymerase chain reaction for *Encephalitozoon* spp. and *Enterocytozoon bieneusi*.

Results: Only one (0.4%) sample from the adult patient was positive for the *Encephalitozoon* species, while no positivity was found in pediatric patients. It was determined as *Encephalitozoon intestinalis* by *ITS rRNA* gene region sequence analysis. A microsporidia species obtained from humans in Türkiye has been characterized for the first time and registered in GenBank.

Conclusion: Our epidemiological results show that the prevalence of renal microsporidiosis in kidney transplant recipients is very low. In addition, as a result of the phylogenetic analysis of the detected isolate, it was observed that it was 100% identical to the isolates reported from dogs in Kayseri, Türkiye. This situation provided essential data regarding the zoonotic transmission dynamics of microsporidia.

Keywords: Renal microsporidiosis, *Encephalitozoon intestinalis*, zoonotic transmission, kidney transplantation, Türkiye

ÖZ

Amaç: Böbrek nakli, son dönem böbrek yetmezliği olan hastalarda en başarılı sağkalım sağlayan renal replasman tedavi seçeneğidir. Ancak böbrek nakli sonrasında uygulanan immün baskılayıcı ajanlar fırsatçı enfeksiyon riskini artırmaktadır. Microsporidialar, immün sistemi baskılanmış hastalarda ölümcül olabilen zorunlu hücre içi patojenlerdir. Bu çalışmada böbrek nakil hastalarında microsporidia prevalansının belirlenmesi ve tespit edilen türlerin moleküler karakterizasyonunun yapılması amaçlandı.

Yöntemler: Böbrek nakli hastalarında renal microsporidiosis prevalansını değerlendirmek için toplam 325 hastadan alınan idrar örnekleri *Encephalitozoon* spp. ve *Enterocytozoon bieneusi* açısından gerçek zamanlı ve nested polimeraz zincir reaksiyonu ile analiz edildi.

Bulgular: Erişkin hastalardan sadece biri (%0,4) *Encephalitozoon* türleri yönünden pozitif belirlendi, çocuk hastalarda ise pozitiflik saptanmadı. *ITS rRNA* gen bölgesi sekans analizi sonucunda tespit edilen türün *Encephalitozoon intestinalis* olduğu görüldü. Bu çalışma ile Türkiye'de ilk kez insanlardan izole edilen bir microsporidia türü karakterize edilerek GenBank'a kaydedildi.

Sonuç: Elde edilen epidemiyolojik sonuçlar, renal transplant hastalarında renal microsiporidiosis prevalansının çok düşük olduğunu göstermektedir. Ayrıca tespit edilen izolatın filogenetik analizi sonucunda Kayseri'de köpeklerden bildirilen izolatlarla %100 benzer olduğu görüldü. Bu çalışma microsporidiaların zoonotik bulaşma dinamikleri açısından önemli bir veri sağlamaktadır.

Anahtar Kelimeler: Renal microsporidiosis, *Encephalitozoon intestinalis*, zoonotik bulaş, böbrek nakli, Türkiye

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Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Microsporidia are spore-forming, single-celled obligate intracellular pathogens that can infect many vertebrates and invertebrate hosts. There are approximately 200 genera and over 1200 species described to date. Fourteen species of eight genera are known to cause infections in humans. *Enterocytozoon* (*E.*) *bieneusi*, *Encephalitozoon* (*E.*) *cuniculi*, *E. hellem* and *E. intestinalis* are the most important species that infect humans and have zoonotic characteristics (1-3). Although these pathogens can also be found in immunocompetent individuals, they are considered one of the most important opportunistic pathogens that cause life-threatening infections, especially in immunocompromised patients (3,4). The first target of the parasite during infection is the small intestinal enterocytes. Therefore, it causes gastrointestinal tract infections and persistent, life-threatening diarrhea and malabsorption, especially in immunocompromised patients. However, infections caused by species of *Encephalitozoon* are not limited by intestinal system diseases. They can also be found in lamina propria macrophages, fibroblasts, and endothelial cells that cause disseminated infections (3,5,6). Microsporidian pathogens are a group that cannot be detected by routine parasitological examinations and require unique methods for diagnosis (3). For this reason, it can easily be missed in routine laboratory tests.

In patients with chronic kidney disease (CKD), kidney replacement therapy requirements can onset since the glomerular filtration rate decreases below 15 mL/min/1.73 m^2 (7). Two treatment options are available at this stage; dialysis or kidney transplantation. Kidney transplantation is the preferred treatment modality due to providing more prolonged survival, a better quality of life, and cost-effectiveness (7). Although the immunosuppressive drugs in kidney transplant recipients (KTR) ensure minimizing acute rejection or chronic allograft nephropathy, they can lead to unfavorable side effects and increase the risk of opportunistic infections. Therefore, KTR should be closely monitored regarding the exceptionally infectious complications in the post-transplant period (8,9).

Because of their lifetime immune suppression, solid organ transplant recipients represent the group of patients at the highest risk for microsporidia infections. Undiagnosed kidney infections can cause proteinuria or deterioration in graft function. In addition, spores are transported from the kidney to the ureters and bladder in the urine, where they can infect the transitional epithelium and cause micro- or macro-hematuria (10). In small case series, it was shown that these pathogens infect the kidney and urinary tract (11,12). However, there is still no large-scale study evaluating the risk factors and clinical features of renal microsporidiosis in KTR.

The present study aimed to determine the prevalence of microsporidia, which can be easily missed in routine follow-ups, in KTR and the molecular characterization of the detected species. It also aimed to evaluate the clinical findings of positive cases to reveal possible conditions that the parasite may cause.

METHODS

Ethics Statement

This study has been approved by the Ethics Committee of the Faculty of Medicine Erciyes University Türkiye (application deadline: 03.03.2021, no: 2021/172). Written informed consent was obtained from every patient before the examination.

Patients and DNA Extractions

Between May and December 2021, urine samples were collected from 325 KTR, 276 (84.9%) adults, and 49 (15.1%) children in the kidney transplantation outpatient clinic. Thirty-four (69.4%) of the pediatric patients were male, 15 (30.6%) were female, and their mean age was 11.7 (±4.64). Of the adult patients, 165 (59.5%) were male, 111 (40.5%) were female, and the mean age was 43.7 (±13.04). The characteristics and symptoms of patients included in the study are shown in Table 1.

When the patients applied to the hospital for their routine follow-up, approximately 50 mL of urine samples were taken and centrifuged at 4000 rpm for 10 minutes. The supernatant was discarded, while the pellet at the bottom was washed twice with 1 mL of sterile PBS following the same centrifugation conditions. After washing, the supernatant was removed, and the pellet was used for DNA isolation. DNA was isolated using the GeneAll® Exgene Cell SV Mini Kit (GeneAll Biotechnology, Seoul, South Korea) according to the manufacturer's recommendation. The DNA isolation was performed with a negative control and a positive control containing ATCC 50506 *E. intestinalis* spores. Acquired DNA was stored at -20 °C.

Molecular Methods

Real-time polymerase chain reaction (PCR) and Nested PCR analyses were performed on all samples. For real-time PCR, primers MsRTf1 and MsRTr1 amplifying the 258-319 bp region of SSU-rRNA were used as previously reported by Polley et al. (13). Amplifications were performed in 20 µL of total volume, containing 8 µL of distilled water, 0.5 µL of both reverse and forward primers (20 µM), 10 µL 2x SYBR-Green Master Mix (Roche Diagnostic, Germany) and 1 µL of genomic DNA. The thermal profile for real-time PCR amplification consists of the following steps: Initial incubation at 98 °C for 3 min., 45 cycles of 98 °C for 15 s., 55 °C for 20 s., and 72 °C for 10 s. followed by 0.5 °C increase in every 2nd between 65-95 °C for melting curve analysis.

The nested PCR protocol reported by Katzwinkel-Wladarsch et al. (14) and Buckholt et al. (15) amplifying the ITS region of *E. bieneusi* and *Encephalitozoon* spp. using microsporidia-specific primers was performed. The tubes were placed in the thermal cycler. The thermal profile was for the first PCR steps at 95 °C for 4 min, 35 cycles at 95 °C for 30 seconds, at 40 °C (*Encephalitozoon* species) at 47 °C (*E. bieneusi*) for 30 seconds, and at 72 °C for 1 minute; it was then set at 72 °C for 10 minutes. The second PCR steps are the same as the first, and amplification will is performed only by setting the annealing degree to 46 °C for the *Encephalitozoon* species and 57 °C for *E. bieneusi* (14,15).

DNA Sequencing and Phylogenetic Analysis

ITS gene regions were sequenced on an Applied Biosystems (ABI) 3500 Sanger sequencing platform using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, MA, USA). Geneious11.0.2 software was used to analyze the sequence reads (16). Sequences were aligned with previously published sequences using the BLASTn. Multiple sequence alignments were performed using the MAFFT server. The DNA sequence of the variant reported in this study was submitted to GenBank with the accession number ON182064. The phylogenetic tree was generated using the ultrafast bootstrap (UFBoot) IQ-TREE web server (17). According to BIC (Bayesian Information Criterion), the most suitable model was F81+F+G4. A bootstrap

Table 1. Demographic, clinical and laboratory parameters of kidney transplantation recipients involved in the study

	Adults (n=276)	Children (n=49)
Age, years, average (range)	43.7 (19-80)	11.7 (2-18)
Sex		
Male	165 (59.5%)	34 (69.4%)
Female	111 (40.5%)	15 (30.6%)
Allograft survival, months, average (range)	84.5 (2-336)	42.6 (4-156)
Etiology of CKD		
Hypertension	105 (38.1%)	-
Diabetes mellitus	26 (9.4%)	-
Primary glomerular disease	31 (11.2%)	11 (22.5%)
Polycystic kidney disease	6 (2.2%)	3 (6.1%)
Nephrolithiasis	10 (3.6%)	1 (2%)
Congenital anomalies of urinary tract	30 (10.9%)	21 (42.9%)
Others	28 (10.1%)	7 (14.3%)
Unknown	40 (14.5%)	6 (12.2)
Dialysis history		
HD	149 (54%)	6 (12.2%)
PD	44 (15.9%)	19 (38.8%)
HD+PD	24 (8.7%)	1 (2%)
Transplantation preempitive	59 (21.4%)	23 (47%)
Comorbidity		
Yes	94 (34.1%)	10 (20.4%)
No	182 (65.9%)	39 (79.6%)
Donor		
Living	238 (86.2%)	42 (85.7%)
Deceased	38 (13.8%)	7 (14.3%)
Immunosuppressive regimen		
CNI+MMF+steroid	247 (89.5%)	33 (67.3%)
CNI+mTORi+steroid	10 (3.6%)	8 (16.3%)
MMF+mTORi+steroid	3 (1.1%)	1 (2%)
CNI+steroid	16 (5.8%)	1 (2%)
CNI+MMF	-	4 (8.2%)
MMF+mTORi	-	2 (4.1%)
Laboratory parameters		
Proteinuria	30 (10.9%)	3 (6.1%)
Hematuria	36 (13%)	3 (6.1%)
Hematuria+proteinuria	20 (7.2%)	1 (2%)
BUN (average, range, mg/dL)	19.6 (7-39.7)	18.6 (5.5-58.5)
Creatinine (average, range, mg/dL)	1.3 (0.6-7.3)	0.9 (0.28-1.84)
Hemoglobin (average, range g/dL)	13.1 (7.2-17.3)	11.93 (8.2-16.7)
Leukocyte (average, range, per µL)	8182 (1390-14000)	8513 (3950-20030)
Lymphocyte (average, range, per µL)	2050 (130-13000)	3674 (660-28400)

CKD: Chronic kidney disease, CNI: Calcineurin inhibitors, MMF: Mycophenolate mofetil, mTORi: Mammalian Target of Rapamycin inhibitors, BUN: Blood urea nitrogen, HD: Hemodialysis, PD: Peritoneal dialysis

test with 1000 repetitions was used to determine the reliability of the trees created by maximum likelihood (ML) analysis.

Statistical Analysis

Since the number of positive patients was only one, statistical analysis could not be performed according to patient characteristics and clinical findings.

RESULTS

According to the results of SYBR green real-time PCR analysis of urine samples, all 49 pediatric patients were negative for microsporidian pathogens. Only one (0.4%) of 276 adult patients was found positive. Also, according to the ITS rRNA PCR analysis result, all the pediatric patients were negative. Amplicons of

approximately 300 bp were detected in an adult patient (real-time PCR positive). The sequence analyses of the PCR product showed 100% identity to *E. intestinalis*. In this study, no *E. hellem*, *E. bieneusi* or *E. cuniculi* infection was found. A 40-year-old female patient with a positive PCR test had developed renal failure after Lupus Nephritis. Peritoneal dialysis was first applied for approximately four years as renal replacement therapy, and then a kidney was transplanted from a living donor eight years ago. She did not experience any rejection attacks in her follow-up. She still has a functional allograft and was on tacrolimus, mycophenolate mofetil, and prednisolone at the time of urine sampling for the study. No additional leukopenia was detected in the hemogram examination. In addition, there is no known further disease other than hypertension.

The DNA sequence of the variant reported in this study was submitted to GenBank with accession number ON182064. In our study, phylogenetic analysis was performed, including our molecularly characterized as an *E. intestinalis* genotype and isolates of various genotypes reported from multiple regions worldwide, as well as some genotypes isolated from animals in Türkiye. Figure 1 shows the results of the phylogenetic analysis of the ITS sequence of *E. intestinalis* isolate from the recovered renal transplant recipients in Kayseri, Türkiye. Multiple alignments of nucleotide sequences of the same isolate are shown in Figure 2. In our study, it was determined that the isolate belonging to the genotype named ERU-RT-Eint, which was first identified from humans in Türkiye, was closely related (99.6-100%) to the isolates of the genotype "ERU-Eint1-3" reported from Kayseri and "DP-3-Samsun" reported from Samsun.

DISCUSSION

In the present study, the prevalence of renal microsporidiosis in KTR was very low. Considering the genetic characteristics of the microorganism, it suggests a zoonotic infection with dogs as a possible source.

It has been known for many years that microsporidia can infect many vertebrates and invertebrates. It can cause severe complications by causing opportunistic infections in HIV-infected and immunocompromised patients for other reasons, such as children, tourists, contact lens wearers, and the elderly (3,4). However, in the last 30 years, it has gained significant importance with reporting many AIDS-related cases and detection in 70% of HIV-infected patients.

Since the first successful human kidney transplant was performed in 1954, solid organ transplantation has increased worldwide. In patients with CKD stage 5, kidney transplantation is the best treatment option for long-term survival. Using lifelong immunosuppressive treatment regimens in the postoperative period has become more potent. Although graft survival has increased after these successful treatment regimens, infections have become an obstacle to disease-free survival, especially in the post-transplant period. In particular, an increase in the incidence and spectrum of opportunistic infections has been observed. Many species of viruses, bacteria, and parasites are among the causative pathogens. Prophylactic treatments are essential for transplantation since these pathogens must be recognized beforehand (8,18).

Some prospective and retrospective studies worldwide have reported microsporidian infections in transplant recipients. Liguory et al. (19) detected *E. bieneusi* in eight transplant patients, and six of these patients were KTR. Similarly, Rabodonirina et al. (20) detected *E. bieneusi* in 23 organ transplant recipients, five of whom were KTR, and Bednarska et al. (21) in 17% of immunocompromised patients, Ghoshal et al. (22) reported that they detected microsporidian pathogens in 5.8% of 272 KTR. In a study conducted in Türkiye, Çetinkaya et al. (23) reported that they detected positivity in 39% of bone marrow transplant patients. The prevalence of intestinal microsporidiosis was investigated in these studies, but the presence of disseminated infections or renal involvement was not investigated.

There are several case reports evaluating renal microsporidiosis, and the common symptoms in these cases seem to be fever and an increase in serum creatinine levels (8,12,24-27). Kicia et al. (11) evaluated renal microsporidiosis in 86 KTR and reported that microsporidian pathogens were detected in 25.5% of the patients.

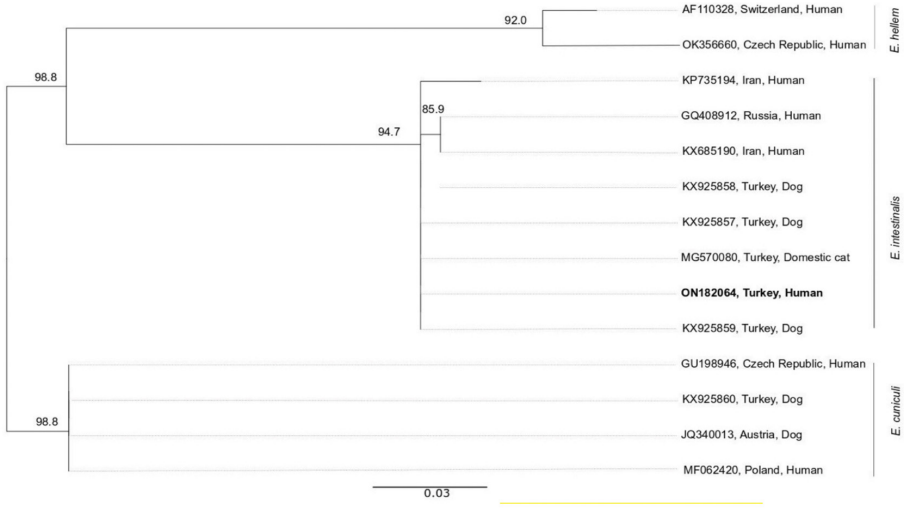

Figure 1. Phylogenetic relationship of ITS sequences of *Encephalitozoon intestinalis* isolate (ON182064, Türkiye, Human) identified from humans in this study and other isolates from people in various regions worldwide and some animals in Türkiye previously deposited in GenBank. Accession number, host, country, and species identified the isolates

They also emphasized that the prevalence is higher in urine samples and that fever and diarrhea are frequently seen in positive patients. In our study, all the renal transplant recipients who applied to the outpatient clinic for routine follow-up, regardless of whether or not they had any clinical complaints, were included in the study, and only urine samples were collected. If we had formed the cohort over the symptomatic patient group instead of randomized selection, our probability of detecting pathogens would have been higher. The clinical findings most associated with microsporidia infections are fever and diarrhea. Kicia et al. (11) reported that 81.8% of positive KTR had a fever, and 59.1% had diarrhea. In this study, only one adult patient had fever and diarrhea (0.3%), while two of the pediatric patients had a fever (0.6%), and two (0.6%) had diarrhea. However, these five patients were negative for microsporidian pathogens. *E. intestinalis* was also detected in a 40-year-old female patient who underwent kidney tranplantation due to Lupus Nephritis.

It is known that spore excretion from infected individuals is not regular and examining only one sample will not reflect the true prevalence. The probability of detecting microsporidia increases with the number of samples tested (28,29). Since the patients who came for their routine follow-ups were included in our study, we did not have the chance to obtain a second sample from many of them. This is thought to be one of the reasons for the low prevalence obtained in our study.

The most detected species in studies with different patient groups on humans are *E. bieneusi, E. intestinalis*, *E. hellem* and *E. cuniculi*. Among these species, *E. bieneusi* and *E. intestinalis* are most commonly associated with intestinal tract infections. *E. intestinalis* is the most frequently detected species in our country. In urinary tract infections, Kicia et al. (11) found that 59% (13/22) of microsporidia-positive patients only had *E. cuniculi* (genotype II), and 23% (5/22) had *E. bieneusi* (genotype D). They also reported that 18% (4/22) were infected with both species simultaneously. They emphasized that they did not detect *E. hellem* and *E. intestinalis* infection in the study (11). Hernández-Rodríguez et al. reported that four (57%) of seven patients with renal involvement had *E. cuniculi*. In other patients, it was reported that the species could not be identified, but it was the genus Encephalitozoon (26). Our study determined that the species isolated from the patient, which was positive due to sequence analysis, was *E. intestinalis*.

Much epidemiological information, such as hosts, life cycle, host specificity, and transmission routes of microsporidia, remains unclear. Recently, the increasing use of molecular methods in diagnosis, especially the sequence analysis of the *ITS* gene region, has contributed to obtaining more information about the genetic diversity, transmission routes, and sources of microsporidia (30,31). It has been reported that four microsporidia species that frequently cause human infection are zoonotic. Various domestic and wild animals can act as reservoir hosts in transmission (31,32). Recent molecular studies of *E. intestinalis* from humans and animals have thus far shown no or very low levels of intraspecific variation in the *ITS* gene. Therefore, it is thought that there is no barrier of transmission between species defined as hosts for *E. intestinalis* (19,33). The isolate we identified as a result of sequence and phylogenetic analysis in our study was genetically close to the isolates reported in dogs and cats in Türkiye.

CONCLUSION

Our results provide current epidemiological data on the prevalence of renal microsporidiosis in pediatric and adult renal transplant recipients. In this study, all KTR who applied to the outpatient clinic for routine follow-up were included, regardless of whether they had any clinical complaints. This study provides the first

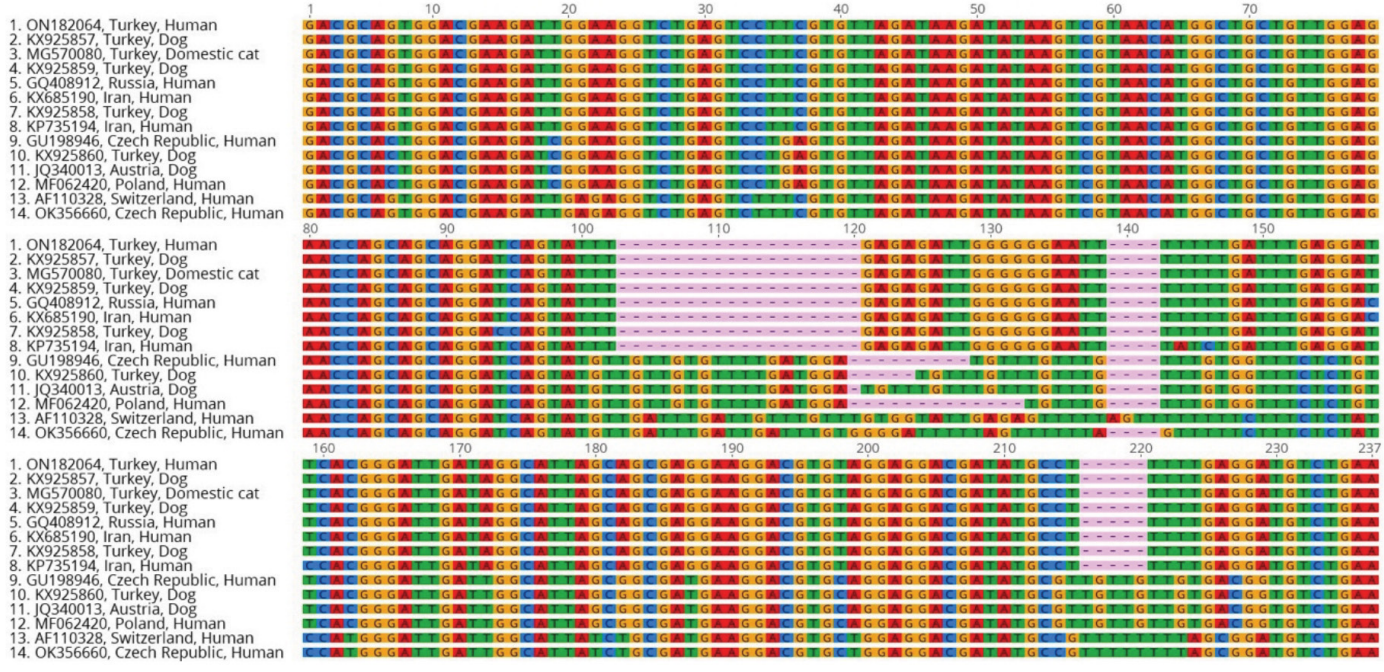

Figure 2. Multiple alignments of ITS sequences of *Encephalitozoon intestinalis* isolate (ON182064, Türkiye, Human) identified from humans in this study and other isolates from people in various regions worldwide and some animals in Türkiye previously deposited in GenBank

genetic data on *E. intestinalis* in humans in GenBank in Türkiye. However, the absence of any clinical complaints in our patients represents the a significant weakness of this study. The genetic similarity between the isolate that we obtained, and the other isolates reported from animals in our region will contribute to developing preventive strategies against microsporidia infections regarding zoonotic transmission.

* Ethics

Ethics Committee Approval: This study has been approved by the Ethics Committee of the Faculty of Medicine Erciyes University Türkiye (application deadline: 03.03.2021, no: 2021/172).

Informed Consent: Written informed consent was obtained from every patient before the examination.

* Authorship Contributions

Concept: Ü.Ç., M.G.Ö., Design: Ü.Ç., M.G.Ö., Data Collection or Processing: Ü.Ç., M.G.Ö., C.U., S.Y., M.B., İ.D., M.H.S., Analysis or Interpretation: Ü.Ç., M.G.Ö., M.B., İ.D., M.H.S., Literature Search: Ü.Ç., M.G.Ö., Writing: Ü.Ç., M.G.Ö., C.U.

Conflict of Interest: No conflict of interest was declared by the authors.

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The First Case of *Menacanthus pallidulus* (Neumann, 1912) (Phthiraptera: Amblycera: Menoponidae) on A Chicken (*Gallus gallus domesticus* Linnaeus, 1758) in Türkiye

Türkiye'de Bir Tavukta (Gallus gallus domesticus Linnaeus, 1758) İlk Menacanthus pallidulus (Neumann, 1912) (Phthiraptera: Amblycera: Menoponidae) Olgusu

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ABSTRACT

This case report was prepared to provide information about *Menacanthus pallidulus* (Neumann, 1912), which was detected for the first time on a domestic chicken in Hatay province of Türkiye. Louse specimens collected from a chicken by a student were brought to Hatay Mustafa Kemal University Faculty of Veterinary Medicine, Department of Parasitology, and sent to Selçuk University Faculty of Veterinary Medicine, Department of Parasitology, for identification of species and microscopic examination revealed the presence of *Menacanthus pallidulus* (Neumann, 1912). Thus, with this study, the presence of *M. pallidulus* on domestic chickens was recorded for the first time in Türkiye.
Keywords: Louse, *Menacanthus pallidulus*, domestic chicken, Türkiye

ÖZ

Bu olgu sunumu, Türkiye'nin Hatay ilinde, evcil bir tavukta ilk kez tespit edilen *Menacanthus pallidulus* (Neumann, 1912) hakkında bilgi vermek için hazırlanmıştır. Bir öğrenci tarafından tavuktan toplanan bit örnekleri Hatay Mustafa Kemal Üniversitesi Veteriner Fakültesi, Parazitoloji Anabilim Dalı'na getirilmiş, tür teşhisi için Selçuk Üniversitesi Veteriner Fakültesi, Parazitoloji Anabilim Dalı'na gönderilen örneklerin mikroskobik inceleme sonucu *Menacanthus pallidulus* (Neumann, 1912) olduğu anlaşılmıştır. Böylece bu çalışma ile evcil tavukta saptanan *M. pallidulus*'un, Türkiye'deki varlığı ilk kez kaydedilmiştir.
Anahtar kelimeler: Bit, *Menacanthus pallidulus*, evcil tavuk, Türkiye

INTRODUCTION

Chewing lice are an important problem for both commercial and small-scale poultry breeders among ectoparasite infestations due to their host specificity, frequent infestations, feeding on feathers and fleece, causing itching, restlessness, weakness, decrease in feed consumption and egg production and underestimated economic loss (1,2).

Menacanthus species are found around the chest, thighs and cloaca of birds, especially poultry (1). *Menacanthus* species, which are mostly common on birds in the order Passeriformes (songbirds), are also seen in poultry in the order Galliformes (landfowl), such as *Gallus gallus* (red junglefowl), *Gallus sonneratii* (gray junglefowl), *Bambusicola thoracica* (chinese bamboo partridge) (3).

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Türkiye PARAZİTOLOJİ Dergisi

Menacanthus cornutus (Schömmer, 1913) and *Menacanthus stramineus* (Nitzsch, 1818) belonging to the genus *Menacanthus* in the family Menoponidae and *Menopon gallinae* (Linnaeus, 1758) in the genus *Menopon* were detected on chickens in studies conducted in Türkiye, but there is no record of *Menacanthus pallidulus* (4). *Menacanthus pallidulus* was first described as *Menopon (Menacanthus) pallidulum* by Neumann (1912), and later this species was accepted as *M. pallidulus* (3,5).

In this report, morphological information will be given about the male and female of *M. pallidulus* found on a domestic chicken in Hatay province.

CASE REPORT

The louse specimens that constitute the material of this study were obtained as a result of the homework given to the students of Hatay Mustafa Kemal University Faculty of Veterinary Medicine. Five louse samples (4♀ 1♂) collected from the chicken were preserved in a tube containing 70% alcohol and sent to Selçuk University Faculty of Veterinary Medicine, Department of Parasitology. The samples were cleared in 10% KOH for 24 hours, then fixed on the slides with Canada balsam. The lice examined under the microscope were identified as *M. pallidulus* according to the relevant literatures (3,6,7).

Morphological Characteristics

Female-the head is triangular and narrowed anteriorly. The width of the head is approximately one and a half times its length. The anterior part is circular, smooth and rounded, the antennae are long and located in the antenna groove. The hooks are quite long and extend to the posterior end of the hypopharyngeal sclerite. The head is very enlarged in the temporal region. Temporal setae are much longer than ocular setae. The hypopharynx is strongly sclerotized, the ocular and occipital nodes are not prominent. The gular plate is distinct, narrowed from anterior to posterior, with four setae on each side. The thorax is longer than the head. The prothorax narrows to connect to the head and pronotum is surrounded by a total of 12 setae on the each side. There are four bristles on the edges of the metathorax, and the lower edge is surrounded by long hairs. On the edges of the femurs there are bristles and a small number of spiny setae on the ventral side of the third pair of legs. Abdomen is oval, elongated, well developed, with large and complete tergites (1-6 segments). Both of the males and females, tergites and sternites have a single row of setae on each abdominal segment and a spine and a bristle at the angles of each segment. There is a long spiny bristle on each side of the genital opening (Figure 1a). Some measurements of male and the female of *M. pallidulus* found on domestic chicken were made (Table 1).

Male-the male is similar to the female (Figure 1b). The prothorax, pterothorax and abdomen are narrower than in the female. Male genital sclerites are short, basal apodem is well hardened, parameres are well developed, externally curved and slender. The penis is short, the posterior margin of the endomeres is shorter than the posterior end of the paramers. The endomers become rounded and narrow towards the tip. Terminal segment is covered with a comb of short marginal hairs (Figure 1c).

DISCUSSION

Due to the economic importance of the domestic chicken *G. gallus domesticus* (Linnaeus, 1758), numerous studies have been conducted on its infectious and parasitic diseases. As a result, more is known about the lice fauna than other bird species, and a total of 22 species of chewing lice have been identified on domestic chickens worldwide (8). *M. gallinae* (Linneaus, 1758), *M. cornutus* (Schommer, 1913), *M. stramineus* (Nitzsch, 1818), *Goniocotes gallinae* (De Geer, 1778), *Goniodes dissimilis* Denny, 1842, *G. gigas* (Taschenberg, 1879), *Cuclotogaster heterographus*

Table 1. Measurements (mm) of *Menacanthus pallidulus* found on domestic chicken

Body parts	Male (n=1)	Female (n=4)
Preocular width	0.37	0.38-0.39
Head length	0.32	0.35-0.36
Head (temple) width	0.50	0.52-0.58
Head index	0.64	0.62-0.67
Prothorax width	0.35	0.43-0.46
Pterothorax width	0.44	0.57-0.61
Thorax length	0.42	0.42-0.46
Abdomen length	0.74	1.10-1.16
Abdomen width	0.55	0.81-0.82
Total length	1.53	1.92-1.97

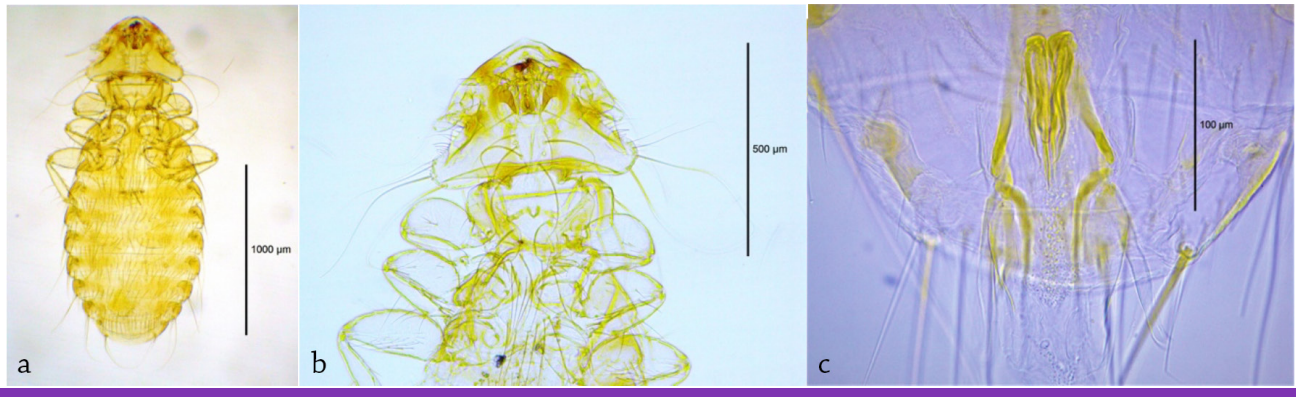

Figure 1. *Menacanthus pallidulus*, **a)** *M. pallidulus* female, **b)** Head and thorax of male *M. pallidulus*, **c)** Male genitalia of *M. pallidulus*

Nitzsch, 1866 and *Lipeurus caponis* Linne, 1758 have been recorded from domestic chickens in Türkiye (4). Besides these species, another Mallophaga species *M. pallidulus*, which has been found on domestic chickens in different countries of the world (2,9-11), has not been reported from Türkiye so far. In this study, 4♀, 1♂ of *M. pallidulus* was detected on a domestic chicken (*Gallus g. domesticus*).

Emerson (7) stated that, *M. pallidulus* has often been misidentified as the immature forms of *M. stramineus* for the reason that two are very similar. *M. stramineus* is larger than other menoponid lice found on chickens and the male genitalia are typical. There is one row of transversal setae on each of the abdominal tergites (3rd and 4th) of the male and female of *M. pallidulus,* and two rows in *M. stramineus*. It is also distinguished from *M. cornutus* by the difference in tergal setae and male genitalia. Moreover *M. gallinae* can be easily differentiated from *M. pallidulus* by the absence of spines on the ventral side of the head and terminates posteriorly in a pointed shape of of the abdomen in the female (7,12).

Menacanthus pallidulus is small size, dark yellow menoponid with a distinct gap between the mandibles and the frontal margin. The ventral spine is long and thick, and the hairs on gular region and the head index with a specific value distinguishing it from other related species on *Gallus g. domesticus*. Posterior femur as in *M. stramineus* but without extra hairs, only a prominent hair is present. Dorsum is covered with one row of setae and ventrum with two rows. The last abdominal segment is rounded and fringed with small hairs and the male genitalia has a very distinctive appearance (6,13). Similar features were observed in the samples obtained in this study.

In previous studies (6,10,14,15), the head length and width of male *M. pallidulus* specimens (0.29-0.31x0.49 mm) are close to the measurements in this study (0.32x0.50 mm). On the other hand, the abdominal length and width (0.91-1.05x0.73 mm) reported in the studies (6,10,14,15) are larger than our measurements (0.74x0.55 mm). The total length of male louse in this study was 0,53 mm which is consistent with previous studies (10,14,15) and smaller than the measurements (1.70-1.84 mm) reported in some studies (6,12).

According to the measurements obtained in previous studies (6,13), the head length and width of female *M. pallidulus* specimens (0.22-0.29x0.45-0.54 mm) were smaller than the measurements in the current study (0.35-0.36x0.52-0.58 mm). The abdominal length and width (0.94x0.54-0.59 mm) reported in the literature (6,13) are much smaller than our measurements (1.10-1.16x0.81-0.82 mm). The total length of female lice in this study (1.92-1.97 mm) was found to be much larger than the measurements (1.46-1.60 mm) in the other studies (6,12,13).

CONCLUSION

Consequently, this is the first report of *M. pallidulus* on domestic chickens in Türkiye. We provide some figures and information about morphological characteristics of *M. pallidulus* in the present study. Poultry breeders need to develop awareness for chewing lice to improve poultry conditions. Further studies are needed to determine the prevalence of this species on domestic chickens and other poultry in Türkiye.

* Ethics

Informed Consent: The louse specimens that constitute the material of this study were obtained as a result of the homework given to the students of Hatay Mustafa Kemal University Faculty of Veterinary Medicine.

* Authorship Contributions

Concept: İ.E., A.Z., B.D., M.Y., Design: F.N.Ş., İ.E., A.Z., B.D., M.Y., Data Collection or Processing: İ.E., A.Z., B.D., Analysis or Interpretation: F.N.Ş., B.D., M.Y., Literature Search: F.N.Ş., B.D., Writing: F.N.Ş.

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Can Parasites be Useful?

Parazitler Yararlı Olabilir mi?

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ABSTRACT

Parasites are commonly associated with harm, but they also have beneficial aspects that are still being discovered. It is important to acknowledge both the harmful and beneficial aspects of parasites. They have been found to have positive effects on non-healing wounds, surgical wounds, obesity, glucose metabolism disorders, nerve repair, cancer treatments, and fertility. Research has shown that helminths, protozoa, and arthropods have the ability to correct, prevent, and cure certain disorders through the use of the parasite itself, its molecules, or even its eggs. This article includes studies on the beneficial aspects of parasites. However, further research is needed to fully understand the mechanisms by which parasites stimulate or affect the immune system and how they can be used therapeutically.
Keywords: Benefits of parasites, maggot therapy, hirudo therapy, parasitomimetics, ascarosides

ÖZ

Parazitler genellikle zararlı yönleri ile bilinen canlılardır. Parazitlerin zararlarının yanında yararlı yönleri de keşfedilmiş ve halen günümüzde keşfedilecek birçok yararlı yönü bulunmaktadır. İyileşmeyen yaralarda, cerrahi operasyon sonrası yaralarda, obezite ve glikoz metabolizması bozukluklarında, sinir onarımında, kanser tedavilerinde hatta doğurganlık üzerine yararlı etkileri olduğu bildirilmiştir. Helmintlerin, protozoonların ve artropodların, parazitin kendisi ya da salgıladığı molekülleri hatta yumurtaları ile belirli bozuklukları düzeltebildiği, engelleyebildiği ve iyileştirebildiği kanıtlanmıştır. Bu yazıda parazitlerin yararlı yönleri ile alakalı çalışmalara yer verilmiştir. Parazitlerin birçoğunun bağışıklık sistemini uyarması ya da etkilemesi ile yarar sağladığı mekanizmaları anlamak ve terapötik amaçla kullanılabilmesini sağlamak için çok daha fazla çalışmaya ihtiyaç vardır.
Anahtar Kelimeler: Parazitlerin faydaları, maggot terapi, hirudo terapi, parazitomimetikler, askarozitler

INTRODUCTION

The term “Parasite” is derived from the Latin words “para” (beside) and “cytos” (food). It refers to a living being that lives off another organism, known as the host. Parasitism is the phenomenon of a living organism living on or in another living organism, temporarily or permanently, to its detriment (1).

However, recent studies have shown that parasites can have both positive and negative effects on their hosts. Research has demonstrated that parasites, including their eggs, developmental forms, and molecules, can provide benefits by affecting certain systems or triggering various mechanisms in humans and animals (2).

While the immune system can expel microorganisms that invade the body, parasites must evolve to maintain their symbiotic relationship with their hosts. Parasites can manipulate the host’s immune system by producing immunomodulatory molecules. They can control specific host immunity and affect the entire immune system (2).

As the host develops its immune system against the parasite, the parasite develops mechanisms to evade immunity. The regulation of host immunity by parasites has been a long-standing research topic. The positive effects of helminths, protozoa, and arthropods have been discussed based on species and grouped according to treatment methods with successful results (2,3).

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Türkiye PARAZİTOLOJİ Dergisi

Maggot Therapy

Myiasis, which originates from the Latin word "Myia" fly, is the name given to the disease caused by the feeding some fly larvae on the tissues of humans and animals (4).

Myiasis is a prevalent disease worldwide. The adult flies responsible for this disease are usually found in Türkiye from April to September. Myiasis is more prevalent during the summer months in areas where sheep and goats are raised (4).

Although myiasis larvae can have harmful effects, they are sometimes used in forensic medicine and wound treatment. The use of live fly larvae to treat wounds is becoming increasingly popular worldwide. These larvae develop in environments where decay and putrefaction occur, and they can be necessary in cases where wound healing is crucial due to microorganisms that are resistant to antibiotics. The treatment is known by several names, such as larval therapy, maggot therapy, biosurgery, and maggot debridement therapy (MDT) (5).

Maggot therapy is a controlled myiasis in open wounds to utilise the positive effects of larvae on necrotic tissue without damaging the intact tissue (6).

Fly larvae suitable for maggot therapy are typically found in the Calliphoridae family (6). The most commonly used species is *Lucilia sericata* (syn. *Phaenicia sericata*). *Lucilia sericata* is preferred due to its ability to feed on dead tissue on the surface of living tissue (7,8).

Cage dressings are the preferred method for maggot therapy (9). Another method, Biobag, has also been used in Maggot therapy applications (10,11).

Maggot therapy is commonly used to treat infected wounds that are unresponsive to treatment. It can also help heal ulcers caused by various factors such as pressure, venous stagnation, nerve disease, surgical operations, trauma, burns, cellulitis, bone marrow inflammation, mastoiditis, thalassemia, polycythemia, Burger disease, necrotic tumors, and crusted or incompletely healed wounds (12).

MDT has found a new role in treating diseases caused by many Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus*, *Pseudomonas aeruginos*a, and methicillin-resistant *Staphylococcus*, due to the increasing incidence of drug resistance in recent years (11).

Kerridge et al. (13) incubated 100 sterile *L. sericata* larvae in 10 mL of bidistilled water overnight. The liquid was collected, centrifuged, filtered, and lyophilised. The resulting secretion material was dried, frozen, and resuspended in bidistilled water. The authors reported that this material exhibited antibacterial effects against MRSA (Methicillin Resistant *Staphylococcus aureus*), *Streptococcus pyogenes*, and *Pseudomonas aeruginosa* under *in vitro* conditions.

Jaklič et al. (14) demonstrated that *in vitro* conditions, larval secretions of *L. sericata* possess antibacterial activity against *S. aureus*, *E. coli*, and *P. aeruginosa*.

Harris et al. (15) discovered that larval secretions containing the enzyme chymotrypsin can inhibits *Staphylococcus epidermidis* biofilm. Additionally, maggot secretions can break down established biofilm and prevent biofilm its formation on both abiotic surfaces such as polyethylene, stainless steel, and titanium, as well as on biotic surfaces.

Bohova et al. (16) conducted a study which found that worm secretions effectively reduced biofilm formation of *Enterobacter cloacae* and *S. aureus*, but not against *Proteus mirabilis*.

Clinical studies conducted in a laboratory have shown that larval secretions promote the migration of fibroblasts and keratinocytes, increase angiogenesis and enhance the migration of vascular endothelial cells (11).

Upon analysis the effect of maggot debridement treatment on antibiotic use, it was found that MDT has a synergistic impact on several antibiotics, including gentamicin, flucloxacillin and daptomycin at high concentrations, rather than inhibiting their antibacterial activity. Furthermore, an enhanced effect was observed when larval wastes and secretions were combined with ciprofloxacin (11).

The human complement system plays a crucial role in activating the inflammatory response to injury. However, inappropriate activation can cause severe tissue damage, as observed in chronic wounds that remain in the inflammatory healing phase. Studies have shown that larval secretions can inhibit pro-inflammatory responses in human neutrophils and monocytes without affecting the antimicrobial activities of phagocytes. The study found that inhibiting complement pathways, cytokines, and degrading complement proteins reduced all complement by up to 99.9% (17,18).

Hirudo (Leech) Treatment

"Leech" is derived from the Anglo-Saxon word "laece", which means doctor. The use of *Hirudo medicinalis* in treatments was described by Linnaeus in 1758 (19).

The medicinal leech *Hirudo medicinalis*, has been approved by the United States Food and Drug Administration as a prescription therapeutic material due to the many protease inhibitors found in its saliva. Molecular studies have revealed that there are at least three species of European medicinal leeches and leeches marketed as *H. medicinalis* are actually *Hirudo verbana*. These findings have prompted a review of decades of biomedical research on the organism and a reassessment of regulatory statutes, regulations, and protective measures (20).

Leeches have been used extensively worldwide in recent years. They have been particularly successful in plastic and reconstructive microsurgery in the UK and Ireland, where they are used to alleviate venous occlusion following flap surgery (21,22).

The Impact of *Trichuris* spp. Eggs on Autoimmune Diseases

Clinical trials have investigated the treatment of allergic rhinitis, multiple sclerosis (MS), ulcerative colitis, Crohn's disease, and inflammatory bowel disease through oral ingestion of the swine nematode *Trichuris suis* parasite eggs. These studies have identified beneficial aspects (23).

In 2010, a study was conducted in Finland to investigate the effectiveness of *T. suis* eggs in treating allergic rhinitis. Experimental studies have shown that they protect against allergic airway inflammation. In some observational studies, they have been associated with a reduced risk of atopy and alleviation of asthma symptoms. Treating allergic rhinitis with *Trichuris suis* eggs results in a significant clinical and systemic immunological response in humans. However, no therapeutic effect has been reported (23).

MS is more common in developed countries, but its prevalence is lower in areas where *Trichuris trichiura* carriage rates exceed 10%. Some studies suggest that this may be due to increased helminth exposure, which could have a protective effect against MS. Furthermore, research has shown that MS patients with helminth infections tend to have a milder disease activity and course than those without (24).

Ulcerative colitis is more common in developed Western countries but less prevalent in developing countries where helminth infections are more frequent. Individuals with helminth infections show a modified immunological response to antigens. In animal experiments, helminths have been shown to prevent or alleviate ulcerative colitis by stimulating regulatory T-cells and modulatory cytokines (25).

Helminths have been suggested to have potential benefits in reducing hyperreactive immune responses. This theory is supported by experimental findings that helminth eggs and live intestinal helminths protect against inflammatory bowel disease in mice and can even cure ongoing disease (25).

Similar to other inflammatory bowel diseases, Crohn's disease is less prevalent in less developed countries but more prevalent in developed countries. Exposure to helminths has been linked to a reduced immune response, which may help prevent or treat Crohn's disease (26).

Summers et al. (26) conducted a study on 29 patients with Crohn's disease who were orally administered 2500 live *T. suis* eggs every three weeks for 24 weeks. The study reported a success rate of approximately 80% at the end of 24 weeks. The treatment was found to be safe even for patients receiving multiple immunosuppressants, such as corticosteroids and azathioprine, without causing any side effects or complications.

The Benefical Effects of *Trichinella* spp.

Trichinella spiralis is a parasite that can survive in both adult and larval forms within two different cell environments in the same host. This immunomodulation ensures the survival of both the host and the parasite. The immune response is modulated by various cells from both the innate and adaptive divisions of immunity, including dendritic cells, T regulatory cells, and alternatively activated macrophages. The parasite can evade the immune system through several processes. Researchers are investigating molecules derived from parasites, including *Trichinella*, for their potential to treat immunopathology in animal models of various inflammatory and autoimmune human diseases. This includes components of their excretory-secretory products (27).

During trichinellosis, the immune system is stimulated by various molecules produced by the parasite. These molecules can be found in the cuticle or excretory-secretory products. The interaction between these molecules and the host's immune system can cause a shift in the immune response from an inflammatory to an anti-inflammatory type (28).

Trichinella-derived molecules have been shown to modify antigen-presenting cells, such as dendritic cells, and reduce adaptive immune responses (29).

Investigating how helminth parasites, such as *Trichinella*, modulate the immune system provides insights into the molecules involved in different parts, elements, and interactions within the immune system. These insights may be translated into future therapeutics. Currently, *Trichinella*-derived molecules, particularly excretory and secretory products, are the focus of intensive research aimed at discovering molecules to re-regulate the immune system in various immune dysregulations (28).

While the mechanism by which helminths treat osteoclastic bone destruction is not fully understood, helminths and helminth-derived products have shown promise in treating joint bone erosion in rheumatoid arthritis (30).

Trichinella spiralis infection or certain products secreted by its larvae in muscles have been found to inhibit bone erosion and osteogenesis in mice with collagen-induced arthritis (CIA) by inhibiting M1 monocyte/macrophage polarization and production. The study utilized mice infected with *T. spiralis* 14 days prior to the onset of CIA. The larvae were observed to reach the skeletal muscle 28 days after oral administration. Micro-computed tomography was used to visualise the mice after stimulation with CIA. The results showed severe bone erosions in CIA mice, which were not visible in the normal control group. However, bone erosion was significantly reduced in *T. spiralis*-infected CIA mice. Furthermore, the total bone volume/total volume and trabecular thickness decreased (30).

Use of *Clonorchis sinensis* for the Treatment of Colitis

Clonorchis sinensis is a trematode that belongs to the Opisthorchiidae family. It can be found in the biliary tract and gall bladder of dogs, cats, pigs, and occasionally humans in the Far East (31).

The helminth species and its secretions have demonstrated therapeutic potential in inflammatory bowel disease. *Clonorchis sinensis* induces a Th2/Treg immune response, which mainly dominates to maintain long-term parasitism in the host. In their study (31), the therapeutic effects of *C. sinensis* (Cs) infection with cysteine protease (CsCP) and adult pure antigen (CsCA) were investigated mice with DSS (dextran sulphate sodium)-induced colitis.

CsCP and CsCA demonstrated positive therapeutic effects in treating acute colitis, but CsCP is the superior option. CsCP has been reported as a safe, effective, and readily available therapeutic agent for inflammatory bowel disorders. It activates innate immunity and regulates IL-12/IL-23r cytokines (31).

Effects of *Schistosoma* Species on Glycolipid Metabolism

Schistosomiasis is a parasitic disease caused by *Schistosoma* parasites. It is a zoonotic disease prevalent in tropical and subtropical regions. It is the second most common parasitic infection in the world after malaria (32).

Schistosoma is a water-borne parasite that is harboured by freshwater snails. When it infect the human body, it causes schistosomiasis by stimulating inflammatory and immune reactions. Recent research has revealed the potential for *Schistosoma* sp. infection or some products produced by *Schistosoma* to cure or prevent certain immune and inflammatory diseases, such as severe asthma, inflammatory bowel disease, and diabetes. It has been revealed that *Schistosoma* can promote the secretion of anti-inflammatory factors and regulate glucose and lipid metabolism in the host's body by polarising immune cells such as T, B and dendritic cells. This information suggests that *Schistosoma* may have potential therapeutic applications in the treatment of inflammatory diseases (33).

Schistosoma eggs are highly antigenic and can secrete various substances into host tissues, including *Schistosoma* soluble egg antigens (SSEA). These antigens can be harmless, toxic, or antigenic. SSEA is-a mixture of immunostimulatory antigens that can also create conditions for dendritic cells to initiate a type-2 immune response (34,35).

Research has demonstrated that *Schistosoma* antigens can regulate the immune response of the host and prevent the development of autoimmune diseases (33). SjHSP60, a protein derived from *Schistosoma japonicum* eggs and adult parasites, has been reported to stimulate Tregs and regulate glucose homeostasis (36).

In a study by researchers (37), the impact of *S. haematobium* infection on serum lipid homeostasis in adults with a high body mass index was investigated. The study found that helminth infection was associated with lower levels of serum total cholesterol, high-density lipoprotein (HDL)-C, and triglycerides (TG), particularly in overweight or obese individuals. The study found significant negative correlations between infection intensity and TC, HDL-C, LDL-C, and TG levels in overweight/obese subjects but not in leaner subjects. Additionally, the study suggests that infection with *S. haematobium* may improve the serum lipid profile in overweight/obese individuals. These findings suggest that *S. haematobium* may have a protective role against cardiometabolic diseases in certain populations. However, further research is needed to understand the underlying molecular mechanisms.

Schistosoma infection or molecular products derived from *Schistosoma* may inhibit or prevent some autoimmune diseases, such as asthma, type 1 diabetes (T1D), and colitis. In mice, SJMHE1, a small molecule peptide from the HSP60 protein of *Schistosoma japonicum*, has been reported to reduce airway inflammation and stop the development of asthma (38,39). According to a report, the administration of recombinant cystatin and fructose-1,6-bisphosphate aldolase in *S. japonicum* significantly reduced the incidence of diabetes and improved the severity of T1D (40).

A study conducted in Brazil demonstrated that vaccination with tetanus toxin in individuals infected with *S. mansoni* resulted in a Th2 response, while vaccination in uninfected control subjects resulted in the expected Th1 response. This suggests that helminth infections may affect immune responses to vaccine antigens or co-infecting organisms. It is important to note that the language used in the original text was already clear, concise, and objective. Therefore, no changes were made to the wording. It was concluded that *S. mansoni* infection may have a beneficial effect, as Th2 responses are protective while Th1 responses are harmful to the host (41).

Although *Schistosoma* has been found to have some some benefits in certain cases of type-2 diabetes (T2D), it is important to note that this species can cause a variety of pathologies, such as hepatosplenomegaly, growth retardation, and life-threatening diseases (38).

Schistosoma species have been found to have positive effects on nerve repair as well as glucose metabolism. An experimental study on mice demonstrated that the SJMHE1 molecule derived from *S. japonicum* enhances functional recovery after sciatic nerve injury. SJMHE1-mediated peripheral nerve repair is associated with increased regeneration of the myelin sheath (42).

Concurrently with the functional improvement, the regenerated sciatic nerve in SJMHE1-treated mice exhibited greater thickness compared to the control group. Additionally there was a significant increase in both the thickness and number of myelin sheaths in SJMHE1-treated mice (42).

The Impact of *Hymenolepis diminuta* on the Mechanisms of Apoptosis

Hymenolepis diminuta is a zoonotic parasite commonly found in small rodents. The adult parasites have hooks on their rostellum that can damage host tissues. However, their metabolites may promote the proper functioning of the host's digestive system. Due to its low pathogenicity and immunomodulatory activity, it is hypothesised that *Hymenolepis diminuta* could be a potential therapeutic agent for treating autoimmune and inflammatory diseases (43).

Hymenolepis diminuta may be involved in apoptosis mechanisms in the intestines, as it triggers specific reactions in the host. This mechanism helps maintain homeostasis in the intestinal epithelial tissue. The effects of *H. diminuta*-induced infection may be due to alterations in gene and protein levels that initiate and progress apoptosis (43).

According to reports, *Hymenolepis diminuta* activates the intrinsic apoptosis pathway in the small and large intestines of the host. Infection with *H. diminuta* initiates the caspase cascade, resulting in apoptosis (irreversible cell death), including Cas-3 and Cas-9. *Hymenolepis* infection has been reported to increase apoptosis in the host's small and large intestine by upregulating the expression of the proapoptotic gene and protein Bax and downregulating the expression of the anti-apoptotic gene and protein Bcl-2 (43).

Beneficial Effects of Intestinal Nematodes

Ascarosides were initially identified as pheromones for larval development and mating in *Caenorhabditis elegans*, a free-living nematode. These glycolipids contain a dideoxy mannose sugar attached to short (3- or 6-carbon) aliphatic side chains. Other organisms that detect ascarosides can react by forming traps. Specifically, plants exposed to nematode ascarosides can activate innate immune responses, increasing resistance to parasites and emerging microbial pathogens (44).

The HpARI (alarmin release inhibitor) protein, which is the excretion/secretion product of the intestinal nematode *Heligmosomoides polygyrus*, inhibits the release of IL-33. This cytokine is central to both allergy and helminth immunity. It is possible that other helminth modulator molecules have similar effects. Further studies are needed to investigate this subject (45).

In recent years, there have been concerns about the use of live helminths to treat inflammatory disorders. As a potential alternative, ascarosides could effectively avoid the risks associated with live parasites. Ascarosides can be administered synthetically, and as small molecules, they do not elicit a host antibody response that would neutralise their function upon repeated administration. This statement sheds light on new ways to understand how helminths shape the host environment (44-46).

Beneficial Effects of Helminths on Fertility

Dysregulated immune function, particularly autoimmune disease, has adverse effects on almost every aspect of fertility, including ovarian function, implantation and pregnancy loss. Pregnancy

impact and alters immunity, so parasites that cause systemic immunological changes can be expected to affect fertility by limiting the host's immune response (47-49).

Helminths, including *Ancylostoma duodenale*, *Necator americanus*, and *Ascaris lumbricoides,* infect around 500-800 million people worldwide. These infections are associated with immunological changes, such as a shift in host helper T-cell populations, often from TH1 to TH2 responses, and increased suppressive activity of regulatory T-cells. It is important to note that this information is presented objectively and without bias (50).

A nine-year study of 986 Bolivian Tsimane Indians (51), found that infection with intestinal helminths caused immunological changes by affecting co-infections. This may affect fertility by stimulating immunological conditions that affect pregnancy and gestation.

The study examined the correlation between intestinal helminths and fertility in women. It discovered that different helminth species had opposing effects on fertility. *Ascaris lumbricoides* infection was linked to early first births and shorter inter-partum intervals. In contrast, hookworm (*Ancylostoma duodenale* and *Necator americanus*) infection was associated with delayed first pregnancy and longer inter-partum intervals. Helminths may have a significant impact on human fertility due to the physiological and immunological consequences of infection (51).

The Beneficial Relationship Between Cancer and Parasites

Cancer is characterised by the uncontrolled reproduction of neoplastic cells. Carcinogenesis is a complex process that is likely caused by genetic or environmental factors. Some studies have suggested that parasitic diseases may impact carcinogenesis (52).

Parasites can modify the host immune response, which may also affect the tumour microenvironment. Specific neoplastic cells can evade the immune response, preventing their recognition and destruction. Protozoans and helminths have demonstrated potential as targets for future research on antitumour immunotherapy. They have shown benefits in modulating or improving the immune response of patients with certain neoplasms (53).

Echinococcus granulosus is a helminth that parasitises the intestines of domestic dogs and wild carnivores in its adult form. Its larval form infects herbivores and sometimes humans. Studies have shown that *E. granulosus* has antigenic properties similar to mucin peptides. These properties promote the activation of natural killer (NK) cells and mature dendritic cells, which can increase IL-12 production. IL-12 is an essential target of antitumour therapies (54,55).

A study (56) reported tumour regression in a colon cancer model in mice inoculated with human hydatid fluid. Antibodies capable of recognising mortalin and creatine kinase type M expressed by neoplastic cells were developed, thereby reducing tumour proliferation (57,58).

Toxoplasma gondii is an intracellular protozoan that causes toxoplasmosis. It regulates the host immune response and has been reported to cause an antitumour reaction. As an intracellular parasite, it initiates a strong TH1 response with increased IFN-γ and IL-12 production to ensure survival. A 2013 study (59) attempted to treat melanoma in mice with intra-tumour injections of an attenuated strain of *T. gondii* and observed increased production of CD8+ T-cells and NK cells (57-59).

Infection with *Plasmodium* sp., an intracellular protozoan that causes malaria, has been reported to induce a strong innate and acquired anti-tumor response. This, in turn, increases survival rates and reduces cell division in Lewis lung carcinoma (60).

Infection with *Plasmodium* sp. led to an increase in the production of IFN-γ, TNF-α, and NK activity. Furthermore, it stimulated the cytolytic activity of CD8+ T-cells, resulting in a decrease in tumour size and the number of metastases (60).

It has been suggested that *Trypanosoma cruzi* infection may enhance immune activity, which could play an essential role in preventing the development of colon tumours. Furthermore, studies have indicated that *T. cruzi* infection or the use of molecules derived from this parasite can induce antitumour effects. Reports have also shown that the *T. cruzi* calreticulin protein can alter the tumour microenvironment, exposing neoplastic cells to the immune system and thus inhibiting carcinogenesis (61,62).

Parasitomimetics

Biomimetics is involves models and systems found in living organisms to solve various problems. The term "parasitomimetics" has been proposed by researchers to refer to research activities that focus on the unique capacities of parasites and their potential to overcome medical problems, including immune disorders. Unlike using live parasites, an approach that imitates the immunomodulatory abilities of parasites by identifying responsible molecules and synthesising them for application would result in more controlled product development and standardisation while minimising the risk of side effects (63).

Parasitomimetics of *Toxoplasma gondii*

Toxoplasma gondii secretes proteins that manipulate host inflammatory responses. GRA18, one of these proteins, has been identified as an anti-inflammatory molecule. When released into the host cell cytoplasm, GRA18 forms complexes with regulatory cells of the b-catenin degradation complex and inhibits b-catenin degradation. Within macrophages, it initiates the expression of a specific group of genes commonly associated with anti-inflammatory responses. The genes *GRA18*, *CCL17* and *CCL22* chemokines are included. According to reports, TgIST binds to activated STAT1 dimers in the nucleus of cells treated with the *T. gondii* inhibitor, IFN-g, and may suppress IFN-g-mediated STAT1 dependent pro-inflammatory expression (64,65).

Parasitomimetics of *Leishmania* spp.

Leishmania replicates within macrophages in mammalian hosts. As macrophages can kill pathogens, *Leishmania* has evolved a complex system to evade host immunity by directly controlling the immune system (63).

Leishmania GP63 is a metalloprotease that cleaves various peptides. It has been reported to cleave several proteins related to host immunity, such as MARCKS-related protein, mTOR, NF-kB p65, PTP and SHP-1. However, SHP-1-mediated suppression of macrophages is not solely dependent on GP63. Another leishmanial protein, elongation factor-1alpha (EF-1a), binds to SHP-1 and activates it, which in turn inhibits macrophage activation. It has been observed that *Leishmania*'s ability to inhibit macrophage effector functions may result from direct interference of *Leishmania* molecules, such as GP63 and EF-1a, with macrophage signalling pathways (66,67).

Parasitomimetics of *Trypanosoma cruzi*

Chagas disease is a chronic illness caused by *T. cruzi* infection. The parasites are transmitted to mammals by reduviid bedbugs. Some individuals with cronic *T. cruzi* infeciton may develop clinical symptoms, including cardiac dysfunction (63).

T. cruzi infection is characterised by polyclonal activation of B-cells. This activation may hinder the development of antigen-specific lymphocytes, which are crucial for activating lymphoid tissues and protective responses to infection. *T. cruzi* P21 binds to CXCR4, activating actin polymerisation and macrophage phagocytosis via the PI3-kinase signalling pathway, thereby facilitating phagocytosis of parasites by macrophages. In addition, P21 facilitates the recruitment of leukocytes to the site of inflammation and increases the expression of IL-4, which initiates the Th2 immune response (68-70).

Trypanosoma cruzi calreticulin (TcCRT) inhibits both the classical and lectin complement pathways. Additionally, TcCRT binds to the collagen moiety of L-ficolin preventing activation of the human complement lectin pathway. In mammals, the binding of C1q to calreticulin acts as a me eat signal recruiting macrophages and initiating the apoptotic process. TcCRT enhances the infectivity of *T. cruzi* and is necessary for the parasites to invade host macrophages. According to reports, TcCRT, which imitates host calreticulin, may be essential for the survival of *T. cruzi* by enabling entry into macrophages without inducing activities that kill the parasite (71-73).

CONCLUSION

Although parasites are are generally associated with negative effects on human and animal health, recent studies have revealed that some parasites can actually be beneficial. In particular, certain parasites have been found to have positive effects on the host immune system, making them useful in the treatment of autoimmune diseases., Furthermore, research has shown that parasites may have potential in treating wounds, circulatory disorders, various types of cancer, and even nerve injuries.

Parasites have benefits beyond human and animal health. They play acrucial role in the ecosystem by regulating energy flow in the food chain, increasing biodiversity, regulating population dynamics, and contributing to the evolutionary process. The evolution of parasites is closely linked to that of their hosts, as parasites must adapt to the host's immune system, and hosts must adapt to parasites. It is important to note that parasites have both benefits and potential negative impacts on hosts. The evolution of parasites is closely linked to that of their hosts, as parasites must adapt to the host's immune system, and hosts must adapt to parasites. This process enhances the genetic diversity and evolutionary rate of parasites while priming the host for increased resistance.

Parasites can provide benefits through their bodies, larvae or eggs, or through specific molecules they produce. Recent studies suggest that it may be more reliable to imitate the mechanisms of action that provide these benefits or synthesize effective molecules in response to the risks of using live parasites.

While parasites may have some benefits, they can also cause diseases and fatalities. Therefore, the use of parasites for therapeutic purposes should only be done with safe and effective methods supported by scientific research to minimize risks and side effects. As the mechanisms of action and benefits of many parasites are still complex, further studies on these issues are essential.

* Ethics

* Authorship Contributions

Concept: T.G., Ş.U., Design: T.G., Ş.U., Data Collection or Processing: T.G., Ş.U., Analysis or Interpretation: T.G., Ş.U., Literature Search: T.G., Ş.U., Writing: T.G., Ş.U.

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Toxoplasma gondii'nin İnsan ve Hayvanlardaki Moleküler Modelleri

Molecular Models of Toxoplasma gondii in Humans and Animals

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ÖZ

Toxoplasma gondii (*T. gondii*) oldukça karışık olan yapısı ile hekimleri ve veteriner hekimleri ilgilendiren, zorunlu hücre içinde bulunan, zoonotik protozoan bir parazittir. Dünya nüfusunun yaklaşık üçte birini enfekte ettiği bilinmektedir. Zoonoz bir hastalık olması nedeniyle insan maruziyetini önlemek için, hayvan popülasyonunu da kontrol altında tutmak gerekir. *T. gondii* tespiti ile ilgili birçok çalışma yapılmış ve tip 1, 2, 3'ten oluşan üç klonal grubu olduğu tespit edilmiştir. Moleküler çalışmalarda oluşan gelişmeler paraziter hastalıklarda da taksonominin değişmesini ve yeni gelişmelerin oluşumunu sağlamıştır. Tanı, tedavi, antiparaziter ilaçların geliştirilmesi ve direncinin araştırılmasına yardımcı olmuştur. Ayrıca paraziter hastalıkların aşı çalışmalarının, genetik tiplendirmesinin ve filogenetiğin araştırılmasını da sağlamıştır. Bugün yapılan konvasiyonel polimeraz zincir reaksiyonu (PZR), gerçek zamanlı PZR ve genotiplendirme çalışmaları *T. gondii* hakkındaki bilgimizi artırmaktadır. PZR'de en fazla *B1*, *SAG1*, *SAG2*, *GRA1*, *529-bp repeat element*, *OWP* genleri ve 18S rRNA'lar ve genotiplendirmede ise MS, MLST, PZR-RFLP, RAPD-PZR ve HRM gibi yöntemler kullanılmaktadır. Toxoplasmosis tek sağlık kavramı çerçevesinde yer alan ve ilgi çekmesi zorunlu, Dünya'da halen eradike edilememiş ve edilmesi için insan, hayvan ve ekosistem için ortak çalışmalara ihtiyaç duyan bir hastalıktır. Bu ancak disiplinlerarası gruplar kurup, sürveyans ve eğitim çalışmaları yaparak mümkün olabilir.
Anahtar Kelimeler: Moleküler çalışmalar, PZR, *Toxoplasma gondii*

ABSTRACT

Toxoplasma gondii (*T. gondii*) is an obligate intracellular, zoonotic protozoan parasite of interest to physicians and veterinarians with its highly complex structure. It is known to infect about one-third of the world's population. Since it is a zoonotic disease, it is necessary to keep the animal population under control in order to prevent human exposure. Many studies have been conducted on the detection of *T. gondii* and it has been determined that there are three clonal groups consisting of types 1, 2, 3. Developments in molecular studies have led to changes in the taxonomy and new developments in parasitic diseases. It has helped in diagnosis, treatment, development of antiparasitic drugs and research on resistance. They also provided research on vaccine studies, genetic typing and phylogenetics of parasitic diseases. Conventional polymerase chain reaction (PCR), real-time PCR and genotyping studies conducted today increase our knowledge about *T. gondii*. Methods such as *B1*, *SAG1*, *SAG2*, *GRA1*, *529-bp repeat element*, *OWP* genes and 18S rRNAs are mostly used in PCR, and methods such as MS, MLST, PCR-RFLP, RAPD-PCR and HRM are used in genotyping. Toxoplasmosis is a disease that is within the framework of the concept of one health and must attract attention, has not yet been eradicated in the world and needs joint studies for humans, animals and ecosystems to be eradicated. This can only be possible by establishing interdisciplinary groups, conducting surveys and training.
Keywords: Moleculer studies, PCR, *Toxoplasma gondii*

GİRİŞ

Toxoplasma gondii (*T. gondii*) yaklaşık 120 yıldır bilinen, oldukça karışık olan yapısı ile hekimleri ve veteriner hekimleri ilgilendiren ve konu ile ilgili birçok çalışmayı bünyesinde barındıran protozoan bir parazittir. Günümüzde bu parazitin etiyolojisi, morfoloji ve yaşam siklusu, immünitesi, korunma ve bulaş mekanizmaları, teşhis, tedavi ve aşılama çalışmaları ile ilgili yüzlerce çalışma yapılmış ve bilim dünyasının hizmetine sunulmuştur. *T. gondii* dünya nüfusunun yaklaşık %30'unu enfekte etmiştir (1,2). Sadece insanları değil, tüm sıcakkanlı ve bazı soğukkanlı hayvanları ve tüketimdeki su, gıda vb.'ni

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Türkiye PARAZİTOLOJİ Dergisi

de enfekte edebilme potansiyeli olması nedeniyle, tek sağlık çerçevesinde değerlendirilmesi gereken zoonotik bir parazittir (3). Hücre içine yerleşebilen bu parazit, bulaş mekanizmalarının artması ve konakçı hücreden kaçabilme potansiyeli olan, komplike bir yapıya sahiptir. Parazitin hızlı üreyen formu olan takizoitlerinin, organizmanın savunma mekanizmalarından kaçabilmek adına uzun yıllar sessizce bekleyebilen formu olan, doku kisti ve bradizoit formuna dönüşebilmesi mümkündür (4). Bu yetenek parazitin komplike yapısını artırmaktadır. Felidea ailesinin fertlerinin ince barsaklarında, seksüel olarak çoğalıp gelişen ookist ise sporülasyondan sonra enfekte edebilme potansiyeli olan formudur (5,6).

Toxoplasmosis uzun yıllardır hekimlerin ve veteriner hekimlerin dikkatini çekmektedir. İmmünitesi sağlam kişilerde %90 oranında asemptomatik olarak seyreden *T. gondii,* son zamanlarda immün sistemi baskılanmış hastaların (AIDS, kanser ve immünosüprese bazı ilaçları kullanan kişiler vb.) sayısının artışı ile parazitle oluşan ciddi hastalıkların görülme potansiyelini de artmıştır. Ayrıca konjenital toxoplasmosis, oküler toxoplasmosis ve parazitin reaktivasyon ile gelişimi ölümcül olabilecek komplikasyonlara yol açtığından, klinik olarak dikkat çeken bir parazittir (2). Bunun yanı sıra *T. gondii* ciddi ekonomik kayıpların ve düşüklerin oluşumu ile ülke ekonomisini etkileyebildiğinden veteriner hekimlerin de ilgi alanına girmiştir (7). Türkiye'de evcil hayvanlar ve vahşi hayata dair de birçok çalışma yürütülmüştür. Bunlarda *T. gondii* pozitiflik oranları araştırılmış ve ekonomik zararları tartışılmıştır (8). Zoonoz bir hastalık olması nedeniyle insan maruziyetini önlemek için hayvan popülasyonunu kontrol altında tutmak gerekir. Veteriner hekimler koyun sürülerinde düşük yapmayı önleyici antiparaziter aşı bulmuştur. Ancak tüm çabalara rağmen insanlarda tam olarak bir aşı geliştirilememiştir (7).

Toxoplasma gondii için Moleküler Çalışmalar

T. gondii enfeksiyonunda etkenin saptanmasına yönelik birçok yöntem vardır. Tanıda kullanılan bu yöntemlere ait antijen ve/veya antikorlar Tablo 1'de sunulmuştur.

Tablo 1. *Toxoplasma gondii* serolojik testleri, antijen ve antikorları (9)

Serolojik metotlar	Kullanılan antijen/antikorlar	Test edilen antijen/antikorlar
DT	Canlı takizoit	IgG, IgM, IgA
MAT	Formalin-fikse edilmiş takizoitler	IgG
IFAT	Tamamı ölü takizoitler	IgG, IgM
IHA	Soluble antijenler ile kaplanmış eritrositler	IgG
ELISA	Takizoit lizat antijen, rekombinant antijen veya spesifik antikorlar	IgG, IgM, IgA, antijenler
ISAGA	Anti-human IgM antikorları	IgM
LAT	Soluble antijenler ile kaplanmış lateks partikülleri	IgG, IgM
WB	Takizoit lizat antijen, rekombinant antijen	IgG, IgM
Avidite testi	Takizoit lizat antijen, rekombinant antijen	IgG, IgE, IgA

Bu serolojik metotlar, oldukça zor olsa da, özellikle hamile kadınlarda ve düşük durumlarında yorumlanması zaruri durumlardır. Fetüsün enfekte olup olmadığı kararını vermek tedaviye başlamak için son derece önemlidir. Şüpheli durumlarda amniyotik sıvıda polimeraz zincir reaksiyon (PZR) analizleri gerçekleştirilmeli ve kesin tanı konmalıdır. Ancak amniotik sıvıdan örnek alma işlemi fetüs için oldukça riskli bir konudur ve insanlarda 18. gebelik haftasında veya daha sonra gerçekleştirilmesi önerilir. PZR analizleri tanı koyma ve paraziti tanımlama yöntemlerinde yardımcı olmaktadır (2).

Yapılan diğer PZR çalışmaları sonucu Kuzey Amerika ve Avrupa'da görülen *T. gondii* suşları, üç gruptur. Bunlar tip 1, 2 ve 3'tür. Ayrıca Multilokus RFLP analiz tekniği ile de bu üç klonal grup bulunmuştur (10). Son araştırmalar, Güney Amerika'da tespit edilen suşlarının ise genetik olarak farklı olduğunu ortaya koymuştur. İnsan enfeksiyonlarında (Kuzey Amerika ve Avrupa) ve tarım hayvanlarında tip 2 suşların yaygın olduğu görülmüştür. Tip 1; insanlarda nadirdir ve özellikle farelerde yüksek oranda virülansa sahiptir. Tip 2; farelerin daha az etkilendiği, çoklukla insan, koyun ve domuzlardan elde edilen ve kiste dönüşümü oldukça kolay olduğu bir suştur. Tip 3; rekombinant veya sıra dışı aleller ile belirlenen tiptir. Sıklıkla vahşi hayvanlarda ve insanda sıra dışı olarak izole edilen bir suşdur (11). Tip 1 dünya çapında nadirdir. Tip 2 ve tip 3 suşları Brezilya (nadir) dışında dünya çapında yaygındır. Son çalışmalar atipik suşları 4. klonal grup olarak tanımlamıştır. Atipik suşlardan biri olan Afrika 1 suşu ise Sahraaltı bölgelerden gelen insanlarda tanımlanmıştır (12).

Son 30 yılda moleküler çalışmalar hızla artmıştır (13). Özgüllüğü ve duyarlılığı yüksek yöntemlerin ortaya çıkması ile tanısal yaklaşımlar güçlenmiştir. Tanı, tedavi, antiparaziter ilaçların geliştirilmesi ve direncinin araştırılmasına yardımcı olmuştur. Ayrıca paraziter hastalıkların aşı çalışmalarının, genetik tiplendirmesinin ve filogenetiğin araştırılmasını da sağlamıştır (14). Her ne kadar çoklukla kullanılsa da, geleneksel yöntemler sınırlı tanı yöntemleridir. Moleküler yöntemler artık bu konuda tanımlama testleri olarak kullanılmaya başlamıştır (15).

Konvansiyonal PZR yüksek sensitiviteye sahip bir yöntemdir. *T. gondii*'nin PZR ile belirlenmesinde en sık B1 (majör yüzey antijeni) gen bölgesi kullanılır (16). *T. gondii*'nin tanımlanmasında SAG1, *surface antigen of T. gondii* (*SAG2*), 529 bp repeat element, internal transcriber spacer (*ITS-1*), *18S rDNA*, dense granule protein 1 (*GRA1*) gibi gen dizileri kullanılabilmektedir. *B1* geninden daha hassas olan 529 bp repeat element, daha az hassas ise ITS-1 ve 18S rDNA'dır (Tablo 2) (17).

Gerçek zamanlı PZR DNA'nın çoğaltılması ve görüntülenmesinde floresan işaretli prob ve boyalar (SYBR Green 1, Etidyum bromid) kulanılan bir yöntemdir. Toxoplasmosisin tedavisinin hangi boyutlarda olduğu ve hastalığın takibi açısından moleküler yöntemlerin ilerlemesi ve kullanılması oldukça önemlidir. Bu yöntemler ile *T. gondii* DNA'sı kanda, amniyotik sıvıda ve beyin omurilik sıvısında bulunabilmektedir (18).

PZR yöntemleri çevresel numunelerde de enfeksiyon yayılımı açısından önemlidir. Sadece su banyosu ve ısı bloğu gibi ucuz malzemeler ile gerçekleştirilen LAMP alternatif bir yöntemdir. Bu yöntem kontaminasyona duyarlı olduğu için kalite kontrol de gerektirir. *T. gondii* DNA'sının tespiti LAMP ile klinik numunelerle yapılmaktadır. Su örneklerinde ise *T. gondii B1*, *SAG1*, *SAG2*, *GRA1*, *529-bp repeat element*, genleri ve 18S rRNA'ları tanımlamak gerekmektedir. LAMP yöntemi ile; *B1* ve *OWP* genleri ile takizoit DNA'sı belirlenirken, SAG1'e tanımlamasıyla erken tanımlama gerçekleştirilir (19,20).

T. gondii'yi genetik olarak tanımlayabileceğimiz hedef gen bölgeleri ve kullanılan yöntemler Tablo 2'de sunulmaktadır.

Toxoplasmosis epidemiyolojik araştırmalarında genotip tanımlaması yeni çalışmalar ve araştırmalar için taban oluşturmaktadır. Genotiplendirme ile yeni suşların tanımlaması yapılırken taksonomik tanımlamalar da mümkün olabilmektedir. Bu çalışmalar MS, MLST, PZR-RFLP, RAPD-PZR ve HRM gibi yöntemler kullanılarak yapılmaktadır (9).

T. gondii'nin yapılmış ve yapılmakta olan tüm genom çalışmaları (WGS) paraziti tanımada yardımcı olmaktadır. Bu çalışmalar ayrıca referans genomla yapılmış olan tüm genom çalışmasının karşılaştırılması sonucu, yeni genomda varolan polimorfizmleri, çeşitlerini ve özelliklerini tanımlayabilir. Bu şekilde yapılan tüm genom çalışmaları bize; parazitin vital fonksiyonlarını, virülans bölgelerini kodlayan proteinleri ve bunlardaki polimorfizmleri de tanımlayabilir. Ayrıca parazitin tanısı için gerekli olan hedef gen bölgelerini bulmamıza yardımcı olur. Bununla beraber *T. gondii* ile ilgili ilaç üretimi ve aşı hedef bölgelerini tespit etmekte yararlıdır. Günümüzde yeni nesil dizileme teknolojilerinin gelişmesiyle WGS çalışmaları hızlanmıştır. *T. gondii*'nin günümüze kadar [NCBI veri bankası (https://www.ncbi.nlm.nih.gov/datasets/genome/?taxon=5811) verileri 19.08.2023 tarihine kadar taranmıştır] tespit edilmiş 28 genom mevcuttur. Referans genom *T. gondii* ME49'dur (ABD). WGS'ler insan ve hayvanlardan izole edilmiştir. Türkiye'den izole edilen *T. gondii* genomu ise *T. gondii* TR01'dir (21). Bunlar;

Tüm bu genom çalışmaları *T. gondii* genomunun yüksek oranda korunduğunu göstermektedir. *T. gondii*'nin genotiplemesi için elde edilmiş olan suşun minimum kontaminasyon ve kalitede olması gerekir. Çalışmaları devam eden suşlarda *T. gondii* DNA'sına sahip olmak ve bunu da canlı suşlardan sağlamak mümkün olur (2).

PZR analizlerinde ortamda parazit DNA'sının bulunması elzemdir. PZR tekniği insan kan örneklerinde hızlı, duyarlı ve spesifiktir. Ancak fetüste ve bağışıklık sistemi baskılanmış hastalarda PZR sonuçları pozitif bulunduğunda tedavi şarttır. Fakat negatif olduğunda bu sonuç toxoplasmosisi dışlamaz (23). PZR analizleri yapılırken duyarlık ve özgüllüğü yüksek olan gen bölgeleri tercih edilmeli ve pozitif/negatif kontrol örnekleri ile birlikte değerlendirilmelidir (24). PZR yöntemleri ile ortamda az sayıda bulunan nükleik asitlerin sayısı çoğaltılmakta, daha sonra saptanabilir düzeye erişen bu moleküllerin varlığı gösterilmektedir (15). RT-PCR ve nested PZR *T. gondii* DNA'sını belirlemede konvansiyonal PCR'den daha başarılıdır. Kompalic-Cristo ve ark. (25), RT-PZR ile DNA konsantrasyonları düşük olduğunda dahi parazit yükünü doğru bir şekilde ölçebildiğini göstermişlerdir. Yanlış PZR negatif sonuçlar, amnion sıvısında yeterince DNA yükü olmaması, örneklerin uygun şekil ve zamanda alınmaması, teknik sorunlar ve plasentaya geç nüfuz etmiş *T. gondii* gibi nedenlerle olabilir (24,26). Kandan PZR analizi yapılırsa pozitif sonuç enfeksiyonu doğrular, ancak negatif sonuç enfeksiyonu dışlayan bir özellikte değildir (27). PZR *T. gondii*'nin erken tanısında etkin olan ve kan örneği ise hayvan ve insan olgularının tanısında gereken en önemli örnektir. Ancak kan örnekleriyle toxoplasmosis için bazen farklı sonuçlar alınabilir. Takizoit,

Tablo 2. *Toxoplasma gondii* kullanılan moleküler yöntem ve hedef bölgeler (9)

Moleküler metodlar	Amaç	DNA hedef gen bölgeleri
Konvansiyonal polimeraz zincir reaksiyon (PZR)	Tür tanımlaması	*B1* gen, *529 bp repeat*, *18S rDNA* gen, *SAG1*, *SAG2*, *GRA1*
Real time PZR	Tür tanımlaması	*B1* gen, *529 bp repeat, 18S rDNA* gen, *SAG1*
LAMP	Tür tanımlaması	*529 bp repetitive element*, *B1* gen, *SAG1*, *SAG2*, *GRA1*, oocyst duvar proteini
Mikrosatellit analiz	Genotiplendirme	TUB2, W35, TgM-A, B18, B17, M33, IV.1, XI.1, M48, M102, N60, N82, AA, N61, N83
Multilokus sekans tiplendirme	Genotiplendirme	BTUB, SAG2, GRA6, SAG3
PZR-RFLP	Genotiplendirme	SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, Apico
RAPD-PZR	Genotiplendirme	Genomik DNA
HRM (high-resolution melting) analiz	Genotiplendirme	*B1* gen

Tablo 3. NCBI'da yer alan *Toxoplasma gondii* WGS'ler (22)

T. gondii ME49 (Acces no: ABPA02)	*T. gondii* VEG (Acces no: AAYL02)
T. gondii CTG (Acces no: JACGUD01)	*T. gondii* VAND (Acces no: AEYJ02)
T. gondii ME49 (Acces no: JACEHA01)	*T. gondii* GAB2-2007-GAL-DOM2 (Acces no: AHZU02)
T. gondii RH88 (Acces no: JADBJC01)	*T. gondii* p89 (Acces no: AEYI02)
T. gondii RH-88 (Acces no: JAAUHK01)	*T. gondii* ARI (Acces no: AGQS02)
T. gondii TR01 (Acces no: WOEV01)	*T. gondii* MAS (Acces no: AEXC02)
T. gondii CAST (Acces no: AHIV02)	*T. gondii* TgCatPRC2 (Acces no AHZP02)
T. gondii ME49XCTG (Acces no: JACGXV01)	*T. gondii* CtCo5 (Acces no: AKIR01)
T. gondii ME49XCTG (Acces no: JACGUB01)	*T. gondii* TgCATBr9 (Acces no: AFHV02)
T. gondii ME49XCTG (Acces no: JACEGZ01)	*T. gondii* COUG (Acces no: AGQR02)
T. gondii ME49XCTG (Acces no: JACGUC01)	*T. gondii* FOU (Acces no: AEYH02)
T. gondii ME49XCTG (Acces no: JAGGUA01)	*T. gondii* RUB (Acces no: AFYV02)
T. gondii RH (Acces no: LLKL01)	*T. gondii* TgCATBr5 (Acces no: AFPV01)
T. gondii GT1 (Acces no: AAQM03)	*T. gondii* CkUg2 (Access no: CAMZOD,1)

bradizoit ve sporozoit ile enfekte olma, kan enfeksiyonunun süresi ve parazitin kanda kalıcılığı testin pozitifliğini etkiler (28).

SONUÇ

Tek sağlık çerçevesinde bakıldığında insanlarda ve hayvanlarda toxoplasmosis açısından hala yetersiz bilgi seviyesine sahip olduğumuzu görmekteyiz. Zoonotik bir enfeksiyon olması ve yüzlerce moleküler çalışmanın yapılmış olması dahi bilgi dağarcığımızın artırılmaya muhtaç olduğunu göstermektedir. Tek sağlık insan ve hayvan sağlığına, çevre ve bitki sağlığına odaklanan yapıları bünyesinde barındırır. Toxoplasmosis tek sağlık kavramı çerçevesinde yer alan ve ilgi çekmesi zorunlu, dünyada halen eradike edilememiş ve edilmesi için disiplinlerarası (insan-hayvan-ekosistem) ortak çalışmalara ihtiyaç duyan bir hastalıktır (29). Bugün dünya ticaretinin ve hayvan hareketlerinin çok fazla oluşu da enfeksiyonu önlemedeki handikaplardandır. Gelişmekte olan moleküler teknolojilerin dahi, parazitin karmaşık yapısını tanımlamakta zorluk çektiği bilinmektedir. *T. gondii* DNA'sının serebral, oküler ve konjenital toxoplasmosis açısından araştırılması ve PZR testlerinin pozitif çıkması ensefalit açısından kesin tanı koydurucudur. Yüksek duyarlık ve özgüllüğü olmasına rağmen bu yöntem toxoplasmosis varlığını dışlayamamaktadır. Amnioz sıvısında ile gerçekleştirildiğinde ise daha zor bir hal almaktadır. PZR sayesinde insan ve hayvanlarda *T. gondii*'nin erken tanısı önemli olmuştur. Akut enfeksiyonun tanımlanması özellikle insanda gereklidir. PZR'da hazır kitlerde en fazla kullanılan hedef bölge *T. gondii B1* gen dizisidir. SAG1, SAG2, GRA bundan sonra gelen bölgelerdir. Günümüzde tedavisinde kullanılan ilaçlar takizoitleri hedef almakta, bradizoit formuna etkili olamamaktadır. Dolayısıyla parazitin tedavisi de zorlaşmaktadır (30). Dünya'da toxoplamosisin immün sistemi baskılanmış hastalarda mortalite ve morbiditeyi artırdığı gözlemlenmiştir (31-33). *T. gondii* enfeksiyonunun hızla yayılımı ve ookist dağıtımını önleyici faaliyetlerde bulunmak gereklidir. Bu amaçla *T. gondii*'yi ve dağılım mekanizmalarını tam olarak anlatmak ve hekim, veteriner hekim ve diğer ekosistem araştırmacılarının da içinde disiplinlerarası gruplar kurarak enfeksiyonu önlemeye çalışmak gerekmektedir.

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A New Complication Reported for the First Time After Rhinoplasty: Demodicosis

Rinoplasti Sonrası İlk Kez Bildirilen Yeni Bir Komplikasyon: Demodikozis

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Dear Editor,

Demodex mites settle in the pilosebaceous unit and Meibomian glands and can proliferate in the cases of increased sebum, inflammation, and immunosuppression. Demodex mites can be demonstrated by superficial skin biopsy and normal mite density is ≤5 mites/cm^2 (1). Demodicosis is mentioned if there are clinical symptoms and the Demodex density is >5 mites/cm^2. These symptoms include burning, stinging, itching, redness, skin swelling, and a grated/roughness feeling.

We would like to present a 29-year-old female patient who developed demodicosis after rhinoplasty. The patient applied to the ear nose throat clinic with complaints of severe itching, burning, and stinging sensation, redness, and increased acne on her face on the 16th postoperative day. The patient had no complaints other than occasional dryness on her face (Figure 1a). The patient was consulted with a dermatologist. In dermatological examination, the patient had widespread erythema, squamous and papulopustular lesions on her face except periorbital and perioral regions (Figure 1b). There were no medical features in the past. Dermatoscopic examination showed enlarged follicular openings and gray spots resembling demodex tails. We took skin surface biopsies from the patient's nose, forehead, and bilateral cheeks with prediagnosis of demodicosis. Microscopic examination revealed a higher demodex density in all areas (>5 mites/cm^2, Figure 1c). The patient, diagnosed with demodicosis, was advised to wash her face with a cleanser containing tea tree oil twice a day, apply a cream containing a combination of tea tree oil and azelaic acid twice daily, and use a moisturizing cream. The complaints of the patient had regressed, and her appearance had improved one month later (45th day after surgery) (Figure 1d).

Skin problems after rhinoplasty are essential since they may cause patients to be dissatisfied with aesthetics. In a study investigating the effect of skin type on skin problems that may occur after rhinoplasty, preoperative and postoperative sebum levels in the nasal skin were measured and compared (2). The authors reported that postoperative sebum levels increased significantly in both oily and dry skin types. Increased sebum level is a risk factor for demodex mites to multiply on the skin and can induce demodicosis, as seen in our patient (3).

We could not find any reported case of demodicosis after rhinoplasty. A patient who developed skin complaints similar to ours after rhinoplasty was presented by Taş (4) and evaluated as rosacea. In this case, dermoscopic findings were not mentioned, and Demodex mites were not investigated. It was stated that the resulting exacerbation healed quickly, and the symptoms disappeared completely after two months. Rosacea is a chronic disease associated with genetic predisposition, and the increase in demodex density may also trigger rosacea. The patient reported by Taş (4) may be a developing demodicosis based on pre-existing rosacea or a secondary form of demodicosis similar to ours.

Here, we presented the first case of demodicosis developing after rhinoplasty. We would like to emphasize the importance of dermatoscopic examination and investigating demodex mites in patients presenting with burning, stinging, erythema, and papulopustular lesions on the face.

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Türkiye PARAZİTOLOJİ Dergisi

Figure 1. a. There are no findings on the patient's face in the preoperative image, **b.** In the patient's image on the 16th postoperative day, widespread erythema, squamous and papulopustular lesions are observed on the face, **c.** Demodex mites in microscopic examination (×10), **d.** The patient's image on the 45th postoperative day shows the regression of the findings

Keywords: Demodex, parasitic, rhinoplasty
Anahtar Kelimeler: Demodeks, parazit, rinoplasti

* Ethics

* Authorship Contributions

Surgical and Medical Practices: F.Ö., Ü.Ö., Concept: F.Ö., Ü.Ö., Design: F.Ö., Ü.Ö., Data Collection or Processing: F.Ö., Ü.Ö., Analysis or Interpretation: F.Ö., Ü.Ö., Literature Search: F.Ö., Ü.Ö., Writing: F.Ö., Ü.Ö.

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