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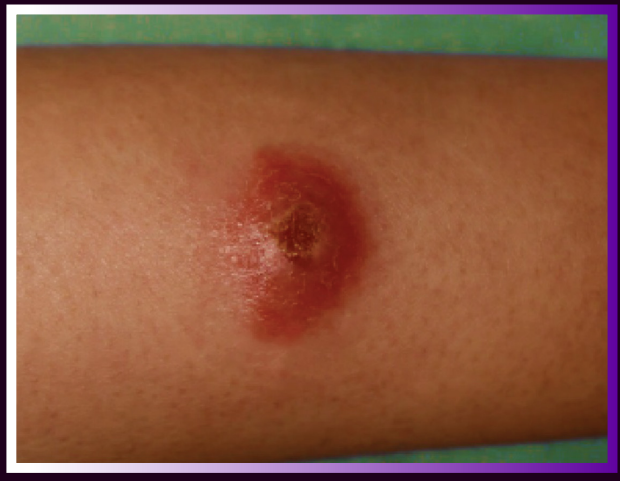

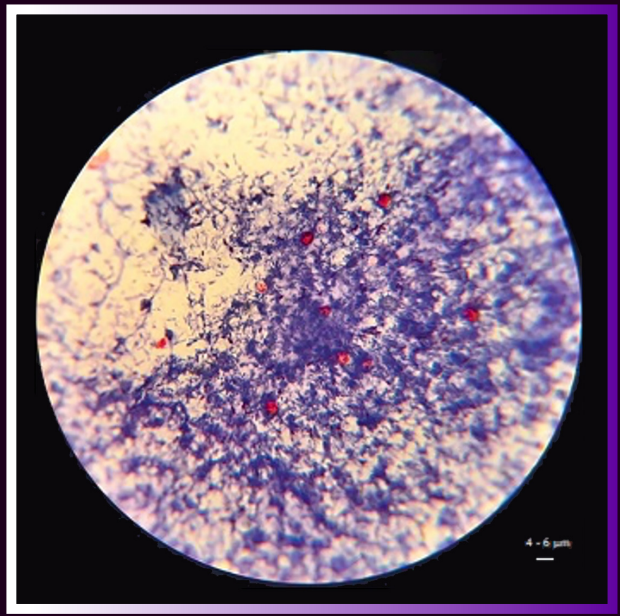

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EDİTÖRDEN

2024 yılının üçüncü sayısını biri yurt dışı kaynaklı 9 özgün araştırma makalesi, 1 olgu sunumu ve 1 derleme ile çıkarmaktayız. Özgün araştırmalarda leishmaniasis, toxoplasmosis ve bağırsak parazitleri ile ilgili çeşitli üniversitelerden yapılan çalışmaları içeren ve ülkemizde sıklıkla rastladığımız *Demodex* enfestasyonu ile ilgili makaleler sunulmaktadır.

Olgu sunumu olarak nadir görülen spinal kistik ekinokokkozus ile ilgili bir olguya yer verilmiştir. Derleme makalelerde ise oldukça ilginç ve gizemli bir konu olan kenelerle ilişkili alpha-gal sendromu hakkında birçok noktayı açıklayan bir makaleye yer verilmiştir.

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Yayınevi İletişim/Publisher Contact

Address: Molla Gürani Mah. Kaçamak Sk. No: 21/1 34093 İstanbul, Türkiye

Telefon/Phone: +90 530 177 30 97

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Contact

Editorial Office

Editor in Chief: Yusuf Özbel, MD, Prof

Address: Ege Üniversitesi Tıp Fakültesi, Parazitoloji Anabilim Dalı, 35100 Bornova-İzmir, Türkiye

Phone: +90 232 390 47 24 / +90 232 373 00 08

Fax: +90 232 388 13 47

E-mail: yusuf.ozbel@ege.edu.tr / yusuf.ozbel@gmail.com

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Antimicrobial Peptides and Their Anti-*Leishmanial* Efficacies on *Leishmania tropica* Promastigotes *In vitro*

Antimikrobiyal Peptitler ve Leishmania tropica Promastigotları ile In vitro Çalışma

Nihan Ünübol[1,2], İbrahim Çavuş[3], Tuba Polat[4], Özgür Kurt[1], Ahmet Özbilgin[3], Tanıl Kocagöz[1]

[1]Acıbadem Mehmet Ali Aydınlar University Faculty of Medicine, Department of Medical Microbiology, İstanbul, Türkiye
[2]Acıbadem Mehmet Ali Aydınlar University Vocational School of Health Services, Medical Laboratory Technician Program, İstanbul, Türkiye
[3]Manisa Celal Bayar University Faculty of Medicine, Department of Parasitology, Manisa, Türkiye
[4]Acıbadem Mehmet Ali Aydınlar University Faculty of Medicine, Department of Medical Biotechnology, Institute of Health Sciences, İstanbul, Türkiye

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ABSTRACT

Objective: Antimicrobial resistance is a real threat to humanity. Pentavalent antimonials are reported non-effective in leishmaniasis treatment today, in countries like India. New treatment options have been assessed worldwide lately. Antimicrobial peptides (AMP) are the leading antibiotic candidates due to their large spectrum, fast efficacy, and low resistance risks. Cathelicidins are the AMP with well-documented antimicrobial activities against bacteria, fungi, and protozoa, over their positively charged membranes. Here, we aim to design cathelicidine-like helical peptides (CLHP), and compare their anti-*Leishmanial* efficacies *in vitro*, with meglumine antimoniate (MA) on *Leishmania tropica*.

Methods: A total of five study [TN-1-5] and two control (MA and non-drug) groups were formed. Cryopreserved *L. tropica* isolate was thawed and cultivated in Novy-MacNeal-Nicolle medium and then in RPMI. Five different CLHPs (TN1-5) were diluted in dimethyl sulphoxide. A total of 150 uL of CLHPs and MA were added into the first wells of the test plaques, followed by serial dilutions that revealed doses within 4 and 512 ug/mL. Then, 100 uL of cultures including $1x10^8$/mL of *L. tropica* promastigotes were added into each well. Viability of promastigotes was checked with XTT, while the parasite count was assessed at 24th and 48th hours.

Results: TN3 was effective at 32 ug/mL. All tested CLHPs exhibited varying degrees of anti-*Leishmanial* activities, except TN5, even at its highest dose.

Conclusion: TN3 showed a particular efficacy against *L. tropica in vitro*. Further studies including *in vivo* testing of the candidate's both efficacy and toxicity are essential.

Keywords: *Leishmania*, antimicrobial peptide, cathelicidin, treatment, Türkiye

ÖZ

Amaç: Antimikrobiyal direnç insanlık için gerçek bir tehdittir. Beş değerlikli antimon bileşiklerinin günümüzde Hindistan gibi ülkelerde leishmaniasis tedavisinde etkili olmadığı bildirilmektedir. Son zamanlarda dünya çapında yeni tedavi seçenekleri değerlendirilmektedir. Antimikrobiyal peptitler (AMP) geniş spektrumları, hızlı etkinlikleri ve düşük direnç riskleri nedeniyle önde gelen antibiyotik adaylarıdır. Katelisidinler, pozitif yüklü membranları üzerinden bakteri, mantar ve protozoonlara karşı iyi belgelenmiş antimikrobiyal aktivitelere sahip AMP'lerdir. Burada, "katelisidin benzeri helikal peptidler" (CLHP) tasarlamayı ve bunların *Leishmania tropica* üzerindeki anti-leishmanial etkinliklerini *in vitro* olarak meglumin antimoniate (MA) ile karşılaştırmayı amaçladık.

Yöntemler: Toplam beş çalışma [TN-1-5] ve iki kontrol antimoniat (MA) ve ilaçsız) grubu oluşturuldu. Kriyoprezerve edilmiş *L. tropica* izolatı çözüldü ve Novy-MacNeal-Nicolle ve ardından RPMI besiyerlerinde kültüre edildi. Beş farklı CLHP (TN1-5) dimetil

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Address for Correspondence/Yazar Adresi: Nihan Ünübol, Acıbadem Mehmet Ali Aydınlar University Faculty of Medicine, Department of Medical Microbiology; Vocational School of Health Services, Medical Laboratory Technician Program, İstanbul, Türkiye
Phone/Tel: +90 (216) 500 44 10 **E-mail/E-Posta:** nihan.unubol@acibadem.edu.tr **ORCID ID:** orcid.org/0000-0003-4644-112X

***Two of the authors of this article (Ö.K. and A.Ö.) is a member of the Editorial Board of this journal. They were completely blinded to the peer review process of the article.**

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sülfoksit içinde seyreltildi. Test plaklarının ilk kuyucuklarına toplam 150 uL CLHP ve MA eklendi ve ardından 4 ve 512 ug/mL arasında dozlar ortaya çıkaran seri seyreltmeler yapıldı. Daha sonra, her bir kuyucuğa 1x10^8/mL *L. tropica* promastigotları içeren 100 uL kültür eklendi. Promastigotların canlılığı XTT ile kontrol edilirken, parazit sayımı 24. ve 48. saatlerde yapıldı.

Bulgular: TN3'ün 32 ug/mL'de etkili olduğu gözlenmiştir. TN5 dışında test edilen tüm CLHP'ler değişen derecelerde anti-leishmanial aktivite sergilerken, TN5'in en yüksek dozunda bile etkisiz kaldığı gözlenmiştir.

Sonuç: TN3'ün *L. tropica*'ya karşı *in vitro* etkinlik gösterdiği belirlenmiştir. Adayın hem etkinliğinin hem de toksisitesinin *in vivo* testleri içeren daha ileri çalışmalarla araştırılması gereklidir.

Anahtar Kelimeler: *Leishmania*, antimikrobiyal peptid, katelisidin, tedavi, Türkiye

INTRODUCTION

Antimicrobial resistance is an urgent global public health problem since it may affect people from all ages or races, as well as the healthcare, veterinary, and agriculture industries. It is estimated that more than 2.8 million antimicrobial-resistant infections occur annually, and 35,000 people die in the United States of America, due to antibiotic resistance in 2019 (1). Main reasons of emerging antimicrobial resistance are inappropriate prescription of antibiotics, their overuse worldwide, both for humans and in agriculture, and availability of few new antibiotics in the market (2). Antimicrobial resistance is not limited to bacterial infections and is documented as an emerging issue for the treatment of parasitic infections as well (3).

Antimicrobial resistance is also an emerging problem for leishmaniasis, as well. Leishmaniasis is a neglected, vector-borne parasitic infection common in tropical and subtropical regions of the world. It is caused by a flagellated protozoan, *Leishmania* sp., and transmitted to humans via the bite of a sandfly (*Phlebotomus* sp. in the Old World and *Lutzomyia* sp. in the New World). *Leishmania* spp. have more than 20 species in nature that are associated with different clinical manifestations in humans. The predominant clinical manifestation is the cutaneous *leishmaniasis* (CL), which is reported in over a million people worldwide annually. Visceral *leishmaniasis* (VL) is seen relatively less common but can be deadly in untreated patients. Leading causative agents are *L. tropica* and *L. major* for CL, and *L. donovani* and *L. infantum* for VL in the Old World. CL has been endemic especially in southeastern Anatolia in Türkiye, which has been reported from western regions as well, lately (3-6).

Treatment of *Leishmaniasis* relies primarily on pentavalent antimonials, which have been commonly used for the treatment of both CL and VL cases worldwide for the last 50 years (4-6). However, due to emerging resistance against them, pentavalent antimonials mostly remain ineffective in leishmaniasis treatment in many countries, such as India today (7). As there is no effective vaccine against leishmaniasis as well as there is an emerging resistance to treatment, there is an urgent need for new drug formulations and treatment options for leishmaniasis. Among these options, both natural and synthetic compounds have been assessed for their anti-leishmanial efficacies *in vitro* and *in vivo* (7,8).

Antimicrobial peptides (AMPs) are positively charged, small peptides with 5-100 amino acid residues, produced in several living organisms as part of the innate immunity, as well as antimicrobial activity. AMPs show large-spectrum anti-microbial efficacy, through either direct elimination of the pathogens (bacteria, viruses, fungi, and parasites) or by modulating the immune response (9-11). Many groups are present within the AMPs; among them, cathelicidins and defensins are the main groups (12). Cathelicidins have well-known antimicrobial activities against not only to bacteria, but also to fungi and protozoa, over their positively charged membranes (10-12).

Many natural AMPs are known to act by disrupting the integrity of cell membranes in protozoa (13). However, some of the AMPs can also interfere with important cellular processes of parasites. AMPs are reported to particularly disrupt Ca2+ distribution on *Leishmania* and consequently disrupt their metabolism. *Plasmodium* is the parasite with which many studies have been carried out with AMPs (14). Some fungal AMPs have an inhibitory effect on histone deacetylase (HDA) in *Plasmodium* species, leading to histone hypermethylation and subsequent alteration of gene expression in the parasite (15). When the effects of AMPs on *Trypanosoma* are evaluated, it is known that they cause the distribution and change of membrane components, stiffness of the cell membrane, and thus cause cell loss (16).

In the light of our previous studies on the antimicrobial activities of antimicrobial peptides, we designed five peptides (named as TN 1 to TN5) inspired by the natural antimicrobial peptide, cathelicidin LL-37; in other words, we designed "cathelicidine-like helical peptides" (CLHP) by imitating the phylogenetically protected sequences of cathelicidins and had them synthesized for our trials. Indeed, using *in vitro* cytotoxicity tests, we observed that the minimum inhibitory concentration values of TN peptides were below HC50 and LC50 on HeLa cells, which indicated their promising roles as antimicrobial drug candidates (17).

In the present study, our aim was to compare the anti-leishmanial efficacies of TN1-5 *in vitro*, with meglumine antimoniate (MA), the current treatment agent, on *L. tropica* promatigotes isolated from a CL patient in Türkiye.

METHODS

Design and Supply of Antimicrobial Peptides

It is well known in the literature that AMPs are generally hydrophobic and positively (+) charged (18). In our study, peptides of 10 to 20 amino acids in length forming α-helix similar to the structure of LL-37 were designed with hydrophobic and positively charged amino acids. The amide group at the C-terminal end causes the peptide to approach the membrane perpendicularly and to be taken up into the cell faster. It is very important that peptides end with an amide group, as this affects the increase in membrane permeability. 3D structures of the designed novel peptides were obtained using the PEP-FOLD3 server (19). The designed peptides were synthesized and purchased from Metabion Company in Germany, according to the guidelines of the Clinical Laboratory Standards Institute (Figure 1).

Leishmania Strains

The *L. tropica* isolate used in this study had been isolated from an 18-year-old female CL patient diagnosed in Manisa Celal Bayar

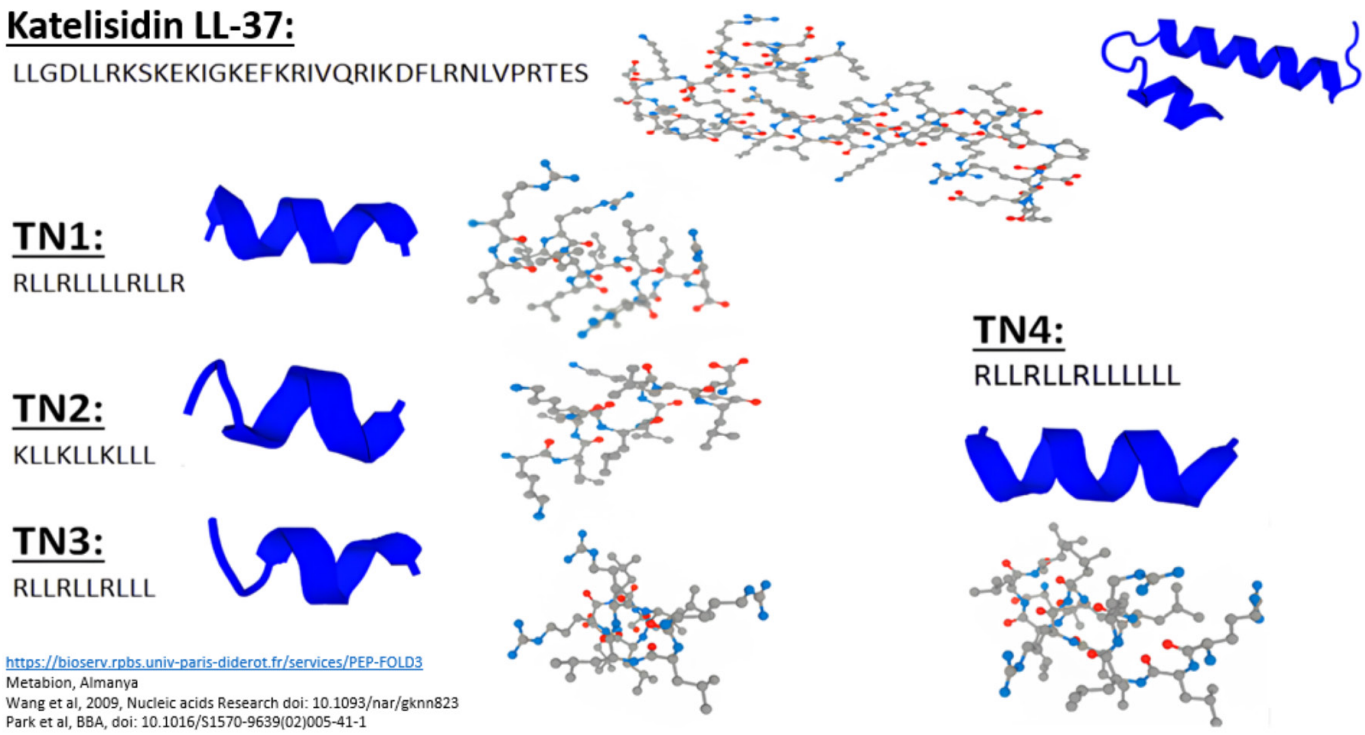

Figure 1. *In silico* figures of cathelicidins and five different cathelicidine-like helical peptides used in the study

University Hospital (MHOM/TR/2011/CBU012). This isolate has been used anonymous (without disclosure of the patient's identity) in similar studies. Initially, the cryopreserved isolate was thawn and inoculated first in Novy-MacNeal-Nicolle medium and then in RPMI medium, including 10% of fetal bovine serum (FBS), penicillin-streptomycin 1% and 0.2% gentamycin. One mililitre of culture medium containing propagated *Leishmania* promastigotes were collected from the culture tubes using fine pipettes and added on a haemacytometer (Neubauer's Thoma slide). Here, the promastigotes seen under the microscope on the four small squares in each corner as well as the ones in the big, central square were counted, multiplied by 10.000 and divided by the number of squares to reach the promastigote number in a mililitre. The final promastigote count was adjusted to 10^8 per mililitre and used for the assessments. A total of 150 uL of CLHPs and MA were added into the first wells of the test plaques, followed by serial dilutions that revealed doses within 4 and 512 ug/mL. Then, 100 uL of cultures including $1x10^8$/mL of *L. tropica* promastigotes were added into each well. Viability of promastigotes was checked with XTT, while the promastigotes were counted under the microscope at 24^{th} and 48^{th} hours.

Assessment of Anti-*Leishmanial* Activity

Activity of AMPs against *L. tropica* promastigotes was assessed by both microscopic counting and the colorimetric cell viability XTT (2,3-bis [2-methyloxy-4- nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide) (Sigma Chemical Co; St. Louis, MO) assay. Promastigotes ($2.5x10^6$/100 mL per well) in the logarithmic growth phase were inoculated into a flat-bottomed 96-well plastic tissue cultured microplates, in triplicate, followed by serial dilutions of each antimicrobial peptide (100 mL). After three days of incubation at 28 °C, 25 mL of XTT (0.2 mg/mL) were added to each well, followed by an additional 3 h of incubation at 37 °C. The optimal density (OD) at 450 nm was measured using an ELISA plate reader. The anti-*leishmanial* activity was further determined by microscopic counting of the live promastigotes for each well and the growth inhibition rate of each concentration was calculated according to the control. The 50% lethal dose (IC50) was evaluated graphically by plotting concentration versus percentage growth inhibition (Figure 2). The anti-leishmanial activity was further determined by microscopic counting of the live promastigotes for each well and the growth inhibition rate of each concentration was calculated according to the control.

Statistical Analysis

Statistical analysis was performed with A Two-Way ANOVA in GraphPad Prism software to calculate the statistical probability. In statistical analyses, difference as $p<0.05$ was considered significant.

RESULTS

The results of the assessments demonstrated that one of the CLHPs, TN3, was effective against *L. tropica* at a lower dose (32 ug/mL) until the end of the 48^{th} hour. All tested CLHPs exhibited varying degrees of anti-leishmanial activities, except TN5 which expressed no efficacy against *L. tropica*, even at its highest dose, neither at 24^{th} nor at 48^{th} hours (Tables 1, 2).

The viability testing of the promastigotes revealed that TN3 managed to kill all promastigotes at the lowest dose (32 ug/mL) at 48^{th} hours. TN4 showed similar efficacy at 128 ug/mL, while TN1 and TN2 at 256 ug/mL. Again, TN5 was found to be ineffective in

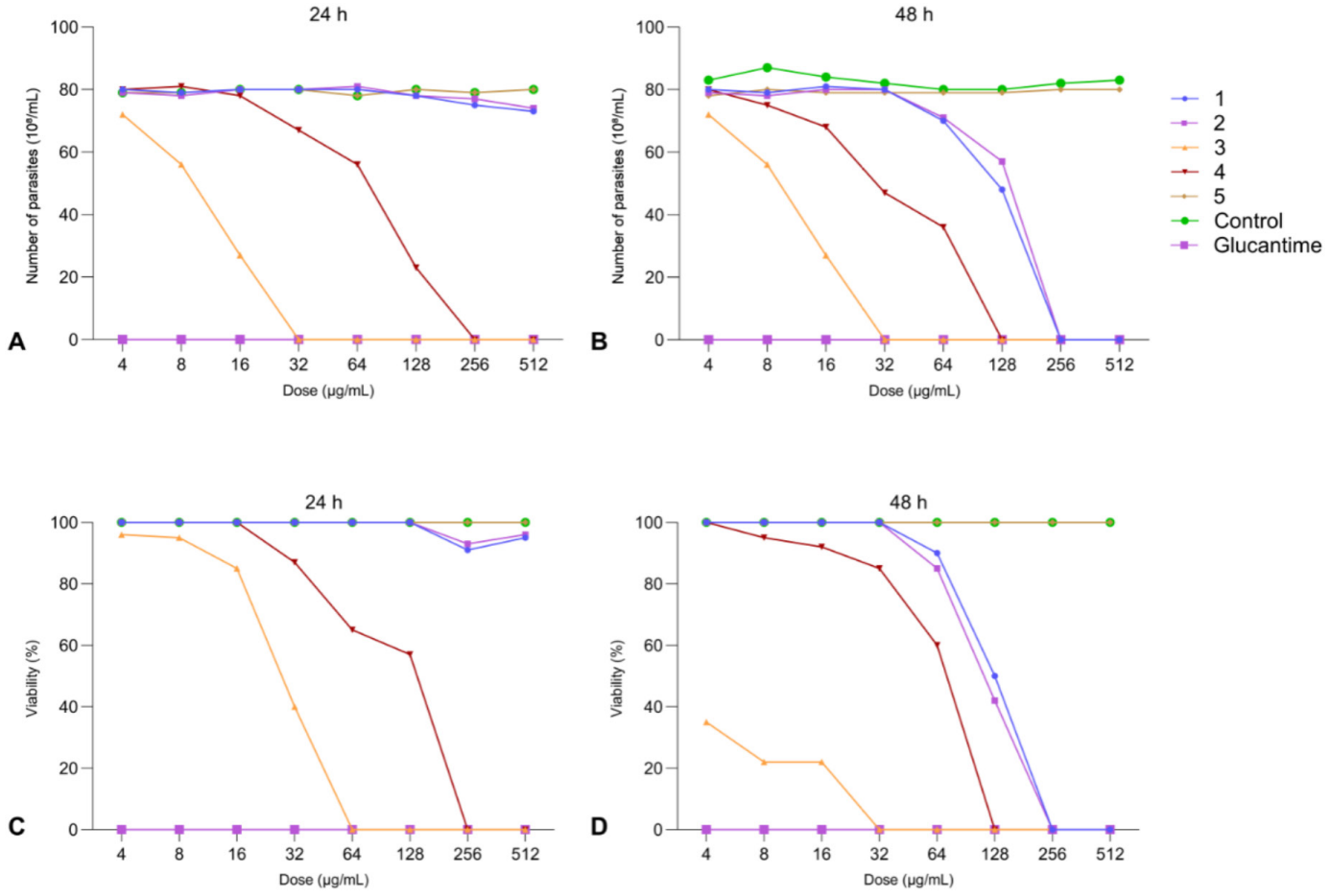

Figure 2. Growth inhibition versus peptide concentration. A and B; Effect of peptide doses on cell number. C and D; Effect of peptide doses on cell viability (%)

Table 1. Live promastigotes count at 24th hour (10^8/mL)

Agents		DOSE (µg/mL)							
		512	256	128	64	32	16	8	4
1.	TN1 RLLRLLLLRLLR	73	75	78	80	80	80	79	80
2.	TN2 KLLKLLKLLL	74	77	78	81	80	80	78	79
3.	TN3 RLLRLLRLLL	0.00	0.00	0.00	0.00	0.00	27	56	72
4	TN4 RLLRLLRLLLLLL	0.00	0.00	23	56	67	78	81	80
5	TN5 RLLLRLLLRLLLR	80.00	79.00	80.00	78.00	80.00	80.00	79	79
6	Control (no drugs)	80.00	79.00	80.00	78.00	80.00	80.00	79	79
7.	Control (Glucantime®)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

killing the parasites even at its highest dose, neither at 24th nor at 48th hours (Tables 3, 4).

DISCUSSION

World Health Organization describes emerging resistance to antibiotics as a global threat for humanity, today (20). It is estimated that antimicrobial resistance (AMR) is directly associated with 1.27 million deaths in the world in 2019, while it will bring an extra cost of health expenditures around 1 trillion USD by the year 2050 (1,20). Main reason of AMR is the abuse and extreme usage of antibiotics, not only in humans but also for animals raised for humans. This abuse of antibiotics may cause not only resistance emergence but also toxicity in humans (2,21,22). AMR is associated with bacteria as well as protozoal infections. For example, pentavalent antimonial compounds which have been used primarily in the treatment of *Leishmaniasis* in the world, are almost non-effective due to emerging resistance in India today, which may have already exceeded 60% (3,7,8). This is also an emerging problem in Türkiye; in addition to unpublished data on

Table 2. Live promastigotes count at 48th hour (10^8/mL)

Agents		DOSE (µg/mL)							
		512	256	128	64	32	16	8	4
1.	**TN1 RLLRLLLLRLLR**	**0.00**	**0.00**	**48**	**70**	**80**	**81**	**79**	**80**
2.	**TN2 KLLKLLKLLL**	**0.00**	**0.00**	**57**	**71**	**80**	**80**	**78**	**79**
3.	**TN3 RLLRLLRLLL**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**27**	**56**	**72**
4	**TN4 RLLRLLRLLLLLL**	**0.00**	**0.00**	**0.00**	**36**	**47**	**68**	**75**	**80**
5	**TN5 RLLLRLLLRLLLR**	**80.00**	**80.00**	**79**	**79**	**79**	**79.00**	**80.00**	**78.00**
6	**Control (no drugs)**	**83.00**	**82.00**	**80.00**	**80.00**	**82.00**	**84.00**	**87**	**83**
7.	**Control (Glucantime®)**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

Table 3. Colorimetric rates of viability of the promastigotes at 24th hour, using XTT method (%) (10^8/mL)

Agents		DOSE (µg/mL)							
		512	256	128	64	32	16	8	4
1.	**TN1 RLLRLLLLRLLR**	95	91	100	100	100	100	100	100
2.	**TN2 KLLKLLKLLL**	96	93	100	100	100	100	100	100
3.	**TN3 RLLRLLRLLL**	0	0	0	0	40	85	95	96
4	**TN4 RLLRLLRLLLLLL**	0	0	57	65	87	100	100	100
5	**TN5 RLLLRLLLRLLLR**	100	100	100	100	100	100	100	100
6	**Control (no drugs)**	100	100	100	100	100	100	100	100
7.	**Control (Glucantime®)**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

Table 4. Colorimetric rates of viability of the promastigotes at 48th hour, using XTT method (%) (10^8/mL)

Agents		DOSE (µg/mL)							
		512	256	128	64	32	16	8	4
1.	**TN1 RLLRLLLLRLLR**	0	0	50	90	100	100	100	100
2.	**TN2 KLLKLLKLLL**	0	0	42	85	100	100	100	100
3.	**TN3 RLLRLLRLLL**	0	0	0	0	0	22	22	35
4	**TN4 RLLRLLRLLLLLL**	0	0	0	60	85	92	95	100
5	**TN5 RLLLRLLLRLLLR**	100	100	100	100	100	100	100	100
6	**Control (no drugs)**	100	100	100	100	100	100	100	100
7.	**Control (Glucantime®)**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

the increase of longer treatment requirements of *Leishmaniasis* patients, there is also an increase in publications on antimonial resistant cases (23-25).

AMPs may become either alternatives or complementary options to conventional antiprotozoal drugs due to their broad-spectrum activity, lower toxicities, and different action mechanisms (26,27). They can exhibit various activities that may directly inhibit the microbial growth or modulate the immune response through the activation of immune cells. They can be totally synthesized or modified chemically, after which they gain higher resistance to proteolytic enzymes (9). Thus, AMPs were initially used to fight the antibiotic resistance of microorganisms, since these compounds were not affected by the mechanisms of bacterial resistance to conventional anti-microbials. Despite their disadvantages such as current high production costs, lower activity in certain conditions (interaction with proteases, etc.) (27,28).

There are almost 3000 natural AMPs identified predominantly from eukaryotes (18,27,28). Among the 990 active registered AMPs today, only 83 of them were assessed as anti-parasitic agents. The leading parasites assessed in AMP studies are *Plasmodium* sp., *Leishmania* sp., *Toxoplasma gondii, Trypanosoma cruzi* and *Cryptosporidium* spp. (26,27).

Previously, various AMPs were assessed for their anti-*Leishmanial* activities, and shown to be effective against clinically-relevant *Leishmania* species, such as *L. amazonensis* (29,30), *L. donovani* (19,31), *L. major* (31), *L. mexicana* and *L. tropica* (32) and *L. infantum* (33). Cathelicidins are important AMPs and human cathelicidin, LL-37, has well-known antimicrobial effects. They interact with the negatively-charged cell membranes of bacteria, fungi and protozoa and kill them, either directly or through pore formation (34). The role of cathelicidins has been investigated in many studies in *Leishmaniasis* as well, mainly using *in vitro* assays, especially in the promastigote stage (34-36). They were found to be involved in the restriction of *Leishmaniasis* in macrophages of CL patients (19), and augmentation of Amphotericin B's macrophage-activating effects (37). In addition, human cathelicidin was shown

to induce an apoptosis-like phenotype in a dose dependent manner in both *L. major* and *L. aethiopica* promastigotes as well as in *L. aethiopica* amastigotes, while they are also involved in the innate immune responses against *Leishmaniasis* in a human primary cell model (34,37).

Here, in the present study, anti-*Leishmanial* efficacy of cathelicidin-like alpha-helical peptides we designed was investigated on *L. tropica* promastigotes *in vitro*. The results of our assessments indicated that one of the assessed AMPs, TN3, showed efficacy against *L. tropica* at a lower dose (32 µg/mL) compared to MA, *in vitro*. Other peptides, TN1, TN2 and TN4 showed efficacy against *L. tropica* as well, but in higher doses, while TN5 exhibited no efficacy in our trial against *L. tropica* even in its highest dose.

In the literature, it is seen that most of the studies conducted with parasites are with natural AMPs such as mellitin, temporin, cathelicidin (10). When the antileishmanial activities in these studies were evaluated, it was seen that melittin inhibited *L. major* at 74.01 mg/mL (34). It has been stated that the antileishmanial effect of cecropin, another antimicrobial peptide, on *L. aethiopica* was greater than 250 mg/mL (35). It was also stated that the antileishmanial effect of temporin antimicrobial peptide was 11.6 uM on *L. major* (36). It has been stated that cathelicidin, another antimicrobial peptide, can kill *L. major* and *L. donovani* by 50% even at high concentrations (37). Here, we observed that TN3 exhibited particular antileishmanial efficacy at a relatively lower dose (32 ug/mL), which is similar to natural derivatives and even more effective than cathelicidin and cecropin.

CONCLUSION

The results of this *in vitro* study indicate TN3 as a promising anti-*Leishmanial* agent. Further studies involving its *in vivo* efficacy and toxicity are warranted to unveil its potential as a treatment option for leishmaniasis in future. Regarding their efficacy in resistant microorganisms, AMPs may soon become the leading weapons of our arsenal against life-threatening microbial agents.

***Information:** This isolate has been used anonymous (without disclosure of the patient's identity) in similar studies.

*Ethics

Ethics Committee Approval: The *Lesihmania* strains used in this article are study materials that have been stored in liquid nitrogen for research purposes for many years, and are research samples that have been stored and used with the identities of the patients from whom they were isolated deleted. In this context, there is no need to obtain ethics committee approval.

Informed Consent: Not necessary.

*Authorship Contributions

Concept: N.Ü., İ.Ç., T.P., Ö.K., A.Ö., T.K., Design: A.Ö., T.K., Data Collection or Processing: N.Ü., İ.Ç., T.K., Analysis or Interpretation: N.Ü., İ.Ç., T.P., Ö.K., A.Ö., T.K., Literature Search: N.Ü., Ö.K., Writing: N.Ü., Ö.K.

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A Comprehensive Bibliometric Analysis of Research Trends About Congenital Toxoplasmosis

Konjenital Toksoplazmoz Hakkındaki Araştırma Eğilimlerinin Kapsamlı Bibliyometrik Analizi

Mustafa Bağcı[1], Özlem Ulusan Bağcı[2]

[1]İzmir Atatürk Training and Research Hospital, Department of Obstetrics and Gynecology, İzmir, Türkiye
[2]Ankara University Faculty of Medicine, Department of Medical Parasitology, Ankara, Türkiye

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ABSTRACT

Objective: *Toxoplasma gondii* is an obligate intracellular protozoon that infects approximately one-third of the human population. The parasite could transmit from mother to fetus in cases of acute infection during pregnancy and cause complications in the fetus. The bibliometric analysis is a popular research area that evaluates all the studies indexed in particular databases on a subject.
Methods: This article puts forth bibliometric review of the literature on maternal and congenital toxoplasmosis research indexed in the Web of Science database between 1945 and 2024. VOS viewer, Web of Science and MS Office Excel 17 programs were used in the study.
Results: The results of the search showed 1476 publications. The countries that most contributed to the literature were France (n=306, 20.73%), the USA (n=229, 15.52%), and Brazil (n=146, 9.89%). The most cited country was also France (n=10271, 35.52%), followed by the USA (n=9113, 31.51%), and England (n=2611, 9.03%). The top three countries by number of citations per document were Denmark (44.88), the USA (39.79) and France (33.57). The five departments with the most publications are Pediatrics (20.26%), General Internal Medicine (18.16%), Infectious Diseases (16.8%), Obstetrics (14.57%), and Immunology (11.86%). Wallon M. (n=57), Peyron F. (n=49), Thulliez P. (n=36) and Vilena I. (n=36) were the leading authors in terms of contribution to the literature. The five most published journals were Pediatric Infectious Disease Journal (3.66%), Journal of Clinical Microbiology (2.78%), Lancet (2.3%), Presse Medicale (1.76%), and American Journal of Obstetrics and Gynecology (1.63%).
Conclusion: France is one of the countries that pays the most attention to congenital toxoplasmosis and compatible with this, in our study, the country with the highest number of studies on congenital toxoplasmosis was France. It is thought that drawing more attention to this issue and conducting more studies in countries where the disease is common might yield successful results, as in France.
Keywords: *Toxoplasma gondii*, toxoplasmosis, congenital, pregnancy, bibliometric

ÖZ

Amaç: *Toxoplasma gondii*, insan popülasyonunun yaklaşık üçte birini enfekte eden zorunlu hücre içi bir protozoondur. Hamilelik sırasında akut enfeksiyon geçirilmesi durumunda parazit anneden fetüse geçebilir ve fetüste birtakım komplikasyonların ortaya çıkmasına neden olabilmektedir. Bibliyometrik analiz, bir konu hakkında veri tabanlarında indeklenen tüm yayınları araştıran popüler bir araştırma türüdür.
Yöntemler: Çalışmamız, 1945 ile 2024 yılları arasında Web of Science veri tabanında indekslenen maternal ve konjenital toksoplazmoz araştırmalarına ilişkin literatürün bibliyometrik bir incelemesini ortaya koymaktadır. Çalışmada VOS viewer, Web of Science ve MS Office Excel 17 programları kullanılmıştır.
Bulgular: Araştırmamız sonucunda 1945 ile 2024 yılları arasında maternal ve konjenital toksoplazmoz konularında toplam 1476 yayın saptanmıştır. Literatüre en çok katkı sağlayan ülkeler Fransa (n=306, %20,73), ABD (n=229, %15,52) ve Brezilya'dır (n=146, %9,89). Aynı zamanda en çok atıf yapılan ülke Fransa (n=10271, %35,52) olurken, bunu ABD (n=9113, %31,51) ve İngiltere (n=2611, %9,03) takip etmiştir. Yayın başına yapılan atıf sayısına göre ilk üç ülke Danimarka (44,88), ABD (39,79) ve Fransa'dır (33,57). En fazla yayın yapılan beş bölüm sırasıyla Çocuk (%20,26), Genel Dahiliye (%18,16), Enfeksiyon Hastalıkları (%16,8), Kadın Doğum (%14,57) ve İmmünolojidir (%11,86). Wallon M. (57), Peyron F. (49), Thulliez P. (36) ve Vilena I. (36) literatüre katkı

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Address for Correspondence/Yazar Adresi: Özlem Ulusan Bağcı, Ankara University Faculty of Medicine, Department of Medical Parasitology, Ankara, Türkiye
Phone/Tel: +90 539 860 03 31 **E-mail/E-Posta:** drozlemulusan@gmail.com **ORCID ID:** orcid.org/0000-0002-9695-5703

Türkiye PARAZİTOLOJİ Dergisi

açısından önde gelen yazarlardır. En fazla yayın yapılan beş dergi sırasıyla Pediatric Infectious Disease Journal (%3,66), Journal of Clinical Microbiology (%2,78), Lancet (%2,3), Presse Medicale (%1,76) ve American Journal of Obstetrics and Gynecology'dir (%1,63).
Sonuç: Konjenital toksoplazmoz konusuna en çok önem veren ülkelerden biri olan Fransa, bununla uyumlu olarak en fazla çalışmanın yapıldığı ülke olmuştur. Hastalığın yaygın olduğu ülkelerde bu konuya daha fazla dikkat çekilmesinin ve daha fazla çalışma yapılmasının Fransa'da olduğu gibi başarılı sonuçlar verebileceği düşünülmektedir.
Anahtar Kelimeler: *Toxoplasma gondii*, toksoplazmoz, konjenital, gebelik, bibliyometrik

INTRODUCTION

Toxoplasmosis is caused by the etiological agent *Toxoplasma gondii* (*T. gondii*), an obligate intracellular protozoon, that could infect all warm-blooded animals. The parasite infects approximately one-third of the human population during their lifespan (1). The Centers for Disease Control and Prevention reported that toxoplasmosis is the second most common cause of death due to foodborne illnesses and the fourth leading cause of hospitalizations based on foodborne diseases (2). The prevalence varies from 10% to 80% between countries across the globe and even between different communities within a country based on geographical changes and the eating habits of humans. Toxoplasmosis is most prevalent in Brazil (77.5%) and followed by Iran (63.9%), Colombia (63.5%), and Cuba (61.8%), respectively (3,4).

The protozoa have a complex life cycle, including cats as definitive hosts and other warm-blooded animals as intermediate hosts. The asexual life cycle occurs in the intermediate hosts, while the cats harbor both sexual and asexual life cycles. Humans could be intermediate hosts in the life cycle of the parasite, and they are most commonly infected by ingesting bradyzoites in undercooked or raw meat or ingesting oocysts in improperly washed vegetables and fruits (5). Many patients infected with *T. gondii* are asymptomatic. On the other hand, it could cause serious complications in immunosuppressed patients, and the parasite could transmit from mother to fetus in terms of acute infection gained during pregnancy. The rate of transmission is low in the first trimester due to the intact structure of the placenta. But in cases of transmission, the outcomes may be very serious, such as spontaneous abortion, stillbirth, or congenital anomalies, including hydrocephalus, intracranial calcification, and retinochoroiditis. Although transition rates increase towards the end of pregnancy, the severity of sequelae decreases (6).

Screening for toxoplasmosis before and during pregnancy and taking necessary precautions by making an early diagnosis in cases of acute infection is very important in preventing congenital toxoplasmosis cases. Therefore, some countries have included toxoplasmosis in their national screening programs. There are two preventive strategies against congenital toxoplasmosis, including prenatal and neonatal. Prenatal programs combine education with serological testing and aim to prevent acute infection during pregnancy, early diagnosis in cases of acute infection, and blockage of transmission from mother to fetus. Prenatal screening programs are conducted on a national scale in France, Austria, and Slovenia. In France, all pregnant women have been screened monthly until delivery since 1992, and a prominent decrease has been achieved in the prevalence of toxoplasmosis. The incidence of acute toxoplasmosis during pregnancy has decreased from 5.4 to 1.6 per 1000 susceptible women (6,7). Prenatal screening is cost-saving in countries where the incidence is high, and this situation also encourages efforts to perform these programs even in countries where the incidence is low (7).

The bibliometric analysis is a popular research area that evaluates all the studies indexed in particular databases and the research tendencies of countries and individuals on a subject. Therefore, by revealing the research tendencies on a subject, one can get an idea of its importance and the level of knowledge on that subject. There are few toxoplasmosis bibliometric analyses available in the literature (8). Nevertheless, bibliometric analysis of publications on congenital and maternal toxoplasmosis indexed in the Web of Science database has not been discovered in any study. With this study, we aim to put forth the research trends about maternal and congenital toxoplasmosis by evaluating all the studies indexed in the Web of Science database and detecting the contribution of countries, authors, or journals to this significant parasitic disease.

METHODS

Data Collection

This research is a bibliometric publication in the fields of maternal and congenital toxoplasmosis, including all the documents indexed in the Web of Science Core Collection database between 1945 and 2024. Using OR between the terms, the advanced search function of the database examined the titles of the documents containing the following terms: "*Toxoplasma* pregnancy", "toxoplasmosis pregnancy", "*T. gondii* pregnancy", "maternal toxoplasmosis", "congenital *Toxoplasma*", and "congenital toxoplasmosis". All the document types were included in the analysis. Excluded from consideration were documents with titles that contained animal names like murine, mouse, cats, rat, pig, etc.

Statistical Analysis

The documents were downloaded to the computer in plain text format as full records, cited references, and transferred to VOSviewer program 1.6.20 for forward analysis (9). Visualizations and graphics were made in both the VOSviewer program and Web of Science. MS Office Excel 2017 was used for the the preparation of the tables and calculation of data frequencies and percentages. The Spearman and Pearson correlation tests were utilized to assess the association between research data using the IBM SPSS Statistics 23 Program.

Ethical approval and informed consent are not required because neither humans nor animals are included in the study.

RESULTS

The results of the search in the Web of Science database showed 1476 publications about maternal and congenital toxoplasmosis between 1945 and 2024. Although the number of publications showed a fluctuating trend since 1945, it gradually increased and peaked in 2010 with 50 documents, followed by 2021 and 2019 with 49 and 45 documents, respectively. One thousand four hundred and seventy six publications have been cited 28917 times by 10135 publications. When self-citations were excluded, the

number of citations was 19707, made by 9044 publications. The average number of citations per publication was 19.59 (Figure 1).

The first document was about congenital toxoplasmosis and was conducted by Ricci HN in 1946 (10). The most indexed document type was the original article (67.07%), followed by letter (9.69%), meeting abstract (8.94%), and review article (6.1%). Most of the publications were published in Science Citation Index Expanded (SCI-E) journals (90.65%), while 6.78% in Emerging Sources Citation Index (ESCI), and 6.03% in Conference Proceedings Citation Index-Science (CPCI-S) journals. The most contributor five departments were Pediatrics (20.26%), General Internal Medicine (18.16%), Infectious Diseases (16.8%), Obstetrics Gynecology (14.57%) and Immunology (11.86%), respectively (Figure 2).

The researches were conducted in 106 different countries. France was the leading country with 306 documents, consisting of 20.73% of all the publications and followed by the USA (15.52%), Brazil (9.89%), Italy (6.1%), and England (5.29%). The most cited country was also France (35.52%), followed by the USA (31.51%), England (9.03%), Brazil (6.54%), and Italy (5.78%). The leading country was Denmark (44.88%), taking into consideration the number of citations per document, followed by the USA (39.79%)

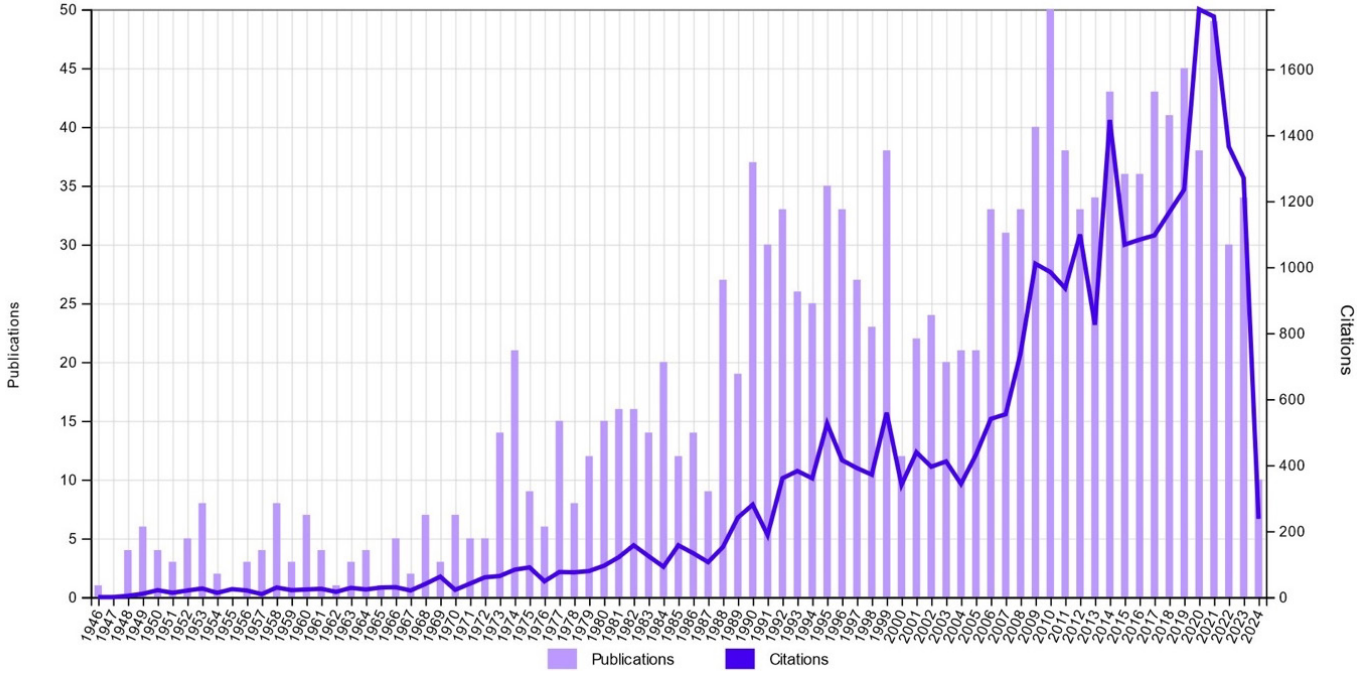

Figure 1. The distribution of publications and citations about maternal and congenital toxoplasmosis between 1945 and 2024

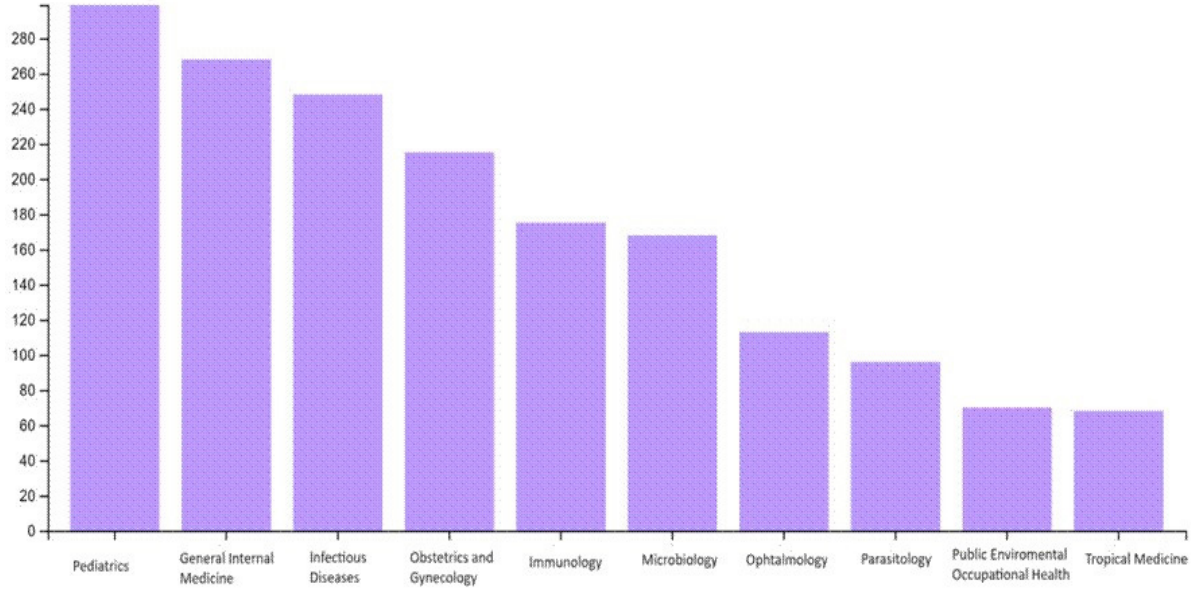

Figure 2. The most contributor 10 departments to the literature about maternal and congenital toxoplasmosis

and France (33.57%), respectively (Table 1). While a strong linear positive correlation was detected between the number of documents published from different countries and the total number of citations (r=0.912, p=0.01), there was no correlation between the number of documents and the average number of citations (p=0.242).

A total of 5065 authors contributed to 1476 publications. The most contributor author was Wallon M with 57 documents, followed by Peyron F, Thulliez P and Vilena I with 49, 36, and 36 documents, respectively (Figure 3). The most published five journals were Pediatric Infectious Disease Journal (3.66%), Journal of Clinical Microbiology (2.78%), Lancet (2.3%), Presse Medicale (1.76%), and American Journal of Obstetrics and Gynecology (1.63%), respectively (Figure 4).

The most cited document was an original article titled "Toxoplasmosis snapshots: Global status of *T. gondii* seroprevalence and implications for pregnancy and congenital toxoplasmosis" conducted by Pappas et al. (3) published in 2009 in the International Journal for Parasitology, with a total number of 672 citations and followed by Desmonts G and Couvreur J, 1974

Table 1. The countries that published at least 23 publications about maternal and congenital toxoplasmosis

Countries	Documents		Citations		Without self-citations		Average citations per document	H-index
	N	%	N	%	N	%		
France	306	20.73	10271	35.52	8636	43.82	33.57	53
USA	229	15.52	9113	31.51	8499	43.13	39.79	50
Brazil	146	9.89	1891	6.54	1539	7.81	12.95	22
Italy	90	6.1	1670	5.78	1591	8.07	18.56	20
England	78	5.29	2611	9.03	2469	12.53	33.47	27
Germany	48	3.25	493	1.70	480	2.44	10.27	12
Switzerland	48	3.25	1204	4.16	1164	5.91	25.08	16
Austria	33	2.24	955	3.30	913	4.63	28.94	15
Denmark	33	2.24	1481	5.12	1420	7.21	44.88	21
Belgium	29	1.97	834	2.88	803	4.07	28.76	15
China	29	1.97	382	1.32	325	1.65	13.17	13
Japan	23	1.56	148	0.51	133	0.67	6.43	6
Scotland	23	1.56	450	1.56	432	2.19	19.57	12

N: Number, percentages are calculated based on total numbers documents (n=1476), citations (n=28917), and without self-citations (n=19707) in WOS database

Figure 3. The most contributor 10 authors in the literature about maternal and congenital toxoplasmosis

(n=479), Montoya and Remington, 2008 (n=424), Torgerson and Mastroiacovo, 2013 (n=396) and Brown et al. (14) (n=356) (Table 2) (3,11-14). The most recurring keywords in the documents were "congenital toxoplasmosis" with 253 repetetives, followed by "*T. gondii*", "toxoplasmosis" and "pregnancy" with 167, 161 and 114 repetetives, respectively (Figure 5).

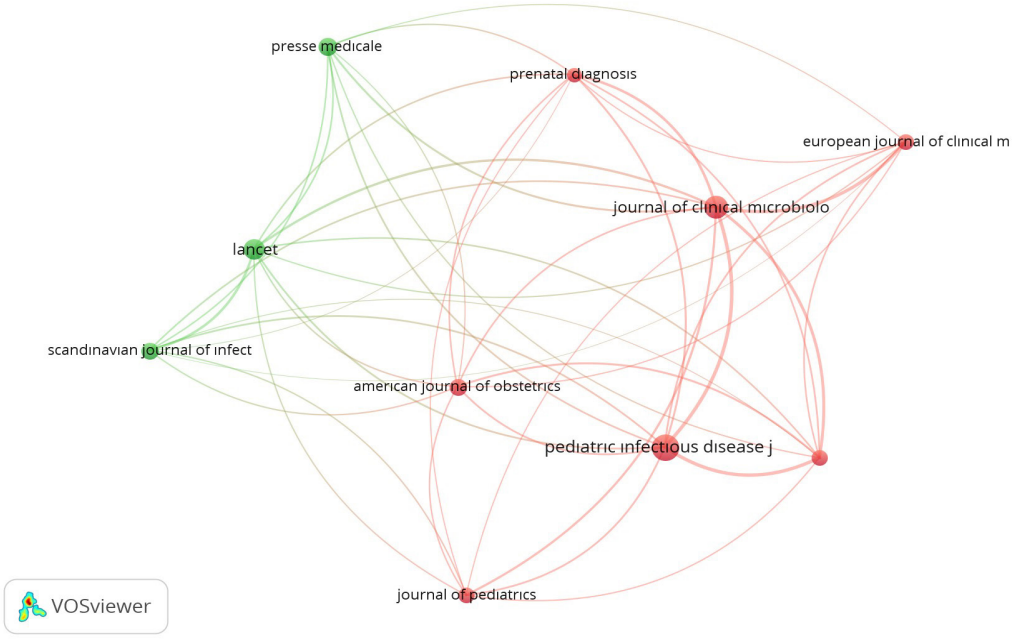

Figure 4. The network visualization of the journals that published at least 17 documents about maternal and congenital toxoplasmosis

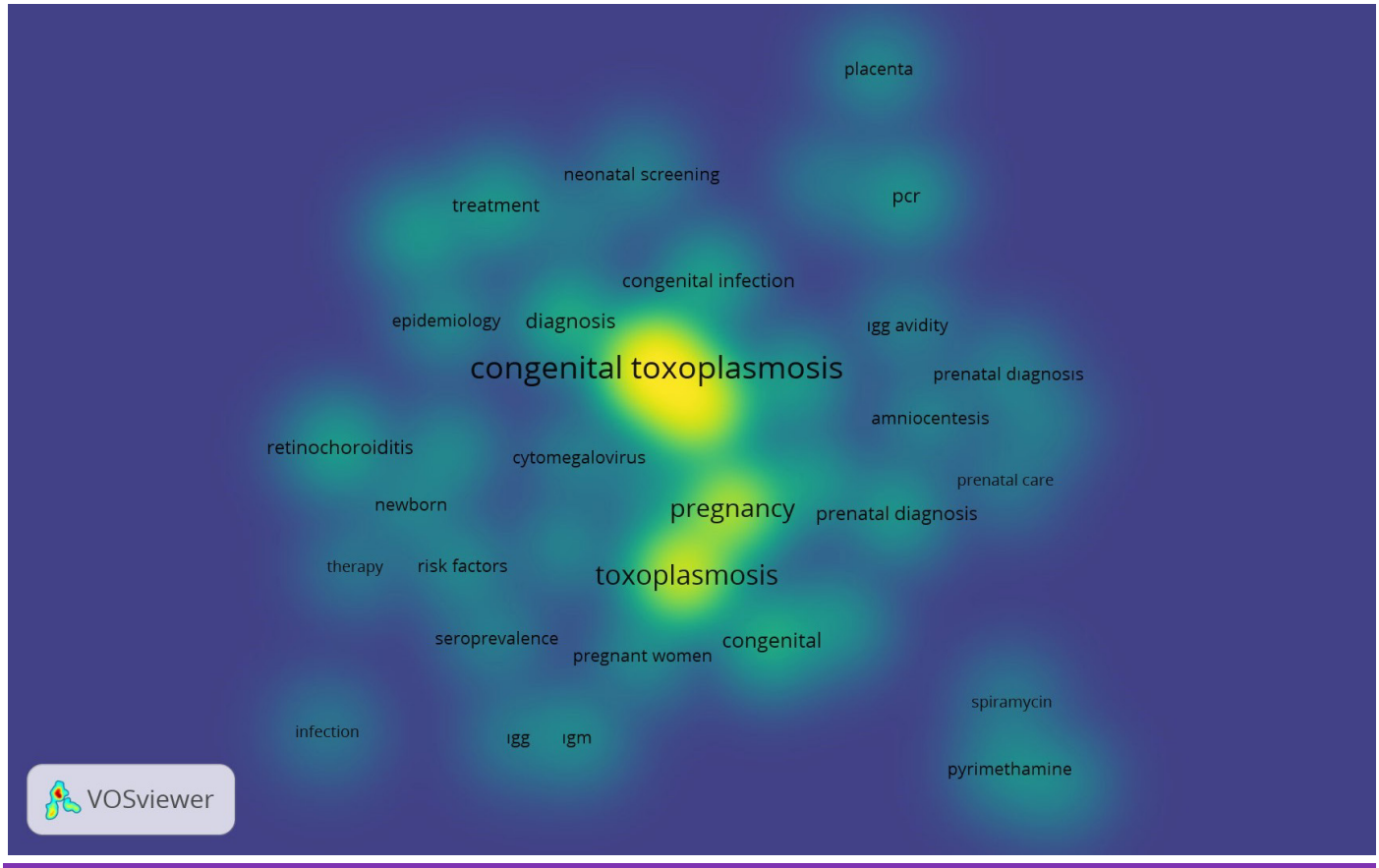

Figure 5. The density visualization of the most repetetive keywords in the literature about maternal and congenital toxoplasmosis

Table 2. The characteristics of the most cited 10 documents about maternal and congenital toxoplasmosis

No	Document title	Authors	Journal	Publication year	Number of citations	Average citations per year	Ref.
1	Toxoplasmosis snapshots: Global status of *Toxoplasma gondii* seroprevalence and implications for pregnancy and congenital toxoplasmosis	Pappas G, Roussos N, Falagas ME	International Journal for Parasitology	2009	672	42	3
2	Congenital toxoplasmosis. A prospective study of 378 pregnancies	Desmonts G, Couvreur J	The New England Journal of Medicine	1974	479	9.39	11
3	Management of *Toxoplasma gondii infection* during pregnancy	Montoya JG, Remington JS	Clinical Infectious Diseases	2008	424	24.94	12
4	The global burden of congenital toxoplasmosis: a systematic review	Torgerson PR, Mastroiacovo P	Bulletin of the World Health Organization	2013	396	33	13
5	Maternal exposure to toxoplasmosis and risk of schizophrenia in adult offspring	Brown AS, Schaefer CA, Quesenberry CP Jr, Liu L, Babulas VP, Susser ES	American Journal of Psychiatry	2005	356	17.8	14
6	Prenatal management of 746 pregnancies at risk for congenital toxoplasmosis	Daffos F, Forestier F, Capella-Pavlovsky M, Thulliez P, Aufrant C, Valenti D, Cox WL	The New England Journal of Medicine	1988	345	9.32	15
7	Genotype of 86 *Toxoplasma gondii* isolates associated with human congenital toxoplasmosis, and correlation with clinical findings	Ajzenberg D, Cogné N, Paris L, Bessières MH, Thulliez P, Filisetti D, Pelloux H, Marty P, Dardé ML	The Journal of Infectious Diseases	2002	336	14.61	16
8	Development of adverse sequelae in children born with subclinical congenital *Toxoplasma* infection	Wilson CB, Remington JS, Stagno S, Reynolds DW	Pediatrics	1980	333	7.4	17
9	Effectiveness of prenatal treatment for congenital toxoplasmosis: a meta-analysis of individual patients' data	SYROCOT (Systematic Review on Congenital Toxoplasmosis) study group; Thiébaut R, Leproust S, Chêne G, Gilbert R	Lancet	2007	304	16.89	18
10	Neonatal serologic screening and early treatment for congenital *Toxoplasma gondii* infection	Guerina NG, Hsu HW, Meissner HC, Maguire JH, Lynfield R, Stechenberg B, Abroms I, Pasternack MS, Hoff R, Eaton RB, Grady GF, and the New England Regional Toxoplasma Working Group	The New England Journal of Medicine	1994	299	9.65	19

DISCUSSION

T. gondii, which infects approximately one-third of the world's population, is an important parasitic agent, especially for patients with suppressed immune systems and pregnant women. In cases of acute infection acquired during pregnancy, there is a risk of the agent passing to the fetus through the placenta and affecting the fetus at varying degrees of severity depending on the week of pregnancy. In this study, a bibliometrical analysis was performed on the maternal and congenital toxoplasmosis publications between 1945 and 2024. The present study may be beneficial for determining the present status of studies on congenital toxoplasmosis around the world.

Some countries have a national screening program to fight against congenital toxoplasmosis, and one of the countries that pays the most attention to this issue is France. There are countries where prenatal screening for toxoplasmosis is compulsory, such as Austria, Belgium, France, Greece, Slovenia, and Slovakia. Screening programs in countries such as Bulgaria, Czechia, Germany, and Hungary are implemented voluntarily. The prenatal screening program that has been organized in France at a national scale, including the implementation of serological

screening of pregnant women starting in the first trimester and follow-up of seronegative women during pregnancy, could be one of the most comprehensive programs in the world (6). While 204 congenital toxoplasmosis cases were diagnosed in 2012, this number decreased to 110 in 2020 in France (20,21). In our study, the country with the highest number of studies on maternal and congenital toxoplasmosis was France, with 306 publications, confirming the importance given to this issue in France. Wallon M. with 57 publications and Peyron F. with 49 publications, both of them from Lyon, France, were the researchers who published the most on congenital toxoplasmosis. France's leadership in publications about maternal and congenital toxoplasmosis may be due to the importance given to national screening programs as well as research funding, infrastructure, and public health policies of the country.

In a bibliometric study investigating toxoplasmosis publications around the world between 2000 and 2016, the country where the most studies were conducted was found to be the USA (8). In another study examining congenital toxoplasmosis publications in the Web of Science database between 1900 and 2012, the USA ranked first in terms of the number of documents and citations, and the number of institutions supporting the studies (22). However, in our study examining congenital toxoplasmosis studies, France ranked first. The difference between the leading countries in terms of the number of studies may be due to the studies covering different time periods and the selection of different keywords.

The USA, where toxoplasmosis causes hundreds of deaths and thousands of hospitalizations and there are an estimated 300-4000 cases of congenital toxoplasmosis each year, is placed in the second rank after France in terms of the total number of publications with 229 documents (23). Brazil placed in the third rank after the USA. The dominance of the two countries could be explained by the fact that the disease is of great interest in both countries, as France has the highest prevalence in Europe due to the habit of consuming undercooked meat, and tropical areas of South America such as Brazil have the highest burden of the parasitic disease worldwide due to the high numbers of infected cats, the presence of highly virulent toxoplasma genotypes, and suitable climatic conditions for oocyst survival (24,25). France (35.52%) and the USA (31.51%) have been the most cited countries for maternal and congenital toxoplasmosis.

Study Limitations

One study limitation might be that only papers indexed in the WOS database were assessed. A publication bias may result from the WOS database's status of only including journals with high impact factors and indexes in SCI-E, ESCI, CPCI-S, Social Science Citation Index, and Book Citation Index-Science.

CONCLUSION

Many studies have been carried out on congenital toxoplasmosis in the world, especially in France, and very important steps have been taken in the fight against toxoplasmosis. Studies in this field have found toxoplasmosis screenings during pregnancy cost-saving when compared with the cost burden of the disease. For this reason, it is thought that it would be beneficial to make more studies about maternal and congenital toxoplasmosis and implement screening programs in other countries.

*Ethics

Ethics Committee Approval: Ethical approval and informed consent are not required because neither humans nor animals are included in the study.

Informed Consent: Ethical approval and informed consent are not required because neither humans nor animals are included in the study.

*Authorship Contributions

Surgical and Medical Practices: M.B., Ö.U.B., Concept: M.B., Ö.U.B., Design: M.B., Ö.U.B., Data Collection or Processing: M.B., Analysis or Interpretation: Ö.U.B., Literature Search: M.B., Ö.U.B., Writing: M.B., Ö.U.B.

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

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Parasitic Infections and Host Tissue Response in Histopathology: A Rare Retrospective Research Study from Rural India

Histopatolojide Paraziter Enfeksiyonlar ve Konak Doku Yanıtı: Kırsal Hindistan'dan Nadir Bir Retrospektif Araştırma

Mani Krishna, Seema Dayal

Uttar Pradesh University of Medical Sciences, Department of Pathology, Etawah, India

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ABSTRACT

Objective: Parasite are living organisms which survive on another living being for their nourishment and survival. When these parasites resides on human body, they bring about inflammatory response. This inflammatory response leads to tissue reaction. Tissue response on microscopy appear as an eosinophilia, abscess and granulomas. This study was planned with the objective to know the frequency of parasite infection, tissue response in parasite infection and its comparison in terms of variables like age, sex and the type of parasite.

Methods: This is a retrospective study, conducted in the department of pathology. A total of 26 cases of parasitic infections in human specimens reported in our department from January 2008 to December 2019 were included in this study. On all archived cases hematoxylin and eosin and where ever required periodic acid schiff was applied. These slides were thoroughly examined and clinicopathological correlation was studied.

Results: Age range of patients was 5 years to 70 years. Maximum number of patients were belonging to 11-20 year age group. Male to female ratio was 1:2. Among the 26 cases, there were 9 cases (34.62%) of hydatid cyst, six cases of *Entamoeba histolytica* (23.07%), four cases of *Enterobious vermicularis* (15.38%), and two cases (7.69%) each of *Ascaris lumbricoides*, filaria and cysticercosis respectively. A specific tissue response seen in cysticercosis having chronic inflammatory cells, palisaded epithelioid cells granuloma and giant cell reaction while other showed inflammatory cells infiltration.

Conclusion: Clinically diagnosis of parasitic infection in each and every case is not possible, similarly radiological investigation is also suggestive only. Histopathology examination is the benchmark investigation to diagnose parasite infection and tissue reaction to the host. Histopathology examination must be implicated in every case to identify parasite and tissue reaction so that the patients can be managed accordingly before the complications rises.

Keywords: Parasite, histopathology, tissue reaction

ÖZ

Amaç: Parazit, beslenmek ve hayatta kalmak için başka bir canlı üzerinde hayatta kalan canlı organizmalardır. Bu parazitler insan vücudunda bulunduklarında iltihabi tepkiye neden olurlar. Bu enflamatuvar yanıt doku yanıtına yol açar. Doku yanıtı mikroskobik olarak eozinofili, apse ve granülomlar şeklinde görülür. Bu çalışma, parazit enfeksiyonu sıklığını, parazit enfestasyonunda doku yanıtını ve yaş, cinsiyet ve parazit türü gibi değişkenler açısından karşılaştırılmasını bilmek amacıyla planlanmıştır.

Yöntemler: Bu çalışma patoloji anabilim dalında yürütülen retrospektif bir çalışmadır. Ocak 2008'den Aralık 2019'a kadar bölümümüze bildirilen insan örneklerinde görülen toplam 26 parazit enfeksiyonu olgusu bu çalışmaya dahil edilmiştir. Arşivlenen tüm olgulara hematoksilen ve eosin ve gerekli durumlarda periyodik asit schiff uygulanmıştır. Bu lamlar ayrıntılı olarak incelenmiş ve klinikopatolojik korelasyon çalışılmıştır.

Bulgular: Hastaların yaş aralığı 5 ile 70 arasında değişmekteydi. En fazla hasta 11-20 yaş grubuna aitti. Erkek/kadın oranı 1:2 idi. Yirmi altı olgu arasında sırasıyla 9 olgu (%34,62) kist hidatik, altı olgu (%23,07) *Entamoeba histolytica*, dört olgu (%15,38) *Enterobious vermicularis* ve ikişer olgu (%7,69) *Ascaris lubricoides*, filaria ve sistiserkoz vardı. Sistiserkozda kronik enflamatuvar hücreler, palizatlanmış epiteloid hücre granülomu ve dev hücre reaksiyonu gibi spesifik bir doku yanıtı görülürken, diğerlerinde enflamatuvar hücre infiltrasyonu görülmüştür.

Sonuç: Her olguda parazit enfestasyonunun klinik olarak teşhisi mümkün değildir, benzer şekilde radyolojik inceleme de sadece düşündürücüdür. Histopatoloji incelemesi, parazit enfestasyonunu ve konakçıya karşı doku reaksiyonunu teşhis etmek için kriter incelemedir. Parazit ve doku reaksiyonunu tanımlamak için her olguda histopatoloji incelemesi yapılmalıdır, böylece hastalar komplikasyonlar artmadan önce uygun şekilde tedavi edilebilir.

Anahtar Kelimeler: Parazit, histopatoloji, doku reaksiyonu

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Address for Correspondence/Yazar Adresi: Seema Dayal, Uttar Pradesh University of Medical Sciences, Department of Pathology, Etawah, India
E-mail/E-Posta: seemadayal5@gmail.com **ORCID ID:** orcid.org/0000-0001-8282-2507

Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

The parasite is a living organism that lives in or on another living organism which is known as host. Parasite obtains nourishment and protection from host and consequently causes infections leading to tissue reaction. Tissue reaction causes inflammatory reaction which occurred due to human parasites. Human parasites were classified in few major divisions, including Protozoa, Fungi, Platyhelminthes (cestodes, trematodes), Nematode and Arthropod (insects, spiders, mites, tick, etc.). The common parasitic infection are like amoeba in the intestine causing amoebic colitis (1), filariasis in scrotum (2), *Echinococcus* causing hydatid cyst in liver (3) and Cysticercosis caused by larval cysts of the tapeworm *Taenia solium* (4).

Histomorphological features are helpful in the diagnosis of human and animal diseases of different etiologies. In many cases, parasitic diseases are not properly recognized on routine laboratory investigations. An insufficient diagnosis often leads to wrong or ineffective treatment. On histopathology, these parasites produce tissue responses which provides clue in the identification of parasites and finally reaching at the correct diagnosis, thus histopathological examination of affected organs or tissues facilitates a concise and accurate diagnosis which is helping in planning the precise and correct treatment (1,5).

Histopathology stains like haematoxylin and eosin and periodic acid schiff are not only fruitful in the identification of the parasite but also in the predicting host tissue response (2,6). As per authors knowledge no such study is performed till date so, this study was planned with the objective to know the tissue response against the parasite infection and its comparison in terms of variables like age, sex, type of parasite.

METHODS

This was a retrospective study, conducted in the department of pathology. All the 26 cases of parasitic infections in human specimens reported in our department from January 2008 to December 2019 were included in this study. All the clinical details like age and sex of patients, and site of lesion along with radiological findings where ever available were noted from the histopathology record register.

All archived H and E stained slides of each case were thoroughly examined for histological identification of the parasite and the various tissue reactions elicited against each parasitic infection. Special stains such as PAS were also performed where ever required to confirm diagnosis, such as in cases of amoebic colitis.

Statistical Analysis

Statistical analysis was done by percentage.

Ethical clearance was taken from Institutional Ethical Committee of Uttar Pradesh University with ethical clearance no: 228/2018.

Consent of patients were not taken, as we had received tissue for histopathology examination and details were obtained from the patients records.

RESULTS

In the present study over a period of 12 years there were 26 cases of parasitic lesions identified on histopathological examination.

Age range of patients was 5 years to 70 years. Nine patients were male while 17 were females with M:F ratio of about 1:2. Females were more infected by parasites.

Maximum number of patients were belonging to 11-20 year age group while minimum cases were belonging to 21-30 year age group (Table 1).

Frequency of various parasitic infections is summarised in (Table 2). Among the 26 cases, maximum cases were of hydatid cyst comprising of 9 cases (34.62%) followed by *Entamoeba histolytica* (23.07%) (Figure 1), *Enterobious vermicularis*

Table 1. Table of age distribution

Age group	No. of cases	% of cases
0-10	2	7.69
11-20	9	34.62
21-30	1	3.85
31-40	5	19.23
41-50	3	11.54
51-60	3	11.54
61-70	3	11.54
Total	26	100

Table 2. Frequency of various parasitic infection

S. N.	Type of parasitic infestation	No. of cases	% of cases
1	Hydatid cyst	9	34.62
2	*Entamoeba histolytica*	6	23.07
3	*Enterobious vermicularis*	4	15.38
4	*Ascaris lubricoides*	2	7.69
5	Filaria	2	7.69
6	Cysticercosis	2	7.69
7	Highly suspicious of parasitic infection; resolving parasitic infection (Filaria on FNA)	1	3.85
	Total	**26**	**100**

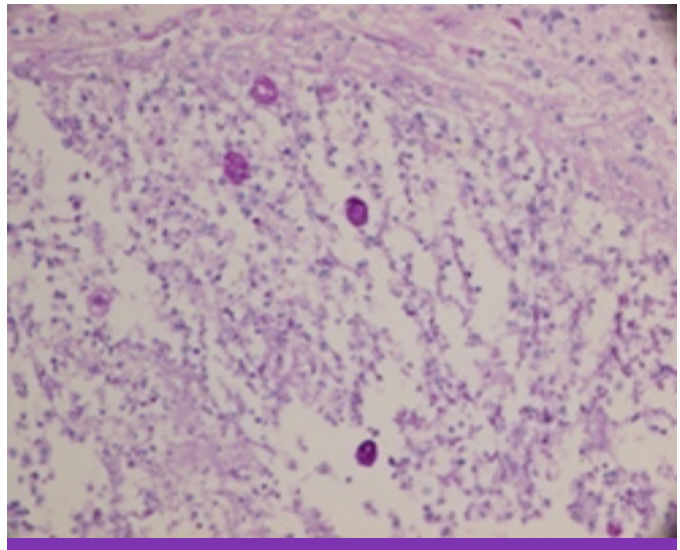

Figure 1. PAS stained section of *Entamoeba histolyica* in intestine

PAS: Periodic acid schiff

(15.38%) (Figures 2, 3), and (7.69%) *Ascaris lubricoides*, (7.69%), filaria (Figures 4, 5) and cysticercosis (Figure 6) respectively.

There was one case (3.85%) in which no fragment of parasite was seen, but tissue reactions strongly raised the suspicion of parasitic infection. In the same case microfilaria was reported on FNA, but it could not appreciated on histopathology because of resolving parasitic infection (Table 2).

Hydatid cyst was most common parasite reported in this study (34.62%). The peak age for the incidence was 11-56 years followed by others.

DISCUSSION

Parasites may infests humans and cause parasitic diseases. In India, Charak Samhita and Sushutra Samhita documented malaria. The Bhrigu Samhita from 1000 BCE had made earliest documentation of amebiasis. The diagnosis of parasitic infection is mandatory to diagnose the disease. The different diagnostic

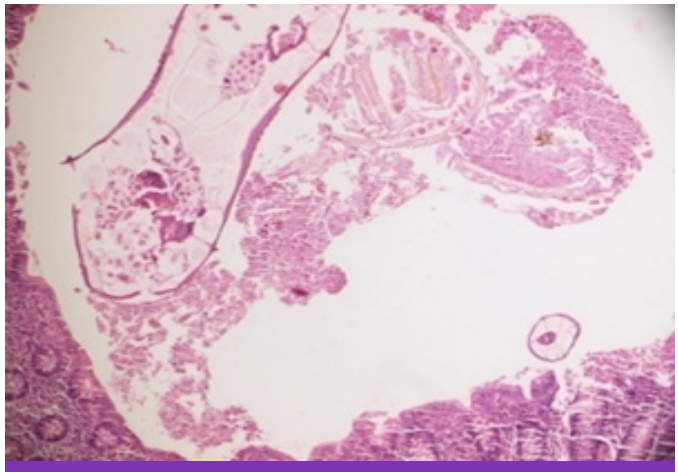

Figure 2. H&E stained section of appendix with *Entrobious vermiculus*
H&E: Hematoxylin and eosin

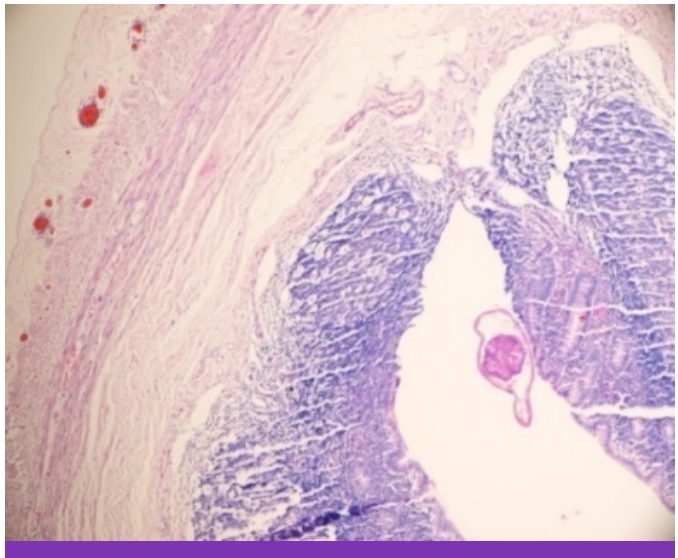

Figure 3. PAS stained section of appendix with *Entrobious vermiculus*
PAS: Periodic acid schiff

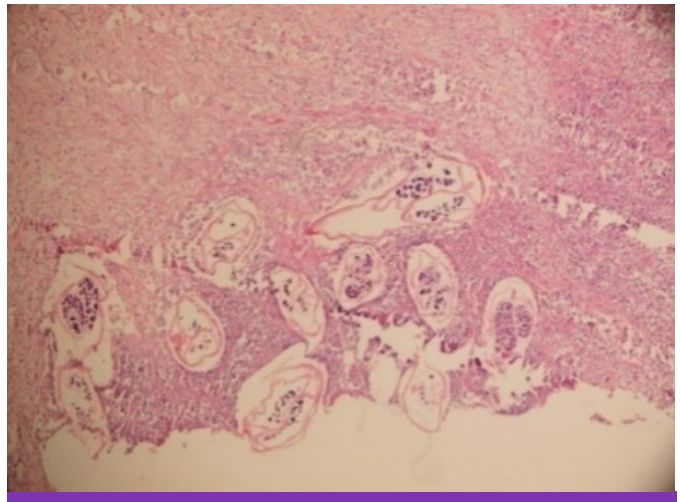

Figure 4. H&E (4x) stained section of lymph node with microfilaria
H&E: Hematoxylin and eosin

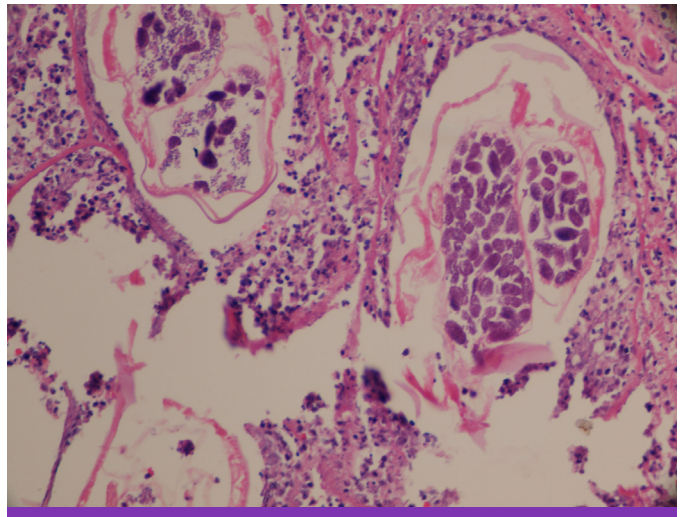

Figure 5. PAS stained section of lymph node with microfilaria
PAS: Periodic acid schiff

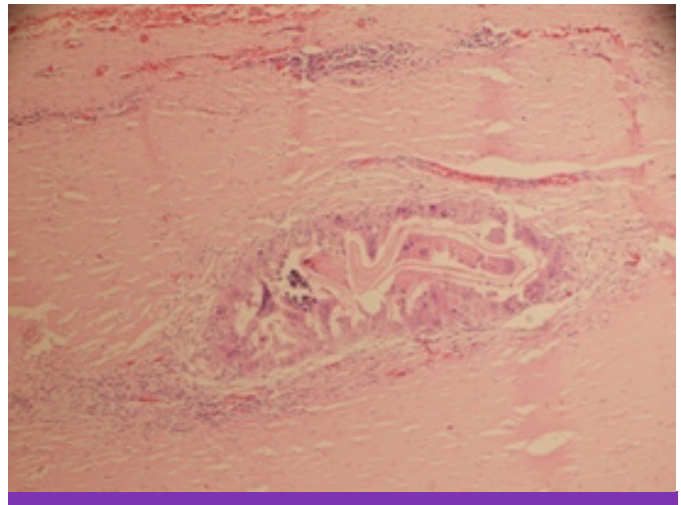

Figure 6. H&E stained section of neurocysticercosis cellulose with tissue response
H&E: Hematoxylin and eosin

tests includes stool examination, endoscopy, blood tests including blood film smearing and serology, radiology investigations and histopathology (7,8). Now a days, PCR is also used for the confirmative diagnosis of parasite and is seen only in higher centres only because it is expensive and requires experience person to manage. Tissue staining with hematoxylin and eosin (H&E) and PAS not only identify parasite but also visualize host tissue reaction (9). So, histopathological examination is the bench mark diagnostic test for identification of various parasitic agents and tissue response.

The government also conducts various policies for the elimination of parasites like filariasis as it causes an important health problem in India (10).

In the present study over a period of 12 years there were 26 cases of parasitic lesions identified on histopathological examination in which parasite was identified in 25 cases and one case there was tissue reactions which strongly raised the suspicion of parasitic infection, as the microfilaria was reported on FNAC in the same patient. Parasite could not visualized on histopathology because of resolving parasitic infection.

Age range of patients was 5 year to 70 year (Table 1) which was comparable with other researchers (9). Most common parasitic infection in our study was hydatid cyst, out of which maximum were located in liver (Table 2). This finding was in concordance with the study done by Rao et al. (11), who reported 72% cases of hydatid cyst in liver.

The difference in incidences of parasitic infection may be because of difference in geographical distribution of various parasitic species.

The peak age range also provide the clue for variant of parasite infection. The youngest patient was seen in peak range of 5-35 years which was reported in ascaris simultaneously the oldest was noted in peak range of 45-70 which was for filaria (Table 3). Age range of the patients diagnosed with hydatid cyst, *Entamoeba histolytica*, and Filaria was in concordant with study done by Manoharan and Sowmya (9), Sabesan et al. (10) and Rayan et al. (12).

As far as the age distribution for *Ascaris lubricoides* and Cysticercosis was reported, it was found lower in comparison to these researchers. This difference could be because of scanty number of parasites cases in their studies.

The parasite commonly encounter in humans are filariasis, ascaris, cysticercosis, amoeba, hydatid cyst and entrobious vermicularis (13-15). The most common location of parasite infection was liver in current study. The site/location of parasitic infection was comparable with other researchers (Table 4). The other researchers reported different sites other than liver this discordance may be due to geographical distribution and due to different parasites harboring at different places.

Tissue response in our study was concordant with the study done by Manoharan and Sowmya (9) (Table 5). In other studies eosinophilia was the most presenting finding for parasite infection but in current study eosinophils were rarely recognized ie, 3 cases out of 26 cases have eosinophil cell in tissue reaction.

In contrast to that here, there was predominance of chronic inflammatory cells infiltration. The reason for the presence of chronic inflammatory cells accumulation might be the presence of persistent parasite leading to chronic immune response (Table 6).

In most cases of hydatid cyst we received only cyst so comment on tissue reaction was not possible. There was only two case of infected hydatid cyst which were found infiltrated by chronic inflammatory cells.

In a case of amoebic colitis initially any amoebic cyst or trophozoite was not appreciated but the mononuclear cell along with eosinophil cells infiltration was so intense which raised suspicion for the parasitic infection. On PAS stain the trophozoites and cyst was well recognized. Similarly Liu et al. (15) applied PAS for the identification of amoebic trophozoite in their study.

PAS staining also enhances the diagnostic efficiency for the identification of parasites. The PAS is a cheaper reagent and the method of PAS staining is equally simple as H&E staining and easily manageable in laboratory. So, it should be applied in

Table 3. Comparison of age group in the present study with other studies (9,10,12)

S.N.	Parasitic infestation	Peak age range (years) present study	Age group in other studies
1	Hydatid cyst	11-56	21-58
2	*Entamoeba histolytica*	22-67	2-65
3	*Enterobious vermicularis*	9-38	-
4	*Ascaris lubricoides*	5-35	15-58
5	Filaria	45-70	35-80
6	Cysticercosis	14-17	20-40

Table 4. Occurrence of common parasites in various studies

S.N.	Authors	Year of study	Site	Occurrence
1	Manoharan and Sowmya (9)	2016	Genital filariasis, hydatid cyst in liver, soft tissue cysticercosis	Each 22.2%
2	Sabesan et al. (10)	2014	Genital filariasis	72.6%
3	Rao et al. (11)	2012	Hydatid cyst in liver	72%
4	Vora et al. (13)	2008	Soft tissue cysticercosis	88%
5	Dhanabal et al. (14)	2014	Intestinal parasites	*Entamoeba coli* (26%) and *E. histolytica* (22%)
6	Present study	2020	Hydatid cyst in liver	34.62%

Table 5. Parasitic lesions and their tissue response comparison in the study by Manoharan and Sowmya (9) and present study

S.N.	Parasite/lesion	Tissue response in study by Manoharan and Sowmya (9)	Tissue response in present study
1	Filariasis	Eosinophilic abscess, fibrosis, calcification, lymphoid aggregate with germinal center formation	Necrosis, acute inflammation chronic inflammation [eosinophils and pigment laden histiocytes and no definite parasitic fragment seen in resolving parasitic cysts (microfilaria was seen in FNAC)]
2	*Ascaris* enteritis	Mucosal ulceration, eosinophilic infiltration, fibrosis, submucosal oedema and congestion	Chronic inflammatory cells infiltration comprising of lymphocytes, plasma cells and histiocytes
3	Cysticercosis	Inflammatory infiltrate, xantho granulomatous reaction.	Chronic inflammatory cells, palisaded epithelioid cells granuloma and giant cell
4	Amoebic colitis	Mucosal ulceration, cytoplasmic vacuolation, congested blood vessels	Mucosal ulceration. Necrotic debris, acute and chronic inflammatory cells infiltration along with eosinophils
5	Hydatid cyst	Mononuclear cell infiltration, fibrosis, hemorrhage, calcification, congested blood vessels.	Mononuclear cells infiltration comprising of lymphocytes, plasma cells, histiocytes and fibroblasts
6	*Entrobious vermicularis*	No case	Acute and chronic inflammatory cells infiltration

Table 6. Percentage of type of tissue reaction in 26 cases

Tissue reaction	No. of cases	Percentage (%)
Acute inflammation	4	15.38%
Chronic inflammation	14	53.85%
Necrosis	3	11.54%
Eosinophils	3	11.54%
Epithelioid granuloma	1	3.85%
Giant cell	1	3.85%

histopathology section in daily routine staining to confirm the parasitic infection.

CONCLUSION

We emphasizes on the application of histopathology along with PAS staining for the diagnosis of parasitic infection which not only reduce the morbidity and mortality but also provide correct way for the management to the infected patients. Among tissue response chronic inflammatory cells infiltration was found more frequent and significant. More studies should be carried out with the same aim and including a more numbers of parasitic infected patients.

*Ethics

Ethics Committee Approval: Ethical clearance was taken from Institutional Ethical Committee of Uttar Pradesh University with ethical clearance no: 228/2018.

Informed Consent: Consent of patients were not taken, as we had received tissue for histopathology examination and details were obtained from the patients records.

*Authorship Contributions

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

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Assessment of the Distribution of Intestinal Parasites Detected in the Parasitology Laboratory of Çukurova University Faculty of Medicine Between 2017 and 2021

Çukurova Üniversitesi Tıp Fakültesi Parazitoloji Laboratuvarı'nda 2017-2021 Yılları Arasında Saptanan Bağırsak Parazitleri Dağılımının Değerlendirilmesi

Mehtap Demirkazık, Eylem Akdur Öztürk, Fatih Köksal

Çukurova University Faculty of Medicine, Department of Medical Parasitology, Adana, Türkiye

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ABSTRACT

Objective: It is known that protozoa and helminths that cause intestinal infections adversely affect human life. Changing climate and demographic and socio-economic factors worldwide necessitate the determination and updating of the incidence of these parasites. Our study aimed to retrospectively examine the distribution of intestinal parasites detected in the Parasitology Laboratory of Çukurova University Faculty of Medicine between 2017 and 2021.

Methods: Parasitological examinations were performed using the native-lugol and formol-ether condensation method. Staining method (Modified Ziehl-Neelsen) and cellophane tape method were then applied to evaluate the specimens considered necessary.

Results: One or more parasites were detected in 33 of 373 patients (8.8%) evaluated in the study. These were *Giardia intestinalis* at a rate of 30.5% (11/36), *Enterobius vermicularis* at a rate of 27.7% (10/36), *Blastocystis* sp. at a rate of 19.4% (7/36), *Entamoeba coli* at a rate of 11.1% (4/36), *Cryptosporidium* spp. at a rate of 8.3% (3/36) and *Taenia saginata* at a rate of 2.7% (1/36). It was determined that two patients were coinfected by *Entamoeba coli* and *Blastocystis* sp. while one patient was coinfected by *Entamoeba coli* and *Giardia intestinalis*.

Conclusion: It is thought that determining the incidence of intestinal parasites, which are an important public health problem, may help guide studies for preventive health services. Although the five-year laboratory data obtained from the study do not reflect our region, it is thought that intestinal parasites maintain their importance

Keywords: Intestinal parasites, Çukurova, Adana

ÖZ

Amaç: Bağırsak enfeksiyonuna neden olan protozoon ve helmintlerin insanlarda yaşamı olumsuz etkilediği bilinmektedir. Tüm dünyada değişen iklim, demografik ve sosyo-ekonomik faktörler, bu parazitlerin görülme oranlarının belirlenmesini ve güncellenmesini zorunlu kılmaktadır. Çalışmamızda Çukurova Üniversitesi Tıp Fakültesi Parazitoloji Laboratuvarı'nda 2017-2021 yılları arasında saptanan bağırsak parazitleri dağılımının retrospektif irdelenmesi amaçlanmıştır.

Yöntemler: Parazitolojik incelemeler nativ-lugol ve formol-eter yoğunlaştırma yöntemi kullanılarak yapılmıştır. Ayrıca gerek görülen örneklere boyama (Modifiye-Ziehl Nielsen) yöntemi ve selofan bant yöntemi uygulanmıştır.

Bulgular: Çalışmada değerlendirilen 373 hastanın 33'ünde (%8,8) bir veya birden fazla parazit saptanmış olup tanımlanan parazitler arasında %30,5 (11/36) oranında *Giardia intestinalis*, %27,7 (10/36) oranında *Enterobius vermicularis*, %19,4 (7/36) oranında *Blastocystis* sp., %11,1 (4/36) oranında *Entamoeba coli*, %8,3 (3/36) oranında *Cryptosporidium* spp., ve %2,7 (1/36) oranında *Taenia saginata* saptanmıştır. İki hastanın *Entamoeba coli* ve *Blastocystis* sp. ile ve bir hastanın ise *Entamoeba coli* ve *Giardia intestinalis* ile koenfekte olduğu tespit edilmiştir.

Sonuç: Önemli bir halk sağlığı sorunu olan bağırsak parazitlerinin çalışmamızda görülme sıklığının belirlenmesi korunmaya yönelik yapılacak çalışmalara yol gösterici olabileceği düşünülmektedir. Çalışmadan elde edilen beş yıllık laboratuvar verileri her ne kadar bölgemizi yansıtmasa da bağırsak parazitlerinin önemini koruduğu düşünülebilir.

Anahtar Kelimeler: Bağırsak parazitleri, Çukurova, Adana

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Address for Correspondence/Yazar Adresi: Mehtap Demirkazık, Çukurova University Faculty of Medicine, Department of Medical Parasitology, Adana, Türkiye
Phone/Tel: +90 533 772 27 33 **E-mail/E-Posta:** mdemirkazik@cu.edu.tr **ORCID ID:** orcid.org/0000-0003-3158-4937

Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

It is known that intestinal parasites have infected humans since prehistoric times. Intestinal parasiticinfections are observed at high prevalence in many regions around the world. Intestinal parasites cause significant health problems in underdeveloped and developing countries, whereas waterborne epidemics have also been encountered in developed industrial countries. The morbidity and mortality rates of intestinal parasitic infections in endemic countries are noteworthy (1,2). Numerous factors, such as appropriate climate and geographical structure, duration of environmental resistance (especially higher resistance of oocysts and nematode eggs), presence of intermediate hosts and vectors, regional social habits, personal hygiene awareness, and education level, play a role in the distribution of parasites in the world. Moreover, people from endemic regions visit areas where parasites are not encountered or contaminated vegetables and fruits are transported to regions where parasites are not observed due to fast and easy transportation, which also affects the distribution of parasites on the earth (1,2). While intestinal parasites spend their obligatory parasitic period in the intestine during their life cycle, they can lead to clinical conditions, such as malnutrition, iron deficiency anemia, and vitamin and mineral deficiency. They adversely affect the quality of life and working strength. Particularly in preschool and school-age children, they can cause mental and physical development retardation due to vitamin and mineral deficiency (3). Furthermore, they can result in significant economic losses due to expenditures on treatment and protection (2,4).

Soil-borne helminths infect one-sixth of the world's population. Consumption of vegetables and fruits in their raw form without sufficiently cleaning them with clean water increases the incidence of helminths. Helminths and protozoa constitute 1/4 of the etiological agents that cause human infection (3). According to the 2008 global distribution map of soil- transmitted helminths of the World Health Organization, Türkiye is among the countries (with a low prevalence (<20%) of soil-transmitted helminths (5).

Since socio-economic and climatic changes are closely related to public health, updating the incidence rates of intestinal parasitic infections (local, countrywide, and worldwide) is of great importance for future studies for preventive health services (2,5).

The present study aimed to examine the distribution of intestinal parasites detected between January 2017 and December 2021 in specimens sent from different outpatient clinics and clinics of Çukurova University Faculty of Medicine Balcalı Hospital to the Department of Parasitology Laboratory due to digestive system complaints.

METHODS

Stool and cellophane tape specimens of 373 patients sent to the department of parasitology laboratory. The cellophane tape method was used to detect *Enterobius vermicularis* (*E. vermicularis*) eggs in 37 of 373 clinically suspected patients.

Procedure

After the stool specimens sent to the laboratory were examined macroscopically, they were examined under a light microscope (x20 and x40) with the saline and Lugol (native-Lugol) methods. Afterward, the formol-ether condensation method was applied to these specimens (6). 1-1.5 g stool specimens were mixed in 15 mL tubes with the help of a baguette, and the suspension was filtered through two layers of gauze into a new tube. Centrifugation was done at 3000 rpm for 3 min. The sample taken from the lowest part was examined under a light microscope (x20 and x40) with Lugol.

The stool specimen required to detect *Cryptosporidium* spp. was stained by the modified Erlich Ziehl-Neelsen (MEZN) staining method (7). The stool specimens spread on the slide were stained with carbol fuchsin, Destain with acid alcohol, stained with methylene blue, and washed. After drying, they were examined under the light microscope (x100) in immersion objective. Cellophane tape preparations were assessed under the light microscope (x20 and x40) for *E. vermicularis* eggs.

Statistical Analysis

The data obtained from the laboratory recording system were analyzed and statistically evaluated via the chi-square test in the SPSS 20.0 software. In all statistical analyses, $p<0.05$ was considered significant.

Ethics committee approval was obtained for our study from Çukurova University Non-Interventional Clinical Research Ethics Committee (decision no: 65, dated: 04.02.2023).

RESULTS

One or more parasites were detected in 33 (8.8%) of 373 patients who presented to the Laboratory of the Parasitology Department of Çukurova University Faculty of Medicine, and a total of 36 parasites were identified in these patients. These patients samples from different outpatient clinics and clinics such as the department of child health and diseases, department of infectious diseases and clinical microbiology, and department of internal medicine were examined for intestinal parasites in a five-year period between January 2017 and December 2021. After the examination, the most common parasite was *G. intestinalis* (*Giardia intestinalis*) at a rate of 30.5% (11/36), whereas other parasites were *E. vermicularis* at a rate of 27.7% (10/36), *Blastocystis* sp. at a rate of 19.4% (3/36), *Entamoeba coli (E. coli)* at a rate of 11.1% (4/36), *Cryptosporidium* spp. at a rate of 8.3% (3/36) and *Taenia saginata* (*T. saginata*) at a rate of 2.7% (1/36). Of the 373 patients included in the study, 155 (41.6%) were female, and 218 (58.4%) were male. Of the patients with parasites, 51.5% (17/33) were female, and 48.5% (16/33) were male. A cellophane tape test was carried out only on 37 patients with suspected clinical symptoms. While a significant (p=0.042) difference was determined in the distribution of the genders of the patients who presented to the laboratory by year (Table 1), no difference was revealed in the distribution of the parasite detection

Table 1. Distribution of patients admitted by years by gender

Years	Female, n (%)	Male, n (%)	Total, n (%)
2017	51 (13.6)	46 (12.3)	**97 (26%)**
2018	43 (11.5)	77 (20.6)	**120 (32.1%)**
2019	39 (10.4)	47 (12.6)	**86 (23%)**
2020	12 (3.2)	26 (6.9)	**38 (10.1%)**
2021	10 (2.6)	22 (5.8)	**32 (8.5%)**
Total	**155 (41.6%)**	**218 (58.4%)**	**373**

rates by gender (p=0.303). *Blastocystis* sp. and *E. coli* were identified in two patients, and the co-infection of *G. intestinalis* and *E. coli* was identified in one patient. Concerning the distribution of parasite detection rates by year, positivity was determined at a rate of 8.2% (8/97) in 2017, at a rate of 11.6% (14/120) in 2018, at a rate of 11.6% (10% 86) in 2019, at a rate of 5.2% (2/38) in 2020, and at a rate of 6.2% (2/32) in 2021. The highest parasite rates were observed in 2018 and 2019 (Figure 1). *G. intestinalis* and *E. vermicularis* were the most common parasites (Figure 2). March and October were the months with the highest number of parasites (Figure 3).

DISCUSSION

In the developing world, insufficient water resources and sanitation, crowded environments, difficulty accessing health services, living and eating habits, and low education levels

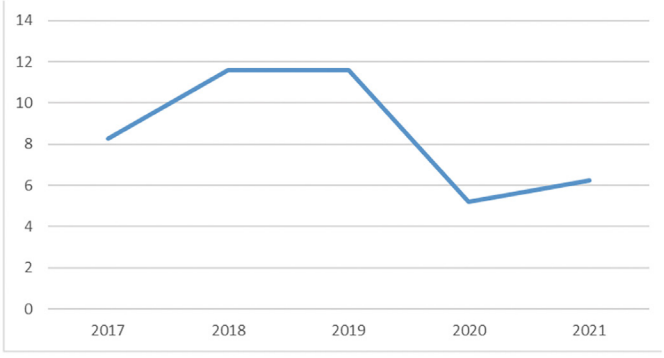

Figure 1. Parasite detection rate by years (%)

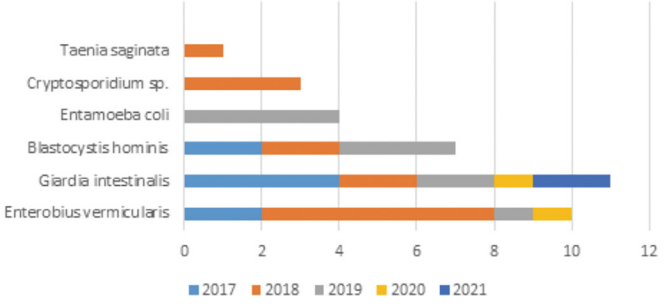

Figure 2. Distribution of detected parasite species by years

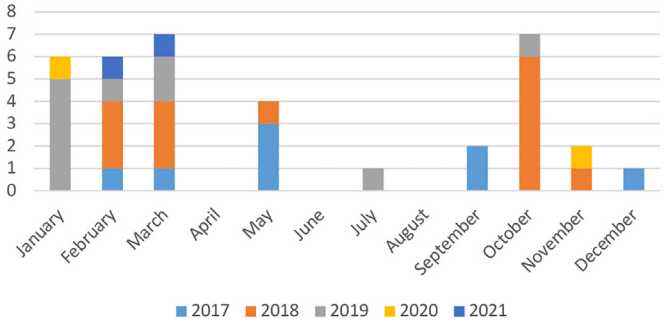

Figure 3. Distribution of parasite species detected between 2017-2021 by months

increase susceptibility to infectious diseases, including intestinal parasites (8).

Numerous studies have been conducted on intestinal parasites in patients who present to parasitology laboratories of medical faculties of universities and health institutions in Türkiye. The distribution of parasites in these studies varies by region and year. In İstanbul, Özyurt et al. (9) detected intestinal parasites at a rate of 5.9% in a training hospital within the four years between 2003 and 2006. Polat et al. (10) detected intestinal parasites in 2.96% of 20,948 patients who presented to the Parasitology Laboratory of İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine between 2012 and 2018. Uzun et al. (11) researched intestinal parasites in five primary schools in the city center of Diyarbakır in 2004. They reported that 490 (52.51%) of 933 stool specimens had parasites and the rate of parasite detection was higher in schools in the region with a low socio-economic level (11). Akpolat et al. (12) examined the distribution of intestinal parasites in patients who presented to Dicle University Faculty of Medicine between 2011 and 2020 and detected parasites in 5.99% of 60.501 stool specimens. A study conducted on children aged 7-14 in Aydın stated that one or more intestinal parasites were detected in 31.8%. The incidence of multiple intestinal parasites was determined to be 29% (13). A study carried out between 2003 and 2012 in the Parasitology Laboratory of Hacettepe University Hospital reported that 3.681 (4.2%) specimens had parasites (14). In the retsrospective results from the Parasitology Laboratory of Hacettepe University Faculty of Medicine, for the period between 2014 and 2019, 7.5% of intestinal parasites were detected in 67.069 specimens (15). Alver et al. (16) reported that one or more parasites were detected in 195 (7.3%) of 2.686 stool specimens at the Parasitology Laboratory of Bursa Uludağ University Health Application and Research Center between 2009 and 2010. Studies conducted in our region stated that parasites were encountered at a rate of 33.08% in a primary school in 1998 (17). In 2003, the prevalence of intestinal parasites was investigated among children in a primary school in Adana, which was located in a region with a low socio-economic level, and one or more parasites were identified in 234 (48.55%) of 482 students whose stool and cellophane tape specimens were examined (18). Before our study, the detection rate of intestinal parasites was reported to be 8.06% in the study conducted in our laboratory between 2003 and 2007 (19). In our current study, one or more parasites were identified in 33 (8.8%) of 373 patients during the five years between 2017 and 2021.

In the study by Okyay et al. (13), the most common parasites were *E. vermicularis* (13%) and *G. intestinalis* (6.1%), and more parasites were observed in individuals living in rural areas compared to those living in urban areas and in individuals without handwashing habits. While studies on intestinal parasites conducted in the same region in different years reported higher rates of parasite density in previous research, Alver et al. (16) stated that the rate of intestinal parasite positivity in stool and cellophane tape examinations of individuals who presented to the Parasitology Laboratory of Bursa Uludağ University Health Application and Research Center between 2009 and 2010 increased compared to the rate of positivity in intestinal parasite examinations performed at the same center in previous years.

In a study carried out by Tanrıverdi and Özcan (17). in a primary school in our region in 1998, *B. hominis* was the most common

parasite at a rate of 13.16%. Aktaş et al. (18) reported parasite distribution by species as *E. vermicularis* at 37.97%, *B. hominis* sp. at 9.33%, *G. intestinalis* at 7.67%, *E. coli* at 4.77%, and *Hymenolepis nana* (*H. nana*) at 0.62%. In the study performed in our laboratory between 2003 and 2007, *G. intestinalis* (19.04%) was the most common parasite, followed by *Cryptosporidium* spp. (11.9%) (17). *G. intestinalis* (30.5%) and *E. vermicularis* (27.7%) were the most common parasites in our study. In the last two years, 2020 and 2021, the number of patients with suspected intestinal parasites or those who presented to the hospital for general check-ups decreased with the measures taken during the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) pandemic. In our laboratory, the number of intestinal parasites decreased in parallel with the decreased number of patients in 2020 and 2021. It is thought that parasites could have been detected at a higher rate than in the previous study conducted in our laboratory if the number of patient presentations in previous years had been reached in these years.

In our current study, parasites were detected at the highest rates in 2018 and 2019 (11.6%), whereas the least (5.5%) were detected in 2020 and 2021.

In the study, the decreased detection rates of parasites in 2020 and 2021 covering the SARS-CoV-2 pandemic period can be associated with the decreased number of patients presenting to the hospital with intestinal parasitic infection complaints during this period. This can also be explained by the fact that people presenting to the hospital during this period paid more attention to personal hygiene rules and were less likely to carry the risks for intestinal parasites.

When the incidence of parasites was assessed statistically according to gender, some publications reported no statistically significant difference in the distribution by gender (20). However, other publications found the incidence of parasites to be statistically significant in female patients (10,21). Some researchers also stated that the incidence of parasites was statistically significant in male patients (12,16). In this study, 51.5% of the patients with parasites were female, and 48.5% were male. No statistically significant correlation was found in the distribution by gender.

When the protozoa and helminth rates among the parasites detected in our study were examined, protozoa were detected at a rate of 97.2%, and helminths were identified at a rate of 2.7%. Polat et al. (10) reported that 84.19% of the parasites identified were protozoa, and 15.81% were helminths. Alver et al. (16) stated that 87.9% of the parasites detected in their study were protozoa, and 12.1% were helminths. Publications suggesting that protozoa are more common in studies conducted in Türkiye are similar to our study (12,15).

Upon evaluating the months or seasons with the highest distribution of intestinal parasites, Usluca et al. (22) expressed that the highest positive parasite incidence in their two-year assessments was observed in summer and autumn. Gürbüz et al. (23) reported that the positive incidence rate increased after spring and reached the highest level in summer. On the other hand, Alver et al. (16) reported that the highest number of parasites was detected in spring and autumn. Polat et al. (10) stated that the month with the highest parasite detection was May, and the month with the lowest parasite detection was July. When the incidence of parasites was assessed by month in our study, March and October were the months with the highest incidence of parasites. It can be thought that digestive system infections are more common in these months since people visit rural areas more often due to air temperature; thus, contamination through water and food sources or contact with sick people is easier.

CONCLUSION

As a result, our retrospective study, which assessed the incidence of intestinal parasites in the Department of Parasitology Laboratory of Çukurova University Faculty of Medicine between 2017 and 2021, showed that intestinal parasites are still important due to their incidence. Hence it is still essential to inform society about an organized infrastructure, access to clean, safe water, eating habits, personal hygiene awareness, and sanitation. We believe that updating the detection rates of intestinal parasites will guide the studies to be conducted on treating intestinal parasitic infections and their prevention.

The male and female percentages given in Table 1 were calculated as the total number.

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*Ethics

Ethics Committee Approval: Ethics committee approval was obtained for our study from Çukurova University Non-Interventional Clinical Research Ethics Committee (decision no: 65, dated: 04.02.2023).

Informed Consent: Retrospective study.

*Authorship Contributions

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Cutaneous Leishmaniasis in Dr. Ersin Arslan Training and Research Hospital After Migration and During the Pandemic (2019-2022)

Dr. Ersin Arslan Eğitim ve Araştırma Hastanesi'nde Göç Sonrası ve Pandemi Sırasında Kutanöz Leishmaniasis (2019-2022)

Ahmet Özkeklikçi

Gaziantep City Hospital, Microbiology/Parasitology Laboratory, Gaziantep, Türkiye

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ABSTRACT

Objective: Cutaneous leishmaniasis is a parasitic skin disease transmitted by the bite of sandflies. In our region, which is endemic for this disease, there has been a great migration from a much more endemic region and population movements from our area to Türkiye and abroad. Afterward, a pandemic was experienced. Due to these two extraordinary events and the possible epidemic potential in our region, it is useful to follow-up on the disease. We aimed to contribute to the evaluation of the disease in these processes by analyzing the data of our laboratory in recent years.

Methods: Between January 2019 and December 2022, samples from patients who came to our laboratory with suspected cutaneous leishmaniasis were taken, stained and examined under a microscope. Patients were evaluated in terms of age, gender, nationality, place of residence, lesion site and duration.

Results: Out of the 144 examined cases, 64 (44.4%) were positive for cutaneous leishmaniasis. Among these positive cases, 40 (62.5%) were women, 24 (37.5%) were men, and 54 (84.3%) belonged to the 0-9 age group. Of those who tested positive, 54 (84.3%) were Turkish citizens and 23 (35.9%) were Syrian citizens. Fifty-four (84.3%) patients had only single lesion. While the number of applications and positivity rates remained within normal levels in 2019 and 2020, a significant decrease was observed in both from 2021 and 2022.

Conclusion: Cutaneous leishmaniasis is carried by migration, decreases in large-scale isolations such as pandemics, and its spread can be prevented with correct diagnosis and treatment. Although the number of patients may change over time and place, cutaneous leishmaniasis is a disease that threatens the health of societies and should always be monitored.

Keywords: Cutaneous leishmaniasis, migration, pandemic, Türkiye, Syria

ÖZ

Amaç: Kutanöz leishmaniasis, kum sineklerinin ısırmasıyla bulaşan paraziter bir deri hastalığıdır. İzi bırakan deri lezyonlarına neden olur. Bu hastalık açısından endemik olan bölgemize çok daha endemik olan bir bölgeden büyük bir göç ve bölgemizden de yurt içine ve yurt dışına nüfus hareketleri olmuştur. Sonrasında bir pandemi yaşanmıştır. Bu iki olağanüstü olay ve bölgemizdeki olası epidemi potansiyeli nedeni ile hastalığın takibinde yarar vardır. Laboratuvarımızın son yıllardaki verilerini analiz ederek bu süreçlerde hastalığı değerlendirmesine katkı sağlamayı amaçladık.

Yöntemler: Ocak 2019-Aralık 2022 tarihleri arasında laboratuvarımıza kutanöz leishmaniasis şüphesiyle gelen hastalardan örnekler alınmış, boyanmış ve mikroskop altında incelenmiştir. Hastalar yaş, cinsiyet, uyruk, ikamet yeri, lezyon bölgesi ve süresi açısından değerlendirilmiştir.

Bulgular: İncelenen 144 olgunun 64'ünde (%44,4) kutanöz leishmaniasis pozitif bulunmuştur. Bu pozitif olguların 40'ı (%62,5) kadın, 24'ü (%37,5) erkek ve 54'ü (%84,3) 0-9 yaş grubuna aitti. Test sonucu pozitif çıkanların 54'ü (%84,3) Türk vatandaşı, 23'ü (%35,9) ise Suriye vatandaşıydı. Hastaların 54'ünde (%84,3) sadece tek lezyon vardı. Başvuru sayısı ve pozitiflik oranları 2019 ve 2020 yıllarında normal seviyelerde seyrederken, 2021 ve 2022 yıllarında her ikisinde de belirgin bir düşüş gözlenmiştir.

Sonuç: Kutanöz leishmaniasis göçlerle taşınmakta, pandemi gibi büyük ölçekli izolasyonlarda azalmakta, doğru tanı ve tedavi ile yayılımı önlenebilmektedir. Hasta sayısı zaman ve mekana göre değişse de kutanöz leishmaniasis toplumların sağlığını tehdit eden ve her zaman takibi gereken bir hastalıktır.

Anahtar Kelimeler: Kutanöz leishmaniasis, göç, pandemi, Türkiye, Suriye

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Address for Correspondence/Yazar Adresi: Ahmet Özkeklikçi, Gaziantep City Hospital, Microbiology/Parasitology Laboratory, Gaziantep, Türkiye
Phone/Tel: +90 530 346 48 18 **E-mail/E-Posta:** ozkeklikci@hotmail.com **ORCID ID:** orcid.org/0000-0003-1619-4156

Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Cutaneous leishmaniasis is a parasitic disease with zoonotic and anthroponotic characteristics. The vector transmits the parasite when biting reservoirs or humans. The lesions are mostly seen on open areas such as the face and extremities where the fly can easily reach. Lesions lasting longer eventually heal by leaving a scar and scar tissue. This scar may cause aesthetics problems in visible areas, especially on the face. Cutaneous leishmaniasis is a common and neglected disease. It has been reported in 98 countries in the world. Low socio-economic level, poor housing and nutrition, population mobility, changes in the environment and climate, and poor health services are the most important risk factors (1-4).

Cutaneous leishmaniasis has been reported in Türkiye since the beginning of the 19th century. It has been known by our people for hundreds of years; it is given different names according to the regions in our country such as oriental boil, Antep boil, Aleppo boil, year boil, beauty sore (5).

Çukurova and Southeastern Anatolia, including Gaziantep, are considered as endemic regions. While the cases were stable in these regions, the Syrian civil war in our nearby geography and migration to our country, followed by population mobility within our country, led to an increase in numbers and prevalence Gaziantep was preferred by migrants due to its proximity to Syrian cities and being an industrial city (5,6). Some of the migrants settled here, some of them stayed here for a while and dispersed to other cities in Türkiye or went to European countries (7).

In our study, we aimed to evaluate the cutaneous leishmaniasis cases admitted to our hospital and the epidemiologic characteristics of these cases during the migration and pandemic process.

METHODS

Between January 2019 and December 2022, patients who applied to the dermatology clinic of our hospital with the complaint of non-healing wounds and were thought to have cutaneous leishmaniasis were referred to our laboratory. At least two samples were taken from all lesions in the patient in accordance with the technique (incision-scraping, aspiration, aspiration following saline injection), fixed with methanol and stained with giemsa stain. After staining, the entire preparation was examined with a x100 objective by the relevant specialist physician. Samples were taken again from very suspicious patients in whom parasites could not be detected and the same procedures were repeated. Samples with amastigotes were considered positive (Figure 1). Patients were recorded in terms of age, sex, nationality, place of residence, site, type and duration of lesion. Patients or their relatives were asked "whether their wounds were associated with a disease they suspected" to understand awareness about the disease.

Statistical Analysis

"Jamovi 2.3.28 Solid" programme was used for statistical analysis. Chi-square test was applied when comparing the data and $p<0.05$ was considered significant.

Ethical approval was obtained from the Clinic Research Ethics Committee of the Gaziantep University (no: 2023/22, date: 12.04.2023).

RESULTS

The total number of patients analysed was 144 and *Leishmania* amastigotes were found in 64 (44.4%) of them. The results of the positive patients were as follows: Twenty-four (37.5%) were male and 40 (62.5%) were female. The age group in which leishmaniasis is most common is 0-9 years old. Fifty-four (84,3%) had a single lesion and 10 (15.6%) had more than one lesion. Positive patients had 39 (48.1%) lesions on the head and neck, 22 (27.1%) on the upper extremities, 14 (17.2%) on the lower extremities and 6 (7.4%) on the trunk. Of the lesions, 50 (61.7%) were papules, 22 (27.1%) nodules, 4 (4.9%) plaques, and 5 (6.1%) other types. Six (9.3%) patients had ulcers and 13 (20%) had crusts (Figure 2). The average time between the onset of lesions and diagnosis was 2.8 months. Forty (62.5%) of the patients were Turkish citizens and 24 (37.5%) were Syrian citizens. Twenty-three (35.9%) were

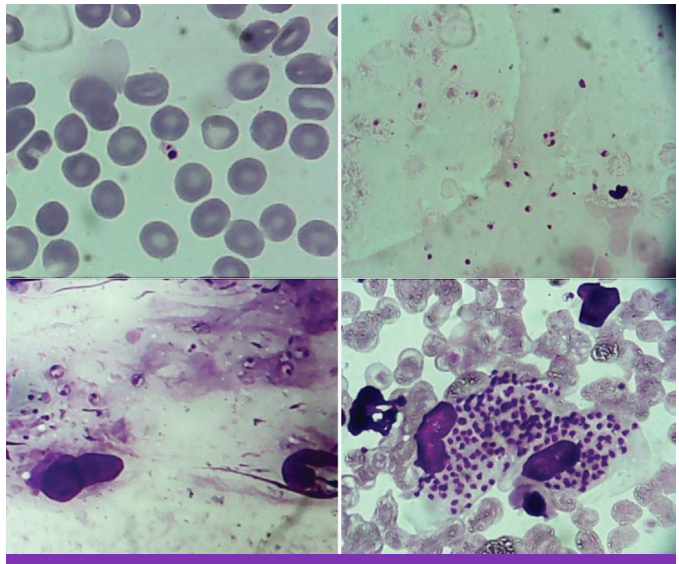

Figure 1. *Leishmania* spp. amastigote forms of in Giemsa staining, x100

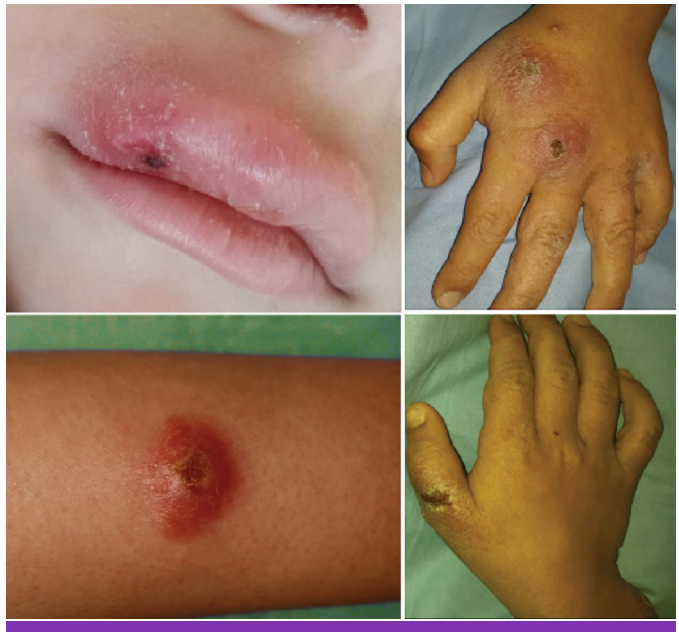

Figure 2. The images of lesions seen in patients

from the centre of Gaziantep and 41 (64.0%) were from the villages. Syrian citizens have no history of travelling to Syria in recent years. In the 4-year period, the highest number of patient applications and positivity was in 2019 and 2020. There was very little positivity in 2022 and none in 2021 (Table 1). Statistically, there was no statistically significant difference between women and men, between 0-9 age group and other age groups, between Turkish citizens and Syrian citizens in terms of positivity rate. Of the 144 applicants we asked about their wounds, 113 (78.4 %) referred to the disease by its local name.

Table 1. Distribution of patients by years

	Turkish	Syrian	Total
2019	30	14	44
2020	6	9	15
2021	0	0	0
2022	4	1	5
Total	40	24	64

DISCUSSION

The leishmaniasis positivity rate of 44.4% in our study was found to be 46% and 50% in previous studies conducted in our city (8,9). Diagnosis rates of stained preparations vary between 30% and 96%. Reasons for this variation include the experience of the examiner, the location and method of lesion acquisition (10). Our study is limited in this respect and additional methods such as culture and polymerase chain reaction should be used for more efficient results.

The number of females and males with *Leishmania* was 40 (62.5) and 24 (37.5), respectively. In previous studies in our province, the rate of female patients was found to be 47.1% by Cömert et al. (11) and Eroglu and Özgöztaşi (6) 53.5% was found. The fact that women are more concerned about physical appearance than men may have increased the number of applicants.

In line with many studies, the highest number of applications and positivity was observed in the 0-9 age group in our study (11,12). As in many infectious diseases, the incidence of leishmaniasis is higher in the pediatric age group. The immune system in children is not fully developed, they cannot protect themselves against the vector, and they spend more time outside during the play-school age (3,5).

The presence of a single lesion in most of the cases and the majority of the lesions on the face are expected results in leishmaniasis. Midges bite exposed areas especially when people are sleeping. Since the face is the most exposed area in all seasons, lesions are mostly seen on the face. The fact that the lesions generally have a papule appearance, papule is an early stage finding and in our opinion, it is related to the awareness of the disease and early presentation. (3,5).

The mean duration of onset of lesions was 2.8 months according to the patients' testimony. This is consistent with the incubation period of the disease. The time of the first appearance of the lesion is based on the patient's statement and generally vague dates are given. Naturally, the duration of lesions is also subjective. However, the fact that the disease is recognized by most of the cases (78.4%) is positive and may help early presentation and early treatment.

The number of Turkish citizens who were positive was 40 (62.5%) and the number of Syrian citizens was 24 (37.5%). The high number and proportion of Turkish citizens is not something we expect. In many previous studies, it is seen that the number of patients with Syrian citizenship is higher than the number of local cases (5,6,8,9,11-14). The fact that Syrian patients are treated in our city (6) may have reduced the rate of anthroponotic transmission and the number of patients. None of the patients had a history of travelling to Syria or abroad in recent years. This indicates that the source of transmission and infection is entirely in Türkiye. Most patients (64%) live in rural areas. This is usual for cutaneous leishmaniasis (5).

The data we evaluated in our study varied over the years. From January 2019 until April 10, 2020, when there was a lockdown due to the pandemic, the number of patients was consistent with previous years. After this date, although outpatient services were provided from time to time, the number of applications and cases decreased significantly. In March 2022, the health system started to work completely, and although there were applications, there was no noticeable increase in cutaneous leishmaniasis cases. This may be due to the fact that all travel between villages, cities and countries has stopped, and people cannot even go outside. Although the pandemic is an unpleasant situation, it is a fact that our cutaneous leishmaniasis cases decreased during and after this period.

CONCLUSION

Migration is expected to increase in the world due to wars, climate crisis, economic and social problems. Cutaneous leishmaniasis, which is one of these diseases, is carried by migration, decreases in large-scale isolations such as pandemics, and its spread can be prevented with correct diagnosis and treatment. Although the number of patients may change over time and place, cutaneous leishmaniasis is a disease that threatens the health of societies and should always be monitored.

***Ethics**

Ethics Committee Approval: Ethical approval was obtained from the Clinic Research Ethics Committee of the Gaziantep University (no: 2023/22, date: 12.04.2023).

Informed Consent: Retrospective study.

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Investigation of Seropositivity of Anti-*Toxoplasma gondii* Antibodies and Possible Risk Factors in Pregnant Women with Diabetes at Risk

Diyabet Tanılı Riskli Gebelerde Anti-Toxoplasma gondii Antikorlarının Seropozitifliği ve Olası Risk Faktörlerinin Araştırılması

Nazlı Aksoy Sanay[1], Neriman Mor[2], Dilek Şahin[3]

[1]Kafkas University Health Sciences Institute, Department of Parasitology, Kars, Türkiye
[2]Kafkas University Faculty of Medicine, Department of Medical Parasitology, Kars, Türkiye
[3]Ankara Bilkent City Hospital, Clinic of Perinatology, Ankara, Türkiye

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ABSTRACT

Objective: Toxoplasmosis is a parasitic infection caused by *Toxoplasma gondii*. Immunocompromised individuals and pregnant women are at risk, with the latter group being susceptible to miscarriages. This study aimed to determine the seropositivity of *T. gondii* antibodies and potential risk factors in pregnant women diagnosed with diabetes mellitus.

Methods: The research was conducted at the Ankara City Hospital Perinatology Clinic between October 2021 and June 2022. The study included 277 pregnant women diagnosed with diabetes mellitus and 277 healthy pregnant women who had given birth. Retrospective analysis of anti-*T. gondii* immunoglobulin (Ig)G and IgM levels was performed for patients between January 2020 and February 2022. Participants were administered an informed consent form and a questionnaire. Data were analysed using SPSS 22.

Results: Among pregnant women with diabetes, IgG seropositivity was 18.4%, IgM was 0.0%, and IgG+IgM was 0.0%. In healthy pregnant women, IgG seropositivity was 12.3%, IgM was 0.4%, and IgG+IgM was 0.4%. Overall, seropositivity rates were 15.3% for IgG, 0.2% for IgM, and 0.2% for IgG+IgM. The difference between the two groups was statistically significant ($p<0.05$). Among pregnant women with diabetes, there was a significant statistical difference ($p<0.05$) in anti-*T. gondii* IgG seropositivity related to education, employment status, number of pregnancies and live births, history of toxoplasmosis diagnosis in children, previous toxoplasmosis diagnosis, hygiene, nutrition, and social habits. Among healthy pregnant women, significant statistical differences were found ($p<0.05$) in IgG seropositivity related to age, income, education level, number of pregnancies and live births, previous toxoplasmosis diagnosis, hygiene, nutrition, and social habits. No invasive interventions were performed on infants born to seropositive mothers, and perinatal data were not available.

Conclusion: The seroprevalence of toxoplasmosis in Ankara appears to be decreasing, but *T. gondii* infections continue to pose a public health concern and are significant in pregnant women with diabetes mellitus.

Keywords: *Toxoplasma gondii*, pregnant, diabetes mellitus, seropositive, risk factors

ÖZ

Amaç: Toxoplasmosis, *Toxoplasma gondii*'nin sebep olduğu paraziter bir enfeksiyondur. Bağışıklığı baskılanmış kişiler ve gebeler risk altında olup gebelerde düşüğe sebep olabilmektedir. Bu çalışma diyabet tanısı almış riskli gebelerde *T. gondii* antikorlarının seropozitifliğinin ve olası risk faktörlerinin belirlenebilmesi amacıyla yapılmıştır.

Yöntemler: Araştırma Ankara Şehir Hastanesi Perinatoloji Kliniği'nde Ekim 2021-Haziran 2022 tarihleri arasında yürütülmüştür. Çalışmanın materyalini, doğum yapmış, 277 diyabet tanısı almış gebe ile 277 sağlıklı gebe oluşturmuştur. Geriye dönük Ocak 2020-Şubat 2022 tarihleri arasındaki hastaların anti *T. gondii* immünoglobulin (Ig)G ve IgM değerlerine bakılmıştır. Katılımcılar bilgilendirildikten sonra anket bilgi formu uygulanmıştır. Elde edilen veriler SPSS 22 programına yüklenerek istatistiksel analizler yapılmıştır.

Bulgular: Çalışmada diyabet tanılı gebelerde IgG %18,4, IgM %0,0, IgG+IgM %0,0; sağlıklı gebelerde ise IgG %12,3, IgM %0,4, IgG+IgM %0,4 olarak tespit edilirken, genel toplamda IgG %15,3, IgM %0,2 ve IgG+IgM %0,2 oranında seropozitiflik tespit

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Address for Correspondence/Yazar Adresi: Neriman Mor, Kafkas University Faculty of Medicine, Department of Medical Parasitology, Kars, Türkiye
Phone/Tel: +90 532 728 23 60 **E-mail/E-Posta:** neriman.mor@kafkas.edu.tr **ORCID ID:** orcid.org/0000-0002-3674-8120

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Türkiye PARAZİTOLOJİ Dergisi

edilmiştir. İki grup arasındaki fark istatistiki olarak anlamlı bulunmuştur (p<0,05). Diyabet tanılı gebelerde; eğitim, çalışma durumu, gebelik ve canlı doğum sayısı, çocuklarında hastalık olma durumu, daha önce toxoplasmosis tanısı alma, hijyen, beslenme ve sosyal alışkanlıkları; sağlıklı gebelerde ise yaş, gelir, eğitim durumu, gebelik ve canlı doğum sayısı, daha önce toxoplasmosis tanısı alma, hijyen, beslenme ve sosyal alışkanlıkları ile anti-*T.gondii* IgG seropozitifliği arasında istatistiki fark anlamlı bulunmuştur (p<0,05). Seropozitif gebelerin bebeklerine herhangi bir invaziv girişim uygulanmamıştır. Dolayısıyla perinatal veriler bulunmamaktadır.

Sonuç: Ankara ilinde toxoplasmosis seropozitifliğinin giderek azaldığı, ancak *T. gondii* enfeksiyonlarının hala halk sağlığı sorunu olmaya devam ettiği ve diyabet tanılı riskli gebelerde önemli olduğu belirlenmiştir.

Anahtar Kelimeler: *Toxoplasma gondii*, gebe, diabetes mellitus, seropozitif, risk faktörleri

INTRODUCTION

Toxoplasma gondii is a eukaryotic parasite with a broad host spectrum, causing a parasitic infection seen in many living organisms (1,2). Approximately one-third of the world's population is exposed to this parasite. Cats are the definitive hosts. It has an obligate or facultative heteroxenous life cycle. It can infect all warm-blooded creatures, including humans (mammals, birds, etc.) (1,3). When acute toxoplasmosis occurs in a pregnant mother, resulting in fetal infection, congenital toxoplasmosis can occur, posing a risk (4,5). Toxoplasmosis, one of the protozoal infections, is seen at a high rate during pregnancy and has been reported to sometimes lead to fetal death (6). Chronic toxoplasmosis is considered a potential risk factor for type 2 diabetes mellitus (T2DM), as it is believed that *T. gondii* directly invades and destroys pancreatic β-cells, potentially triggering pancreatitis and, more importantly, diabetes (7). Particularly, researchers have highlighted that the protozoan parasite *T. gondii* might influence the risk and development of T2DM, potentially inducing low-grade inflammation (8). There is a belief that a possible relationship between toxoplasmosis and diabetes could shed light on the complex pathogenesis of diabetes and lead to significant clinical outcomes. Indeed, it has been suggested that toxoplasmosis increases susceptibility to becoming a diabetic and, conversely, that diabetic patients are more vulnerable to opportunistic infections such as *T. gondii* (7).

Acute toxoplasmosis contracted during pregnancy can be lethal for the fetus, and due to its high reported incidence, it is believed that this parasite may lay the groundwork for the development of diabetes. There is also a consideration that there is a connection between the two conditions. Reduced cellular immunity in pregnant women and diabetic patients may increase the susceptibility to infection. Therefore, despite studies on diabetic patients in Türkiye, there has been a lack of research specifically focusing on pregnant women diagnosed with diabetes. This study was conducted with the aim of determining the seropositivity of *T. gondii* antibodies in pregnant women diagnosed with diabetes at risk and identifying potential risk factors that could affect its epidemiology. In Türkiye, despite studies involving diabetic patients, no research has been found to focus on pregnant women diagnosed with diabetes. Hence, this study is aimed at investigating the seropositivity of *T. gondii* antibodies and identifying possible risk factors that could impact the epidemiology of the infection among pregnant women diagnosed with diabetes. It's important to consider the potential implications of such research, as acute toxoplasmosis in pregnant women and its relationship to diabetes could have significant health implications for both the mothers and their unborn children.

METHODS

The research was conducted at the Ankara City Hospital Department of Obstetrics and Perinatology between October 2021 and June 2022. A total of 277 pregnant women with diabetes who gave birth in the hospital, had anti-*T. gondii* immunoglobulin (Ig)G and IgM values, and were diagnosed with 7.2% (n=20) type-1 diabetes mellitus (T1DM), 19.5% (n=54) type-2 diabetes mellitus (T2DM), and 73.3% (n=203) gestational diabetes mellitus (GDM) constituted the patient group, while 277 healthy pregnant women without chronic disease constituted the control group. None of the women in the control group had been diagnosed with gestational diabetes and were not using insulin. Afterward, a "questionnaire information form" consisting of 26 questions and an "informed consent form" were administered and obtained from the participants. *Toxoplasma* IgG and IgM values were recorded by checking the system.

Statistical Analysis

The participants' responses to the questionnaire and laboratory results were then entered into the Statistical Package for Social Sciences (SPSS) program. Descriptive statistics such as mean (mean), count (n), and percentage (%) were used to present the data for the variables. To determine the relationship between categorical variables, the chi-square (χ^2) test was employed. In cases where the frequency was less than 5, Fisher's Exact chi-square test was applied. Seropositivity values were presented with a 95% confidence interval (95% CI) and a significance level of $p<0.05$ was considered statistically significant.

Ethical Statement

While the study was receiving ethics committee approval, the necessary permission was obtained by applying to the Ministry of Health Provincial Health Directorate Ankara City Hospital No. 2 Clinical Research Ethics Committee (protocol number: E2-21-948, date: 27.10.2021).

For the research, the necessary institutional permission was obtained by applying to Ankara Governorship Provincial Health Directorate Ankara City Hospital Gynecology Hospital Chief Physician (date: 14.10.2021).

RESULTS

The research compared the demographic characteristics and pregnancy histories of diabetic and healthy pregnant women. Among diabetic pregnant women, the highest percentage (38.3%) was in the age group of 35 and above, whereas among healthy pregnant women, the highest percentage (37.9%) was in the age range of 25-29. Both groups had the highest proportion of urban residents, with percentages of 90.6% and 88.8% for diabetic and healthy pregnant women, respectively. Furthermore, both groups had a significant proportion (46.6% for diabetic and 44.4% for

Table 1. Seropositivity of *T. gondii* antibodies in diabetic and healthy pregnant women (n=554)

Groups		Diabetic n/(%)	Healthy n/(%)	Total n/(%)	p	χ^2
IgG	**Positive**	**51 (18.4)**	**34 (12.3)**	**85 (15.3)**	**0.045**	**4.016**
	Negative	226 (81.6)	243 (87.7)	469 (84.7)		
IgM	**Positive**	0 (0.0)	**1 (0.4)**	**1 (0.2)**	1.000	1.002
	Negative	277 (100.0)	276 (99.6)	553 (99.8)		
IgG + IgM	**Positive**	0 (0.0)	1 (0.4)	**1 (0.2)**	1.000	1.002
	Negative	277 (100.0)	276 (99.6)	553 (99.8)		
Total		**277 (50.0)**	**277 (50.0)**	**554 (100.0)**		

Ig: Immunoglobulin

healthy pregnant women) with a high school education level. Regarding pregnancy histories, the number of pregnancies and live births ranged from 1 to 3 in both groups. However, the rates of miscarriage and premature birth were higher in diabetic pregnant women compared to healthy pregnant women. These findings suggest that diabetes may have a potential impact on pregnancy outcomes.

According to the study, anti-*T. gondii* IgG seropositivity was detected in 18.4% of diabetic pregnant women (according to diabetes types; 5.0% T1DM, 13.0% T2DM and 21.2% GDM) and 12.3% of healthy pregnant women, resulting in an overall IgG seropositivity rate of 15.3%. The difference in anti-*T. gondii* IgG seropositivity between diabetic and healthy pregnant women was statistically significant ($p<0.05$) (Table 1).

In this study, when the comparison was made in terms of anti-*T. gondii* IgM seropositivity, it was determined that a total of 1 (one) pregnant woman showed seropositivity (0.4%). The avidity test of this pregnant woman, who was among the healthy pregnant women, was negative. In addition, when compared according to socio-demographic characteristics, possible risk factors such as social, nutritional, hygiene habits, pregnancy status, blood transfusion and previous diagnosis of toxoplasmosis, no statistically significant difference was observed between the groups in terms of IgM seropositivity ($p>0.05$).

In Table 2, potential risk factors that could lead to toxoplasmosis in diabetic and healthy pregnant women are presented. These risk factors include socio-demographic characteristics, pregnancy history, social, dietary, and hygiene habits, and statistically significant findings are shared ($p<0.05$).

In healthy pregnant women, a significant relationship has been detected between age groups and income levels with anti-*T. gondii* IgG seropositivity. It was determined that low-income healthy pregnant women have a higher risk group with a rate of 28.6% ($p<0.05$).

In the diabetic group, according to educational status, illiterate and primary school graduate pregnant women were found to be 2.22 times more at risk compared to high school and university graduates (odds ratio=2.22, 95% CI: 1.200-4.108, $p<0.05$). In the same group, 44.4% of pregnant women who indicated that they had a chronic illness in their living children were found to be anti-*T. gondii* IgG seropositive ($p<0.05$).

When both groups were compared in terms of anti-*T. gondii* IgG seropositivity, although the majority answered positively to questions about potential risk factors, it was determined that a higher rate of seropositivity was found in diabetic diagnosed pregnant women and the difference between the groups was statistically significant ($p<0.05$) (Table 3).

DISCUSSION

According to scientific assessments, it is estimated that the number of diabetic patients will reach 522 million by the year 2030 (9). Recent research has suggested a potential relationship between infectious agents such as *Helicobacter pylori*, Coxsackie B4 virus, and diabetes. Similarly, *T. gondii* has been proposed as a possible cause for diabetes (10). Indeed, there are indications of an indirect relationship between toxoplasmosis and specific types of diabetes (7,9-11). A study examining the potential relationship between latent toxoplasmosis and blood glucose levels found that pregnant women with toxoplasmosis had significantly higher blood glucose levels during oral glucose tolerance testing (OGTT). Elevated glucose levels and increased GDM incidence could lead to significant clinical effects such as metabolic syndrome and T2DM development in women infected with *T. gondii* (11).

Diabetes, a metabolic disorder, has a widespread distribution, especially among individuals with high-calorie diets. While insulin deficiency is associated with type 1 diabetes mellitus (T1DM), improper response to insulin in target cells is known as T2DM, resulting in hyperglycaemia. Genetic factors, autoimmune processes, and environmental factors have been suggested as causes (7). *T. gondii* can infect and replicate in any nucleated cell, including pancreatic cells. Insulin, a hormone secreted by the pancreas, plays a role in regulating blood sugar. Theoretically, toxoplasmosis could play a role in the development of T1DM (9). In fact, Nassief Beshay et al. (9) reported that anti-*Toxoplasma* IgG seropositivity was 86.4% in T1DM, 66.7% in T2DM, and 60.0% in the control group. The difference was statistically significant compared to the control group, with T1DM patients having a 4.2 times higher seroprevalence. In a study conducted in China, as diabetes prevalence increased, *T. gondii* seroprevalence was found to be 16.5% in T1DM, 23.5% in T2DM, and 21.3% in GDM patients. Each type of DM patients had significantly higher *T. gondii* seroprevalence compared to control subjects (10). Another study in China found significantly higher rates of anti-*T. gondii* IgG seropositivity in GDM women compared to non-GDM women, but no statistically significant difference was found in terms of anti-*T. gondii* IgM (12). Similarly, in a study conducted in Trabzon, Türkiye, the seroprevalence of *T. gondii* was found to be significantly higher in diabetic individuals compared to non-diabetic individuals (13).

Table 2. Anti-*T. gondii* IgG seropositivity according to potential risk factors in diabetic and healthy pregnant women

Survey questions		Diabetic pregnant women			Healthy pregnant women		
		n	IgG positive n/(%)	p	n	IgG positive n/(%)	p
Educational level	**Illiterate-primary education**	103	**27 (26.2)**	**0.010**	100	18 (18.0)	**0.029**
	High school and beyond	174	24 (13.8)		177	16 (9.0)	
Number of pregnancies	**1-3**	186	28 (15.1)	**0.039**	222	21 (9.5)	**0.004**
	4-6+	91	**23 (25.3)**		55	13 (23.6)	
Number of live births	**1-3**	247	41 (16.6)	**0.026**	261	28 (10.7)	**0.002**
	4-6+	30	**10 (33.3)**		16	6 (37.5)	
	No	259	43 (16.6)				
Contact with cats	**Yes**	17	**7 (41.2)**	**0.012**	25	12 (48.0)	**0.000**
	No	260	44 (16.9)		252	22 (8.7)	
Feeding cats at home	**Yes**	11	**6 (54.5)**	**0.002**	3	2 (66.7)	**0.041**
	No	266	45 (16.9)		274	32 (11.7)	
Feeding cats in the garden	**Yes**	31	**12 (38.7)**	**0.002**	19	11 (57.9)	**0.000**
	No	246	39 (15.9)		258	23 (8.9)	
Engaging in garden or field work	**Yes**	30	**13 (43.3)**	**0.000**	15	10 (66.7)	**0.000**
	No	247	38 (15.4)		262	24 (9.2)	
Consumption of unwashed fruits and vegetables	**Yes**	18	**7 (38.9)**	**0.020**	16	7 (43.8)	**0.000**
	No	259	44 (17.0)		261	27 (10.3)	
Consumption of raw or undercooked meat	**Yes**	15	**6 (40.0)**	**0.027**	6	4 (66.7)	**0.02**
	No	262	45 (17.2)		271	30 (11.1)	
Consumption of raw or undercooked meat	**Yes**	66	**24 (36.4)**	**0.000**	63	19 (30.2)	**0.000**
	No	211	27 (12.8)		214	15 (7.0)	
Consumption of raw milk	**Yes**	4	**4 (100.0)**	**0.001**	3	2 (66.7)	**0.041**
	No	273	47 (17.2)		274	32 (11.7)	
Consumption of processed foods in their raw form	**Yes**	126	**37 (29.4)**	**0.000**	92	21 (22.8)	**0.000**
	No	151	14 (9.3)		185	13 (7.0)	
Do not use the knife used for cutting raw meat to also cut cooked meat or raw fruits/ vegetables intended for consumption	**Yes**	127	**34 (26.7)**	**0.001**	116	24 (20.7)	**0.000**
	No	150	17 (11.3)		161	10 (6.2)	
Grand total		**277**	**51 (18.4)**		**277**	**34 (12.3)**	

Ig: Immunoglobulin

Toxoplasmosis studies conducted in diabetic individuals have also yielded contradictory results. Indeed, a study in Iran found anti-*T. gondii* IgG seropositivity rates of 69.0% in T1DM, 63.0% in T2DM, and 59.0% in the control group. However, no statistically significant differences were observed in terms of toxoplasmosis among the studied groups (14). A study in Durango, Mexico, concluded that there was no serological evidence of a relationship between *T. gondii* infection and diabetes. Similarly, despite limitations such as a small number of studies, a systematic analysis by Majidiani et al. (7) suggested that chronic toxoplasmosis could be a potential risk factor for T2DM. However, using a random-effects model, no statistically significant relationship between *T. gondii* and T1DM was found. Similarly, a study conducted in Sivas, Türkiye, found that anti-*T. gondii* seropositivity rates were 40.5% in diabetic patients and 38.2% in healthy individuals in the control group, and *Toxoplasma* IgM was negative in both groups. Consequently, there was no statistically significant relationship reported in terms of *Toxoplasma* IgG seropositivity between the groups (15). Another study in Egypt investigated different types of diabetic vascular complications and glycosylated haemoglobin

Table 3. Comparison of anti-*T. gondii* IgG seropositivity in diabetic and healthy pregnant women according to possible risk factors

Groups		n	Diabetic	n	Healthy	Total	p	χ^2
			Positive n/(%)		Positive n/(%)			
Cat contact	No	260	44 (16.9)	252	22 (8.7)	66 (12.9)	**0.006**	**7.650**
Feeding stray cats in the garden	No	246	39 (15.9)	258	23 (8.9)	62 (12.3)	**0.018**	**5.620**
Working in garden and field	No	247	38 (15.4)	262	24 (9.2)	62 (12.2)	**0.032**	**4.605**
Drinking water source	Purifier	67	13 (19.4)	66	3 (4.6)	16 (12.0)	**0.014**	**6.935**
Consumption of unwashed fruits and vegetables	No	259	44 (17.0)	261	27 (10.3)	71 (13.7)	**0.027**	**4.867**
Consumption of raw or undercooked meat	No	262	45 (17.2)	271	30 (11.1)	75 (14.1)	**0.043**	**4.107**
Consumption of raw or undercooked eggs	No	211	27 (12.8)	214	15 (7.0)	42 (9.9)	**0.046**	**3.995**
Handwashing before and after cooking	Yes	270	**50 (18.5)**	275	**34 (12.4)**	84 (15.4)	**0.047**	**3.959**
Total		**277**	**51 (18.4)**	**277**	**34 (12.3)**	**85 (15.6)**	**0.45**	**4.016**

Ig: Immunoglobulin

(HbA1c) levels, yet no significant relationship between *T. gondii* infection and diabetes was identified. Despite the high prevalence of anti-*T. gondii* IgG among diabetic patients, the researchers concluded that there was no association with diabetic complications and glycaemic control (16).

In this study, anti-*T. gondii* IgG seropositivity was determined to be 18.4% in pregnant women diagnosed with diabetes, while it was found to be 12.3% in healthy pregnant women. The difference between the groups is statistically significant ($p<0.05$). In the study, *T. gondii* IgG seropositivity rates were 5.0% in pregnant women diagnosed with T1DM, 13.0% in pregnant women diagnosed with T2DM, and 21.2% in pregnant women diagnosed with GDM. However, no statistically significant differences were observed in terms of anti-*T. gondii* IgG seropositivity between pregestational and gestational diabetes types or between insulin-using and non-insulin-using pregnant women ($p>0.05$).

In a cross-sectional study conducted on subjects with T1DM and T2DM referred to diabetes centres in Iraq, it has been reported that *T. gondii* seropositive diabetic individuals have a higher likelihood of being obese compared to seronegative diabetic individuals (8). In a study conducted in Korea, *T. gondii* IgG seropositive cases were found to have a higher seroprevalence in terms of various diseases in order of frequency, including malignant neoplasms, diabetes mellitus (DM), arthritis, chronic hepatitis B, chronic kidney diseases, schizophrenia, and acute lymphadenitis, compared to the control group. Furthermore, the study suggested that individuals with weakened immune systems due to chemotherapy-related drugs, as well as cancer, chronic hepatitis, or metabolic disorders related to diabetes, might have a higher risk of contracting infectious diseases such as toxoplasmosis. The potential association of *T. gondii* seropositivity with neoplasms, DM, and other chronic infections has been highlighted (17).

When looking at studies conducted on pregnant women in Ankara, Güngör et al. (18) conducted research involving 245 participants using the Sabin Feldman and ELISA methods, reporting a *T. gondii* IgG seropositivity rate of 41.6%. In a study by Saraçoğlu and Şahin (19), involving 231 pregnant women, a *T. gondii* IgG seropositivity rate of 38.1% was reported. In a study conducted by Oral (20) on healthy pregnant women, a *T. gondii* IgG seropositivity rate of 27.4% was reported. In this study conducted at Ankara City Hospital Women's Health and Birth Tower, a total of 554 pregnant women showed a *T. gondii* IgG positivity rate of 15.9%. When examining all these studies conducted in Ankara, it can be observed that *T. gondii* seropositivity is decreasing gradually. This decline may be explained by factors predominant in Ankara, where urbanization is prominent, such as reduced contact with soil, decreased engagement in gardening and farming, a lower presence of stray cats, increased education levels, and greater awareness over the years. Similarly, in Fas, when studies conducted in 2007, 2014, and 2021 are compared, a decrease in *T. gondii* IgG seroprevalence is reported, with rates of 51.0%, 47.0%, and 43.0% respectively in the studied regions (21).

In some studies, it has been observed that *T. gondii* seroprevalence increases proportionally with age, and this is attributed to the elevated exposure to *Toxoplasma* with aging (22). Indeed, the reason for the increase in quantitative titers with age is thought to be the higher likelihood of an individual coming into contact with one of the routes of transmission. Many studies support this phenomenon (13,17,19,21,23-26). In a study conducted in Muş, while no significant difference was found between age and *Toxoplasma* IgM seropositivity, a linearly significant increase in *Toxoplasma* IgG seropositivity with age was observed. Researchers attributed this to the higher risk of encountering the pathogen as age progresses in an area where contact with both large and small livestock is common (27). Additionally, it has been reported that there is no significant relationship between age increase and infection (28-32). In this current study, while no statistical significance was found between seropositivity and age in pregnant women diagnosed with diabetes, a significant relationship was observed in healthy pregnant women ($p<0.05$).

Researches revealing the relationship between the number of pregnancies and anti-*Toxoplasma* IgG seropositivity have been encountered (20,33,34). While two studies conducted at different times found no relationship between the number of pregnancies and anti-*Toxoplasma* IgG seropositivity (33,34), another study reported a proportional increase in *Toxoplasma* IgG seropositivity

with an increasing number of pregnancies (20). In this study, it was determined that as the number of pregnancies and live births increased, the proportional increase in anti-*Toxoplasma* seropositivity was significant ($p<0.05$). Education level has contributed to awareness in many diseases and has also added the skill of being conscious about toxoplasmosis. There are studies indicating an inverse relationship between education level and seropositivity. Changes in lifestyle related to development, hygiene measures, and higher education levels can contribute to reducing the prevalence of infection (13,20,21). In this study, when the education levels of pregnant women diagnosed with diabetes and healthy pregnant women were categorized as "illiterate, primary education, secondary education, university and above", it was observed that as education levels increased in both groups, *T. gondii* IgG seroprevalence rates decreased, and a significant relationship was found ($p<0.05$). This suggests that educated pregnant women act more consciously, and the increase in knowledge of personal hygiene could explain the lower incidence of infection.

The final host of the disease, cats, are known to play a significant role in the spread of toxoplasmosis. Regions with a high cat population and activities involving contact with cats or cat litter have been found to be significantly associated with *T. gondii* seropositivity (5,28,35). It has been reported that both *Toxoplasma* IgG and IgM seropositivity are higher in individuals with pet animals (36,37). A study conducted in Ethiopia found that living with pet cats increased the infection rate by five times by *T. gondii* (38). A study in China reported that keeping cats at home was a significant risk factor for diabetes patients (10). Another study found a significant association between feeding cats in the garden and seropositivity (31). A seroprevalence study conducted in Kars, Türkiye, found that although pregnant women who had cats at home or in the garden had higher seropositivity rates, there was no statistically significant relationship (26). In this study, a significant relationship was observed between anti-*Toxoplasma* IgG seropositivity and keeping cats at home and in the garden, as well as contact with cats ($p<0.05$).

CONCLUSION

This study has once again highlighted potential risk factors for toxoplasmosis in both pregnant women with diabetes and healthy pregnant women. Pregnant women who are in contact with cats, which is one of the most important potential risk factors for transmission, may be less likely to transmit the disease if their cats are treated with internal parasite medication every three months. Increasing awareness and knowledge could play a pivotal role in disease control. Educational sessions during prenatal classes and regular follow-ups at healthcare centres could offer informative insights about toxoplasmosis and its associated risk factors. Displaying informative posters in relevant centres could effectively raise awareness. Moreover, nationwide screening programs should be implemented to ensure broader coverage and prevention of congenital toxoplasmosis, ultimately contributing to the overall health of the population. The development and implementation of preventive and control programs for the disease are both cost-effective and feasible. This study could serve as a guide for future research and control policies among pregnant women diagnosed with diabetes.

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*Ethics

Ethics Committee Approval: While the study was receiving ethics committee approval, the necessary permission was obtained by applying to the Ministry of Health Provincial Health Directorate Ankara City Hospital No. 2 Clinical Research Ethics Committee (protocol number: E2-21-948, date: 27.10.2021).

Informed Consent: Informed consent was obtained.

*Authorship Contributions

Surgical and Medical Practices: D.Ş., Concept: N.M., D.Ş., Design: N.M., D.Ş., Data Collection or Processing: N.A.S., Analysis or Interpretation: N.A.S., N.M., Literature Search: N.A.S., N.M., Writing: N.A.S., N.M.

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Investigation of Factors Associated with Gut Microbiota in *Demodex*-associated Skin Conditions

Demodeks ile İlişkili Deri Hastalıklarında Bağırsak Mikrobiyotasına İlişkin Faktörlerin Araştırılması

Fatmagül Gülbaşaran[1], Seray Sarımustafa[1], Özlem Özbağçıvan[1], Şükran Köse[2], Emre Avcı[1]
[1]Dokuz Eylül University Hospital, Department of Dermatology, İzmir, Türkiye
[2]Dokuz Eylül University Hospital, Department of Infectious Diseases and Clinical Microbiology, İzmir, Türkiye

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ABSTRACT

Objective: This study describes the relationships of factors related to gut microbiota and skin conditions associated with *Demodex*, including demodicosis, rosacea, and perioral dermatitis.

Methods: A total of 113 patients from Dokuz Eylül University Hospital Dermatology Department answered a cross-sectional questionnaire. They consisted of 42 cases of *Demodex*-related skin diseases and 71 healthy controls. Demographic data and medical history, dietary and lifestyle habits, and gastrointestinal symptoms were recorded. Statistical analysis included descriptive statistics, chi-square tests, Fisher's Exact tests, independent samples t-tests, and logistic regression methods.

Results: Our findings identified alcohol consumption [odds ratio (OR)=11.13, 95% confidence interval (CI): 4.11-17.22, p<0.01] and smoking (OR=10.32, 95% CI: 2.47-21.57, p<0.01) as strong risk factors for *Demodex*-related conditions. Low water intake (0-1 liter per day) (OR=3.39, 95% CI: 2.08-5.57, p=0.03) and infrequent exercise (less than 1 hour per week) (OR=4.87, 95% CI: 2.70-12.54, p=0.02) were also significant risk factors. Additional factors associated with increased *Demodex* risk included reduced bowel movements (OR=2.71, 95% CI: 1.45-4.06, p=0.01) and higher pet ownership (OR=2.85, 95% CI: 2.13-4.27, p=0.03). Although vegetarian and high-fat diets showed some associations, they were not independently significant.

Conclusion: This study demonstrates key environmental and lifestyle factors, such as low water intake, infrequent exercise, reduced bowel movements, higher pet ownership, alcohol consumption, and smoking, that are significantly associated with *Demodex*-related skin conditions. These factors, related to gut microbiota, may provide valuable insights for managing these skin conditions and suggest promising directions for future research.

Keywords: *Demodex*, gut microbiota, rosacea, skin conditions, gut-skin axis

ÖZ

Amaç: Bu çalışma, demodikoz, rosacea ve perioral dermatit gibi *Demodeks* ile ilişkili deri hastalıklarına ve bağırsak mikrobiyotasına bağlı faktörlerin ilişkilerini tanımlamaktadır.

Yöntemler: Dokuz Eylül Üniversitesi Hastanesi Dermatoloji Bölümünden toplam 113 hasta kesitsel bir anketi yanıtladı. Bu hastalar, 42 *Demodeks* ile ilişkili deri hastalığı olgusu ve 71 sağlıklı kontrol grubundan oluşmaktaydı. Demografik veriler, tıbbi geçmiş, beslenme ve yaşam tarzı alışkanlıkları ve gastrointestinal semptomlar kaydedildi. İstatistiksel analizler, betimleyici istatistikler, ki-kare testleri, Fisher'ın kesin testleri, bağımsız örneklem t-testleri ve lojistik regresyon yöntemlerini içerdi.

Bulgular: Bulgularımız, alkol tüketimini [olasılık oranı (OO)=11,13, %95 güven aralığı (GA): 4,11-17,22, p<0,01) ve sigara içmeyi (OO=10,32, %95 GA: 2,47-21,57, p<0,01) *Demodeks* ile ilişkili hastalıklar için güçlü risk faktörleri olarak belirlemiştir. Düşük su tüketimi (0-1 litre gün başına) (OO=3,39, %95 GA: 2,08-5,57, p=0,03) ve seyrek egzersiz (haftada 1 saatten az) (OO=4,87, %95 GA: 2,70-12,54, p=0,02) de önemli risk faktörleri olarak bulunmuştur. Artmış *Demodeks* riskiyle ilişkili diğer faktörler arasında azalmış bağırsak hareketleri (OO=2,71, %95 GA: 1,45-4,06, p=0,01) ve yüksek evcil hayvan sahipliği (OO=2,85, %95 GA: 2,13-4,27, p=0,03) bulundu. Vejetaryen ve yüksek yağlı diyetlerin bazı ilişkiler gösterdiği görülmüş olmasına rağmen, bağımsız olarak anlamlı bulunmadı.

Sonuç: Bu çalışma, düşük su tüketimi, seyrek egzersiz, azalmış bağırsak hareketleri, yüksek evcil hayvan sahipliği, alkol tüketimi ve sigara içme gibi *Demodeks*-ilişkili deri hastalıkları ile önemli ölçüde ilişkili olan çevresel ve yaşam tarzı faktörlerini göstermektedir. Bağırsak mikrobiyotası ile ilişkili bu faktörler, bu deri hastalıklarının yönetiminde değerli bilgiler sunabilir ve gelecekteki araştırmalar için umut verici yönler önerebilir.

Anahtar Kelimeler: *Demodeks*, bağırsak mikrobiyotası, rosacea, deri hastalıkları, bağırsak-deri ekseni

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Address for Correspondence/Yazar Adresi: Fatmagül Gülbaşaran, Dokuz Eylül University Hospital, Department of Dermatology, İzmir, Türkiye
Phone/Tel: +90 532 479 63 80 **E-mail/E-Posta:** drfatmagulgulbasaran@gmail.com **ORCID ID:** orcid.org/0000-0002-7550-6052

Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Demodex mites are microscopic ectoparasites that mainly inhabit the hair follicles of mammals (1). In humans, two species are common: *Demodex folliculorum* and *Demodex brevis,* often called eyelash or face mites (1). Generally, they exist in small numbers, without visible problems. However, when overpopulated or penetrating deeper into the skin, they can cause pityriasis folliculorum, rosacea, and perioral dermatitis (2-6).

Demodex folliculorum resides in facial skin, and its overgrowth causes itching, redness, and scaling of the face. This condition is described as demodicosis (7). Rosacea, a chronic inflammatory disorder of the central face (8), often initiates with transient facial redness that may progress into fixed erythema and papules, pustules, and telangiectasia (8). This condition is most commonly seen in persons between 30 and 50 years of age and may also present with eye symptoms (8). Perioral dermatitis is another chronic inflammatory condition characterized by red papules and vesicles around the mouth, nose, and eyes; women were predominantly affected between the ages of 16 to 45 years (9).

Recent studies have established the critical role of gut microbiota in the maintenance of skin health and in modulating immune responses (10,11). Diversity in short-chain fatty acids produced by gut microbiota might affect the composition of skin microbiota and hence influence cutaneous immune responses (11-13). Besides, perturbations in gut microbiota have been shown to weaken skin barrier functions and lead to skin disorders through systemic inflammation (10,11).

Treatments for diseases such as rosacea (14), perioral dermatitis (15), and demodicosis (16) typically involve a strategy of reducing *Demodex* mites and minimizing environmental factors like sun exposure and spicy diet. A rising body of evidence points to the role of factors influencing gut microbiota -for example, dietary habits, lifestyle, and medical history- that might also impact the course of these skin diseases (17-22), but the data are still limited.

In this regard, this study investigates the possible relationships between several factors, including medical history, lifestyle, eating habits, and gastrointestinal symptoms related to gut microbiota, with *Demodex*-associated skin conditions, including demodicosis, rosacea, and perioral dermatitis. The identification of these relationships will be useful in attaining better management for those suffering from these diseases.

METHODS

Study Design and Participants

We carried out a cross-sectional web-based survey among adults who had been diagnosed with demodicosis, rosacea, or perioral dermatitis at Dokuz Eylül University Hospital's Dermatology Department within the past two years. Participants were recruited through a screening process and invited to complete a web-based survey. The study included a total of 113 participants, comprising 42 patients with the aforementioned conditions and 71 healthy controls.

Inclusion criteria for the patient group were adults with a confirmed diagnosis of demodicosis, rosacea, rhinophyma, or perioral dermatitis based on ICD-10 codes as determined by a dermatologist. Exclusion criteria included individuals under 18 years old, pregnant or lactating women, those with a history of malignancy or who were undergoing chemotherapy or radiotherapy, individuals with prior gastrointestinal surgery, those with severe infectious or immunosuppressive diseases, and those with significant cognitive impairments.

Ethical approval for the study was obtained from the Dokuz Eylül University Ethics Committee (approval number: 2024/20-20, date: 05.06.2024).

Data Collection

Participants who consented to join the study were given access to an anonymous web-based survey via a provided link. Informed consent was required at the start of the survey, and only the responses from participants who completed the entire survey were included in the analysis.

The survey gathered a variety of information, including demographic details such as age and sex, along with data on dermatological diagnoses to confirm conditions like demodicosis, rosacea, rhinophyma, or perioral dermatitis. Additionally, participants provided information about their medical history, current medications, family history of autoimmune diseases, and any known allergies.

The survey also collected details about dietary habits and lifestyle factors, such as alcohol and tobacco use, exercise routines, and the use of nutritional supplements. Furthermore, participants reported their psychological stress levels and any gastrointestinal symptoms they experienced.

Statistical Analysis

Descriptive statistics, including the mean, standard deviation, frequencies, and percentages, were calculated. Categorical variables were analyzed using chi-square (χ^2) tests and Fisher's Exact tests, while independent samples t-tests were used for continuous variables. Logistic regression analysis was performed to evaluate the impact of various risk factors on the diagnosed conditions. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. A p-value of less than 0.05 considered statistically significant. All analyses were performed using SPSS version 22.0.

RESULTS

Among patients, 73.8% had rosacea, 14.3% had perioral dermatitis, and 9.5% had demodicosis. Age and gender distributions between the control and patient groups were similar (p=0.08 and p=0.06, respectively) (Table 1).

Regarding clinical characteristics, patients had significantly fewer weekly bowel movements compared to controls (5.12±2.41 vs. 7.41±3.79; p=0.02). Additionally, controls had a higher prevalence of known allergies compared to patients (40.8% vs. 21.4%; p=0.01) (Table 1).

There were no significant differences between the control and patient groups regarding immunosuppressing conditions, significant stress or life events, or known infections (all p>0.05). However, patients reported significantly higher levels of physical intensity or fatigue compared to controls (p=0.04). No participants had a history of inflammatory bowel disease or family history of it (Table 2).

Comparing gastrointestinal symptoms, no significant differences were found between groups in terms of constipation, post-meal bloating, abdominal pain, gallbladder issues, and reflux (Table 3). Patients, however, reported higher regular medication use

Table 1. Comparison of demographic and clinical measurements between patients with dermatologic conditions related to *Demodex* and controls

Measure	Control (n=71) mean ± SD	Patients (n=42) mean ± SD	p-value
Age	33.66±12.25	38.21±13.82	0.08
Sex			0.06
- Male	37 (52.1%)	14 (33.3%)	
- Female	34 (47.9%)	28 (66.7%)	
Clinical characteristics			
Comorbidities	24 (33.8%)	15 (35.7%)	0.94
Medication for other diseases	16 (22.5%)	11 (26.2%)	0.92
Annual antibiotic use	1.85±1.95	1.45±1.66	0.19
Average annual diarrhea episodes	9.18±15.01	10.83±5.72	0.51
Average weekly bowel movements	7.41±3.79	5.12±2.41	**0.02***
Stress severity	2.25±0.67	2.19±0.59	0.37
Medical history			
Duration of breastfeeding (months)	12.26±9.14	11.50±9.22	0.54
Delivery method			**0.03***
- Vaginal delivery	44 (62.0%)	31 (73.8%)	
- Cesarean section	27 (38.0%)	11 (26.2%)	
Autoimmune disease	9 (12.7%)	2 (4.8%)	0.13
Autoimmune disease in family	20 (28.2%)	9 (21.4%)	0.44
Known allergy	29 (40.8%)	9 (21.4%)	**0.01***
Family history of allergy	19 (26.8%)	14 (33.3%)	0.12

*: p<0.05 indicates significant differences, SD: Standard deviation

Table 2. Clinical characteristics related to immunity

Characteristic	Control (n=71)	Patients (n=42)	p-value
Immunosuppressing conditions or medications	1 (1.4%)	0 (0.0%)	0.92
Significant stress or life events	21 (29.6%)	11 (26.2%)	0.77
Physical intensity/fatigue	24 (33.8%)	17 (40.5%)	**0.04***
Known infections (HIV, hepatitis, syphilis)	2 (2.8%)	1 (2.4%)	0.92

*: p<0.05 indicates significant differences, HIV: Human immunodeficiency virus

compared to controls (p=0.04). Post-antibiotic diarrhea incidence was similar in both groups (p=0.93).

Regarding dietary habits, there were no significant differences between groups in weekly consumption of vegetables, probiotic foods (such as homemade yogurt, pickles, and vinegar), fiber-rich foods, fermented foods, sugary foods, packaged foods, and processed foods (Table 4). However, a carbohydrate-based diet was more prevalent among controls, while a vegetarian diet and a high-fat diet were more common among patients, with these differences being statistically significant (p=0.04).

The analysis of lifestyle characteristics revealed several significant differences between the healthy controls and patients (Table 5). Alcohol consumption was notably higher among patients (61.9% vs. 32.4% in controls, p<0.01), as was tobacco use (69.0% vs. 52.1% in controls, p<0.01). Pet ownership was also more common among patients (35.7% vs. 19.7% in controls, p=0.02). Patients consumed less water daily, with 23.8% drinking 0-1 liter compared to 14.1% of controls (p=0.01). Moreover, patients engaged in less physical activity, with 52.4% reporting no exercise versus 26.8% of controls (p=0.01). There was a borderline significant difference in the proportion of patients who reported regularly getting adequate sleep (54.8% vs. 43.7% of controls, p=0.04), though sleep problems were similarly reported in both groups (p=0.89).

The most frequently used supplements among patients were vitamin D, multivitamins, omega-3, vitamin B12, magnesium, iron, zinc, and protein powder. No significant differences were found between patients and controls regarding the use of these supplements (all p>0.05).

In multivariate analysis (Table 6), alcohol consumption exhibited a strong association with *Demodex*-related skin conditions, with an OR of 11.13 (95% CI: 4.11-17.22, p<0.01). Smoking also had a significant association, with an OR of 10.32 (95% CI: 2.47-21.57, p<0.01).

Lack of regular exercise (less than 1 hour per week) was a notable risk factor, with an OR of 4.87 (95% CI: 2.70-12.54, p=0.02). Low

Table 3. Digestive characteristics

Digestive characteristics	Control (n=71)	Patients (n=42)	p-value
Constipation	22 (31.0%)	14 (33.3%)	0.68
Post-meal bloating	32 (45.1%)	22 (52.4%)	0.24
Abdominal pain	21 (29.6%)	15 (35.7%)	0.17
Gallbladder issues	4 (5.6%)	2 (4.8%)	0.91
Reflux	21 (29.6%)	14 (33.3%)	0.18
Regular medication use	9 (12.7%)	10 (23.8%)	**0.04***
Post-antibiotic diarrhea	10 (14.1%)	6 (14.3%)	0.93

*: p<0.05 indicates significant differences

Table 4. Examination of nutritional characteristics

Nutritional characteristics: Weekly frequency of consumption	Control (n=71) mean ± SD	Patients (n=42) mean ± SD	p-value
Vegetables	3.11±0.69	3.07±0.89	0.78
Homemade yoghurt, pickles, vinegar	2.39±1.10	2.69±1.07	0.17
Fiber-rich foods	0.70±0.72	0.90±0.82	0.18
Fermented foods	0.66±0.67	0.60±0.66	0.61
Sugary food consumption	0.86±0.87	0.71±0.83	0.39
Packaged food consumption	0.56±0.71	0.33±0.57	0.08
Processed foods	0.17±0.53	0.24±0.53	0.51
Nutritional habit definition			
Mediterranean diet	29 (40.8%)	18 (42.9%)	**0.04***
Meat-based diet	21 (29.6%)	13 (31.0%)	
Carbohydrate-based diet	20 (28.2%)	6 (14.3%)	
Vegetarian diet	0 (0.0%)	3 (7.1%)	
High-fat diet	1 (1.4%)	2 (4.8%)	

*: p<0.05 indicates significant differences, SD: Standard deviation

water intake (0-1 liter per day) had an OR of 3.39 (95% CI: 2.08-5.57, p=0.03), indicating its importance.

Pet ownership was also significantly associated with increased risk, with an OR of 2.85 (95% CI: 2.13-4.27, p=0.03). Fewer bowel movements per week were associated with an OR of 2.71 (95% CI: 1.45-4.06, p=0.01). Known allergies did not significantly impact the outcome (p=0.11), while vegetarian and carbohydrate-dominant diets showed no significant associations after adjustment (p=0.62 and p=0.51, respectively).

DISCUSSION

This study investigates the associations between gut microbiota-related factors and *Demodex*-associated skin conditions. Pathophysiology of rosacea, a prototypical condition among *Demodex*-related skin conditions, characterized by immune system imbalances and inflammation, is linked to variations in skin microbiota, such as increased *Proteobacteria* and *Firmicutes* (23). Although studies on gut microbiota's role in rosacea are limited, differences in bacterial genera like *Acidaminococcus* and

Table 5. Examination of lifestyle characteristics

Lifestyle characteristic	Control (n=71)	Patients (n=42)	p-value
Alcohol consumption	23 (32.4%)	26 (61.9%)	**<0.01***
Tobacco use	37 (52.1%)	29 (69.0%)	**<0.01***
Regularly getting adequate/quality sleep	31 (43.7%)	23 (54.8%)	**0.04***
Sleep problems	28 (39.4%)	16 (38.1%)	**0.89**
Keeping pets at home	14 (19.7%)	15 (35.7%)	**0.02***
Daily water consumption			
0-1 liter	10 (14.1%)	10 (23.8%)	**0.01***
Liters	35 (49.3%)	18 (42.9%)	
More than 2 liters	26 (36.6%)	14 (33.3%)	
Weekly exercise duration (hours)			
None	19 (26.8%)	22 (52.4%)	**0.01***
1 hour	16 (22.5%)	5 (11.9%)	
Hours	27 (38.0%)	12 (28.6%)	
4-6 hours	9 (12.7%)	3 (7.1%)	

*: p<0.05 indicates significant differences

Megasphaera in rosacea patients suggest a potential connection via the gut-skin axis (24,25).

Our study identified alcohol consumption as the strongest risk factor for rosacea (RR=11.13, 95% CI: 4.11-17.22, p<0.01), consistent with a meta-analysis showing a 4.17-fold increased risk of phymatous rosacea among alcohol drinkers (95% CI =1.76-9.91) (26). While the exact mechanism linking alcohol and rosacea remains unclear, potential pathways include direct effects of alcohol metabolites on skin vasculature and indirect effects on both the skin and gut microbiomes (19,20).

Our study showed a significant association between smoking and rosacea (OR=10.32, 95% CI: 2.47-21.57, p<0.01). However, the relationship between smoking and *Demodex*-related conditions is complex and potentially paradoxical. While some studies suggest smoking might initially reduce rosacea symptoms (potentially through vasoconstriction, anti-inflammatory effects, and skin barrier disruption) (17), it could ultimately contribute to *Demodex* proliferation and rosacea development through its impact on the skin, immune system, and gut microbiota (18). This complexity is highlighted by research indicating that former smokers may have an increased risk of rosacea compared to active smokers (17).

Our study identified that infrequent bowel movements (three or fewer weekly) were significantly associated with 2.71 times the risk of *Demodex*-related dermatological conditions (95% CI: 1.45-4.06, p=0.01). This finding aligns with the understanding that constipation, often linked to bacterial overgrowth in the gut (27), can influence skin health. Treating bacterial overgrowth has been shown to improve rosacea symptoms (28), suggesting a potential pathway through which reduced bowel movements and associated gut microbiome changes could contribute to *Demodex*-related skin conditions.

Our study identified that infrequent exercise (less than 1 hour per week) as a significant risk factor for *Demodex*-related skin disorders (OR=4.87, 95% CI: 2.70-12.54, p=0.02). The literature on exercise and rosacea is limited, but a cross-sectional study with 110 rosacea patients found that increased muscle mass, a

Table 6. Identification of multiple risk factors

Variable	Univariate OR (95% CI)	p-value	Multivariate OR (95% CI)	p-value
Alcohol	1.61 (1.10-2.12)	**<0.01***	11.13 (4.11-17.22)	**<0.01***
Smoking	1.45 (0.94-1.96)	**<0.01***	10.32 (2.47-21.57)	**<0.01***
Lack of regular exercise (<1 hour/week)	1.43 (0.92-1.94)	**<0.01***	4.87 (2.70-12.54)	**0.02***
Low water intake (0-1 L/day)	1.17 (0.66-1.68)	**0.02***	3.39 (2.08-5.57)	**0.03***
Pet ownership	1.28 (0.77-1.79)	**0.03***	2.85 (2.13-4.27)	**0.03***
Weekly bowel movements (3 and less)	1.21 (0.89-1.56)	**0.01***	2.71 (1.45-4.06)	**0.01***
Known allergy	0.63 (0.38-1.04)	**<0.01***	0.46 (0.18-1.19)	0.11
Vegetarian diet	1.07 (0.56-1.58)	**0.04***	0.61 (0.14-4.33)	0.62
Carbohydrate-dominant diet	0.92 (0.41-1.43)	**0.04***	0.93 (0.22-2.35)	0.51
Regular medication use	1.02 (2.55-3.57)	**0.02***	0.94 (0.57-3.64)	0.44
Normal delivery method	1.05 (2.64-3.66)	**0.03***	0.96 (0.48-2.82)	0.74
Experiencing physical fatigue	1.01 (2.51-3.53)	**0.03***	0.99 (0.77-1.17)	0.16

*: p<0.05 indicates significant differences, OR: Odds ratio, CI: Confidence interval

proxy for regular exercise, was associated with reduced rosacea severity (29). It has been suggested that insufficient exercise may contribute to the development of rosacea by altering the gut microbiome and disrupting immune function, thereby increasing susceptibility to skin disorders (30). However, more comprehensive research is needed to draw reliable conclusions about the effects of exercise and muscle mass on *Demodex*-related conditions. This is especially important because microbiota, which can have significant immune effects throughout the body, is influenced by various personal factors such as urban versus rural living, geographical location, alcohol and tobacco use, stress, mental health status, and exercise (31).

Low water intake (0-1 liter per day) was significantly associated with 3.39 times the risk of having *Demodex*-related cutaneous disease (95% CI: 2.08-5.57, p=0.03), highlighting its importance. The protective effect of water consumption in *Demodex*-related skin conditions could be attributed to its role in maintaining skin hydration and normal physiological processes (32,33). Studies have shown that low skin moisture correlates with higher mite density, though results have not always been significant (34). Water intake is also a critical factor influencing gut microbiota (35), which may indirectly affect skin microbiota diversity and resistance to diseases.

Pet ownership was significantly associated with *Demodex*-related skin disorders in our multivariate analysis (OR=2.85, 95% CI: 2.13-4.27, p=0.03). While *Demodex* mites are common in animals and can cause demodicosis, they are not typically transmissible to humans through normal contact (36). A study involving 96 healthy volunteers found no significant relationship between pet ownership and the presence of *Demodex* mites (36). The increased risk observed in our study may not solely be related to the direct effect of having pets on *Demodex* mites. Numerous findings have identified relationships between the microbiota characteristics of pet owners and their pets (37). For example, one study found that patients with rosacea who also had higher rates of pet ownership exhibited gut microbiome dysbiosis, with a significant decrease in the abundance of *Ruminococcaceae* and *Blautia* and an increase in *Prevotellaceae*. This suggests that environmental factors like pet ownership might contribute to microbial imbalances that influence *Demodex* proliferation and inflammation, rather than direct mite transmission (38).

This study observed a higher frequency of *Demodex*-related skin conditions in patients reporting vegetarian or high-fat diets, though these associations were not independently significant in multivariate analyses. Previous research has linked specific enterotypes, influenced by long-term dietary habits, to rosacea and other conditions (39). Differently from the reults of the present study, one study found a higher prevalence of carbohydrate-associated Enterotype III in rosacea patients (39), while fiber-rich, plant-based Enterotype II was less common (39). However, the study also noted Enterotype I, associated with animal products and saturated fats, as most prevalent in both patient and control groups, wich they concluded as potentially because of regional common dietary patterns (39).

The lack of significant dietary associations in our study might be attributed to our single-center design and limited sample size, potentially restricting dietary variations. Additionally, the inconsistencies observed across the literature underscore the complex interplay of factors influencing diet's impact on skin health, emphasizing the need for more standardized research methodologies. Future research on cutaneous *Demodex* and dietary habits should incorporate rigorous, objective assessment methods to clarify the potential links between environmental factors and these conditions.

Study Limitations

This retrospective study has inherent limitations. Recall bias may have influenced survey responses, and the relatively small sample size could limit statistical power, potentially obscuring the impact of environmental factors. Despite these limitations, this study offers valuable preliminary findings regarding the connection between gut microbiota and *Demodex*-associated skin conditions, informing future research directions.

CONCLUSION

This study uncovers important links between gut microbiota-related factors and *Demodex*-associated skin conditions. Key findings include the strong association of alcohol consumption

with *Demodex* disorders, likely due to its effects on the skin and gut microbiomes. Smoking is also linked, though its effects on *Demodex* and rosacea are complex. The research highlights the significance of hydration and physical activity, with low water intake and lack of exercise identified as risk factors. Additionally, reduced bowel movements and higher pet ownership are associated with increased risk. Future research should involve larger, diverse populations and objective dietary assessments to better understand the interplay between environmental factors, gut microbiota, and *Demodex*-related conditions, potentially leading to improved management and therapeutic strategies for affected individuals.

*Ethics

Ethics Committee Approval: Ethical approval for the study was obtained from the Dokuz Eylül University Ethics Committee (approval number: 2024/20-20, date: 05.06.2024).

Informed Consent: Informed consent was required at the start of the survey, and only the responses from participants who completed the entire survey were included in the analysis.

*Authorship Contributions

Concept: F.G., S.S., Design: F.G., S.S., Data Collection or Processing: S.S., E.A., Analysis or Interpretation: F.G., S.S., Ö.Ö., Ş.K., E.A., Literature Search: F.G., Writing: F.G., S.S., Ö.Ö., Ş.K., E.A.

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Retrospective Evaluation of Intestinal Protozoa Parasites in Patients Presenting to Kafkas University Health Research and Application Hospital Between 2019-2022

2019-2022 Yılları Arasında Kafkas Üniversitesi Sağlık Araştırma ve Uygulama Hastanesi'ne Başvuran Hastalarda Bağırsak Protozoon Parazitlerinin Retrospektif Olarak Değerlendirilmesi

Hilal Bedir, Neriman Mor, Ahmet Deniz, Mükremin Özkan Arslan
Kafkas University Faculty of Medicine, Department of Medical Parasitology, Kars, Türkiye

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ABSTRACT

Objective: This study aimed to retrospectively evaluate the prevalence of protozoan parasites in stool samples collected from patients presenting with various gastrointestinal complaints to the Medical Parasitology Laboratory of Kafkas University Research and Application Hospital between 2019 and 2022.

Methods: Stool samples were initially examined using the native-Lugol method for the detection of protozoan parasites, followed by the formol-ethyl acetate sedimentation method, Giemsa, and carbol fuchsin staining methods. Specific immunochromatographic card tests were used for the diagnosis of *Entamoeba histolytica*, *Cryptosporidium* spp., and *Giardia intestinalis*.

Results: Of the 2.267 stool samples examined over the four-year period from January 2019 to December 2022, 7.63% were found to contain one or more protozoan parasites. Among these parasites, *Entamoeba histolytica* was detected at the highest rate of 4.06%. The other parasite species were identified as follows: *Blastocystis* spp. 1.15%, *Entamoeba* spp. and *Entamoeba coli* each 0.52%, *Giardia intestinalis* 0.48%, *Endolimax nana* 0.17%, and *Entamoeba histolytica/dispar* 0.08%.

Conclusion: This study indicates that despite a decrease in the prevalence of intestinal protozoan infections in the Kars region, these infections remain a significant public health issue. Therefore, improvements in hygiene and sanitation conditions, increased public health education, and the widespread implementation of early diagnosis and treatment methods are necessary. Special measures should be taken to protect vulnerable groups, particularly children and the elderly.

Keywords: Protozoan parasites, microscopic examination, Kars

ÖZ

Amaç: Bu çalışmada, 2019-2022 yılları arasında Kafkas Üniversitesi Araştırma ve Uygulama Hastanesi Tıbbi Parazitoloji Laboratuvarı'na çeşitli gastrointestinal şikayetlerle başvuran hastalardan alınan dışkı örneklerinde protozoon parazit prevalansının retrospektif olarak değerlendirilmesi amaçlanmıştır.

Yöntemler: Protozoon parazitlerin tespiti için dışkı örnekleri öncelikle Nativ-Lugol yöntemiyle incelenmiş, ardından formol etil asetat çöktürme yöntemi, Giemsa ve karbol fuksin boyama yöntemleri uygulanmıştır. *Entamoeba histolytica*, *Cryptosporidium* spp. ve *Giardia intestinalis*'in tanısında spesifik immünokromatografik kart testleri kullanılmıştır.

Bulgular: Ocak 2019 ile Aralık 2022 tarihleri arasındaki dört yıllık dönemde incelenen 2267 dışkı örneğinin %7,63'ünde bir veya birden fazla protozoon parazit tespit edilmiştir. Bu parazitler arasında %4,06 ile *Entamoeba histolytica* en yüksek oranda saptandı, diğer parazit türleri ise sırasıyla *Blastocystis* spp., %1,15 *Entamoeba* spp. ve *Entamoeba coli* %0,52 *Giardia intestinalis* %0,48, *Endolimax nana* %0,17 ve *Entamoeba histolytica/dispar* %0,08 oranında tespit edildi.

Sonuç: Bu çalışma, Kars bölgesinde bağırsak protozoan enfeksiyonlarının prevalansının azalmış olmasına rağmen, bu enfeksiyonların hala önemli bir halk sağlığı sorunu olduğunu ortaya koymaktadır. Bu nedenle, hijyen ve sanitasyon koşullarının iyileştirilmesi, halk sağlığı eğitimlerinin artırılması ve erken tanı ve tedavi yöntemlerinin yaygınlaştırılması gerekmektedir. Özellikle çocuklar ve yaşlılar gibi savunmasız gruplara yönelik özel önlemler alınmasına ihtiyaç vardır.

Anahtar Kelimeler: Protozoan parasitler, mikroskobik inceleme, Kars

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Address for Correspondence/Yazar Adresi: Hilal Bedir, Kafkas University Faculty of Medicine, Department of Medical Parasitology, Kars, Türkiye
Phone/Tel: +90 506 420 89 40 **E-mail/E-Posta:** bedirhilal@gmail.com **ORCID ID:** orcid.org/0000-0002-1583-3385

Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Intestinal protozoan infections constitute a significant global public health concern in developing countries, where water and sanitation facilities are often inadequate (1). In developing countries, factors such as low hygiene standards, environmental fecal contamination, and insufficient infrastructure have been reported to increase the prevalence of these infections (2). Although individuals of all ages are at risk, the most vulnerable groups include young children, individuals with low socio-economic status, those with limited education, the elderly, individuals with chronic health conditions, and migrants (3).

Transmission of intestinal protozoan parasites primarily occurs through contaminated water and food (4) and is clinically characterized by diarrhea. Diarrhea can be chronic or severe and is often associated with symptoms such as nausea, vomiting, low-grade fever, abdominal cramps, loss of appetite, fatigue, and weight loss. Prolonged infections can lead to serious anemia or malnutrition and may impair psychomotor development in children (5).

Enteric protozoan parasites are among the leading causes of the 1.7 billion annual cases of diarrhea worldwide, resulting in 842.000 deaths annually, making it the second leading cause of death among children under five years old. The most common etiological agents are *E. histolytica*, *G. intestinalis*, and *Cryptosporidium* spp. (6). These parasites are transmitted primarily through direct contact with infected individuals or animals or indirectly through the consumption of contaminated water or food; transmission typically occurs via the fecal-oral route (7). *E. histolytica* is the pathogenic species responsible for amebiasis worldwide (8). According to estimates from the Global Burden of Disease Study 2010, amebiasis accounts for 22 million disability-adjusted life years and approximately 55.500 deaths annually (1). *G. intestinalis* is a significant cause of chronic infectious diarrhea in both developed and developing countries, causing approximately 200-300 million clinical cases annually worldwide (9). It is also suggested to be responsible for ~15% of childhood diarrhea cases in developing countries (10). Data from the Global Enteric Multicenter Study indicate that *Cryptosporidium* spp. is a leading cause of moderate-to-severe diarrhea among children under two years old in developing countries (11).

Although there are many studies on the prevalence of intestinal protozoa in the literature, data from the Kars region of Türkiye is limited. The last significant study conducted in the region was in 2008, which reported a high prevalence of protozoan infections (12). This study aims to retrospectively evaluate the prevalence of protozoan parasites in stool samples collected from patients presenting with various gastrointestinal complaints at the Medical Parasitology Laboratory of Kafkas University Research and Application Hospital between January 2019 and December 2022.

METHODS

A retrospective analysis was conducted on stool samples from 2.267 patients presenting with various gastrointestinal complaints at the Kafkas University Medical Faculty Health Research and Application Hospital Parasitology Laboratory between January 2019 and December 2022. The inclusion criteria encompassed all patients who provided stool samples within the study period, while exclusion criteria included incomplete patient records or insufficient sample quality. Since our study is retrospective, patient consent was not required.

Stool samples were initially examined macroscopically, followed by microscopic evaluation using native-Lugol and formalin-ethyl acetate concentration techniques. Samples suspected of containing protozoa were stained and evaluated using Giemsa and Carbol Fuchsin staining methods. Prepared native-Lugol smears were examined under a light microscope at 40x magnification, concentration method smears at 10x magnification, and permanently stained smears at 100x magnification. For the qualitative detection of *Giardia lamblia*, *Entamoeba histolytica*, and *Cryptosporidium* spp., rapid chromatographic immunological tests were used: *Giardia lamblia* Rapid Test Cassette (feces) (Acro Biotech Inc., USA, BGL 602), *Entamoeba histolytica* Rapid Test Cassette (feces) (Acro Biotech Inc., USA, BEH 602), and *Cryptosporidium* Antigen Rapid Test Cassette (feces) (Acro Biotech Inc., USA, BC 602). All procedures were conducted in accordance with the manufacturer's instructions and standard laboratory protocols.

This study was approved in Research Committee of Kafkas University approval no: 10, date: 28/12/2022.

Statistical Analysis

All cases in which intestinal protozoan parasites were detected were statistically evaluated in terms of age, gender, and presence of parasites over the years. The statistical analysis of the data obtained from the research was performed using the IBM SPSS Statistics for Windows Version 23.0 (Statistical Package for the Social Sciences, IBM Corp., Armonk, NY, USA) software program, and Pearson's chi-square test was utilized. In all evaluations, a p-value of <0.05 was considered significant.

RESULTS

During the four-year period from January 2019 to December 2022, our study analyzed a cohort of 2.267 patients, identifying protozoan parasites in 173 individuals (7.63%). Of these cases, 91.2% were infected with a single parasite species, while 8.1% harbored multiple species. The most frequently detected protozoan was *E. histolytica*, present in 4.05% of the cases, followed by *Blastocystis* spp. at 1.14%, *E. coli* and *Entamoeba* spp., each at 0.52%, *G. intestinalis* at 0.48%, *En. nana* at 0.17%, and *E. histolytica/dispar* at 0.08%. The species of parasites identified and their prevalence rates are presented in Table 1.

An analysis of the annual distribution of parasite detection rates revealed that parasites were identified in 8.13% of 1.242 cases in 2019, 6.99% of 486 cases in 2020, 7.67% of 352 cases in 2021, and 5.9% of 187 cases in 2022 (Table 1). There was a notable decline in parasite prevalence from 2019 onward, though no statistically significant differences were observed between the years ($p>0.675$). However, a significant seasonal variation was noted, with higher rates of parasite detection during the autumn-winter months compared to the spring-summer months ($p=0.02$) (Figure 1).

The overall study population had a mean age of 23.23±21.95 years, ranging from 0 to 98 years, with a male prevalence of 56.6% and female prevalence of 43.4%. Among the parasite-positive patients, 54.9% were male and 45.1% were female, with a mean age of 11.24±14.33 years (ranging from 0 to 79 years). There was

Table 1. Number of patients by year, rates of positive cases, and distribution of intestinal protozoan species

IPP species	2019 (n=1242)		2020 (n=486)		2021 (n=352)		2022 (n=187)		Total (n=2267)	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Single infection										
Entamoeba histolytica	67	(5.39)	19	(3.9)	5	(1.42)	1	(0.53)	92	(4.05)
Blastocystis spp.	5	(0.4)	2	(0.41)	10	(2.84)	9	(4.81)	26	(1.14)
Entamoeba spp.	8	(0.48)	3	(0.61)	3	(0.85)	-	-	14	(0.61)
Entamoeba coli	10	(0.8)	2	(0.41)	-	-	-	-	12	(0.52)
Giardia intestinalis	5	(0.4)	4	(0.82)	2	(0.56)	-	-	11	(0.48)
Endolimax nana	3	(0.24)	1	(0.2)	-	-	-	-	4	(0.17)
Double infection										
Entamoeba histolytica + *Giardia intestinalis*	-	-	-	-	3	(0.85)	-	-	3	(0.13)
Giardia intestinalis + *Blastocystis* spp.	-	-	1	(0.2)	1	(0.28)	-	-	2	(0.08)
Entamoeba spp.+ *Blastocystis* spp.	-	-	-	-	1	(0.28)	-	-	1	(0.04)
Giardia intestinalis + *Entamoeba* spp.	-	-	-	-	1	(0.28)	-	-	1	(0.04)
Entamoeba histolytica + *Blastocystis* spp.	1	(0.08)	-	-	-	-	-	-	1	(0.04)
Entamoeba histolytica + *Endolimax nana*	1	(0.08)	-	-	-	-	-	-	1	(0.04)
Entamoeba coli + *Blastocystis* spp.	-	-	-	-	-	-	1	(0.53)	1	(0.04)
Entamoeba coli + *Endolimax nana*	1	(0.08)	-	-	-	-	-	-	1	(0.04)
Giardia intestinalis + *Iodamoeba butschlii*	-	-	1	(0.2)	-	-	-	-	1	(0.04)
Triple infection										
Entamoeba spp.+ *Blastocystis* spp. + *Chilomastix mesnili*	-	-	1	(0.2)	-	-	-	-	1	(0.04)
Entamoeba histolytica + *Giardia intestinalis* + *Cryptosporodium* spp.	-	-	-	-	1	-	-	-	1	(0.04)
Total	**101**	**(8.13)**	**34**	**(6.99)**	**27**	**(7.67)**	**11**	**(5,9)**	**173**	**(7.63)**

IPP: Intestinal protozoa parasite

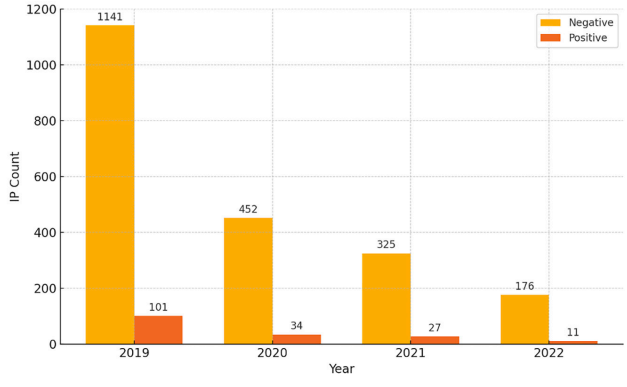

Figure 1. The total number of cases and the distribution of positive samples by year

no statistically significant difference in the prevalence of parasites between genders (p=0.345) (Table 2).

Further age stratification showed that parasites were most prevalent in individuals aged 0-15 years (72.3%), followed by those aged 16-30 years (9.9%), 31-45 years (7.6%), 46-55 years (2.9%), and over 56 years (7.6%). The highest frequency of parasitic infections was observed in the 0-15 year age group, with significantly higher infection rates in the 46-55 and over 56 age groups compared to other age groups (p=0.0001) (Table 3).

Table 2. Distribution of patients with and without detected parasites by gender

		n	%	Age average	Age range	p*
Positive	Male	95	54.9	11.24±14.33	0-79	0.345
	Female	78	45.1			
Negative	Male	1189	56.8	23.23±21.95		0.345
	Female	905	43.2		0-98	
Total		2267	100	11.24±23.23	0-98	

p*: p<0.05

In reviewing the annual parasite distribution, no statistically significant differences were found in the numbers of detected parasites from the onset of the study in 2019 through 2022 (p>0.675). Nevertheless, a significant increase in parasite numbers was observed during the autumn-winter months compared to the spring-summer months (p=0.02) (Figure 2).

DISCUSSION

Recent assessments of disease burden and epidemiological studies have highlighted the role of intestinal protozoa as significant etiological agents in low- and middle-income countries. Insights

Table 3. Prevalence of positive samples and negative samples by age

Age group	Negative number	Negative (%)	Positive number	Positive (%)	Total
0-15	1044	49.85%	125	72.25%	1169
16-30	362	17.28%	17	9.82%	379
31-45	315	15.04%	13	7.51%	328
46-55	129	6.16%	5	2.89%	134
>56	244	11.65%	13	7.51%	257
Total	2094	100.0%	173	100.0%	2267

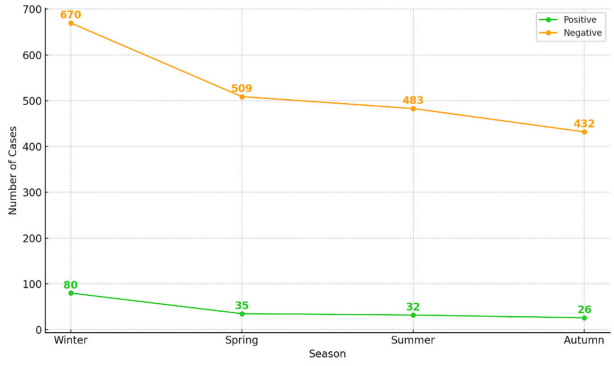

Figure 2. Distribution of total cases and positive samples by seasons

from the second decade of the twenty-first century have brought to light both the unexpected and severe global health impacts of these organisms (1).

In our study, in addition to the traditional method of microscopic examination for the diagnosis of intestinal protozoan parasites, immunochromatographic tests (ICTs) were used as an alternative, particularly for the detection of significant pathogenic intestinal parasites such as *E. histolytica*, *G. intestinalis*, and *Cryptosporodium* spp., which are among the most common etiological agents of diarrhea in humans. While microscopic examination allows for the direct morphological identification of parasites, ICTs operate by detecting specific antigens or antibodies. Microscopy can identify a wide range of parasites, whereas ICTs focus only on specific parasite species. The advantages of microscopy include its broad application and low cost, while ICTs are distinguished by providing rapid results and showing higher sensitivity in detecting low-intensity infections (12).

In our study, the overall prevalence rate of intestinal protozoan parasites was 7.63%, a significant reduction from the 34.1% reported in 2008 (13). This finding indicates that intestinal protozoan infections continue to be a significant public health issue in the region. The observed decrease in prevalence compared to previous years suggests that improvements in infrastructure and sanitation, as well as public health interventions, have been effective across the province. This study aligns with global efforts to mitigate the impact of protozoan parasites and emphasizes the need for ongoing interventions to sustain and improve public health outcomes.

The prevalence of protozoan parasites in our country shows significant geographical variation. Recent studies demonstrate that the incidence of these parasites fluctuates by year and region. The overall prevalence of protozoan infections in our study is consistent with the rates reported by Manisa Celal Bayar University Hafsa Sultan Hospital at 9.72% (14), Sivas Cumhuriyet University Faculty of Medicine at 8.65% (15), and Dicle University Faculty of Medicine at 5.64% (16). These rates are lower than those reported by Ege University Faculty of Medicine at 10.23% (17) and Gaziantep University Faculty of Medicine at 14.61% (18), yet higher than those found at Cerrahpaşa Faculty of Medicine at 2.49% (19), Yüzüncü Yıl University Faculty of Medicine at 2.62% (20), Fırat University Faculty of Medicine at 1.21% (21), and Hacettepe University Faculty of Medicine at 4.30% (22). These findings underscore the ongoing significance of protozoan parasite infections, which are influenced by geographical and socio-economic factors. Variations in prevalence rates may also stem from differences in stool examination techniques employed.

Since 2020, our research has documented a consistent decline in both the number and prevalence of parasitic infections. Lifestyle changes during the Coronavirus disease-2019 pandemic, such as improved hand hygiene, social distancing, and quarantine measures, have contributed to a reduction in intestinal parasite infections (23). It is likely that heightened public awareness during the pandemic has led to a reduction in fecal-oral transmission of pathogens, which correlates with the observed decrease in the quantity of samples analyzed and the number of parasites detected. This trend underscores the impact of behavioral health interventions on controlling infectious diseases.

The global molecular prevalence of *Entamoeba* species in humans is estimated at 3.5%, with *E. histolytica* and *E. dispar* comprising 81.7% of this prevalence, with *E. dispar* being significantly more prevalent than *E. histolytica* worldwide (24). In Kars, the prevalence of *Entamoeba* species has been reported with *E. histolytica/dispar* at 10.1% and *E. coli* at 8% (12). Our study confirms the presence of both pathogenic and non-pathogenic *Entamoeba* species, specifically *E. histolytica* at 4.06%, *E. coli* at 0.52%, other *Entamoeba* spp. at 0.52%, and *E. histolytica/dispar* at 0.08%. Accurate diagnosis of *E. histolytica* is essential to avoid unnecessary treatment of individuals infected with non-pathogenic *Entamoeba* species (25). The risk of *E. histolytica* infection is higher among individuals living in households with a middle socio-economic status (26). Our findings indicate the hygiene and sanitation conditions in the province, suggesting that individuals with lower socio-economic status are at a higher risk, underscoring how socio-economic disadvantages can contribute to the spread of *E. histolytica*.

G. intestinalis, a significant pathogenic intestinal protozoan, is one of the most common causes of waterborne disease outbreaks related to drinking water (27). In a previous study, the prevalence of *G. intestinalis* was reported to be as high as 10.9% (12). In contrast, our study found the prevalence rate of *G. intestinalis* to be significantly lower at 0.48%. The low prevalence of *G. intestinalis* observed in our study may reflect improvements in public health measures, such as enhanced management of water quality and increased awareness of personal hygiene.

In our study, the second most commonly encountered parasite was identified as *Blastocystis* spp., with a prevalence rate of 1.15%. *Blastocystis* spp. is known as the most frequently detected unicellular parasite in human fecal samples worldwide. The highest reported prevalence of this parasite was 100% in a child population in Senegal (28). Although the majority of individuals infected with *Blastocystis* spp. do not show intestinal symptoms,

infections are associated with non-specific gastrointestinal symptoms such as diarrhea, abdominal pain and urticaria (29). Studies conducted in Türkiye have reported significant positive rates for *Blastocystis* spp., varying between 6.63% and 71.6% (14,15,17,18,22). Higher prevalence rates of *Blastocystis* infections have previously been reported among individuals in close contact with animals, especially animal caretakers (30). The Kars Plateau, an area with extensive agriculture and animal husbandry, constitutes a high-risk environment for *Blastocystis* spp. infections, particularly among farmers. This suggests a high likelihood of transmission between humans and animals in rural settings. The low prevalence observed in our study may not be generalizable to the broader population, but could reflect improvements in public health measures. Research into the sources and transmission routes of *Blastocystis* spp. is essential to prevent the spread of infections between humans and animals.

Regarding co-infections with pathogenic and non-pathogenic parasites, our results are comparable with other studies (13,14). Co-infections with protozoan species demonstrate their opportunistic and recurrent nature, which may proliferate under favorable conditions, leading to intestinal disorders.

Regarding the risk factors associated with protozoan infections, our data analysis has shown that protozoan parasites can infect all age groups. Age is a potential risk factor for intestinal infections (31). In our study, the high prevalence rate observed among children aged 0-15 years (72.03 %) is likely due to this age group's immature immune systems, consumption of unwashed vegetables and fruits, reluctance to wash hands before meals and after using the toilet, nail-biting, and poor personal hygiene habits. Previous studies have identified *G. intestinalis* and *E. histolytica/dispar* as common species in child patients (13). These species are among those detected in this age group in our study. Family size, source of drinking water, handwashing habits before meals, wearing shoes, and nail cleanliness are strongly associated with the presence of intestinal parasitic infection (32). The possible explanation for this relationship is that as family size increases, overcrowding, malnutrition, and problems with personal and household hygiene may arise; these conditions create ideal environments for parasite transmission and increase the susceptibility of family members to parasitic infections. Additionally, the prevalence of parasites was found to be higher in the 46-55 age groups and those over 56 years. This can be associated with the increasing prevalence of chronic diseases and the weakening of the immune system with age (33).

When all cases were evaluated by gender, 54.9% of the patients were male and 45.1% were female. This situation can be associated with the increased exposure to environmental factors and infection risk due to men spending more time outdoors. Men involved in outdoor activities such as agriculture, construction, and animal husbandry are more exposed to environments where parasites are present. Although some studies have reported gender differences, in general, parasitic infections are not directly related to gender, and hygiene and sanitation conditions are more determining factors (14,20,34). Consequently, our study did not observe a significant difference between genders in the frequency of parasitic infections, which is consistent with other literature findings. The management of protozoan infections should focus on improving hygiene and sanitation conditions rather than targeting specific genders. Additionally, raising public awareness among those involved in outdoor and animal husbandry activities is important to reduce the prevalence of these infections.

In our study, a significant increase in the number and prevalence of parasites was observed during the autumn-winter seasons compared to the spring-summer seasons. This increase can be attributed to factors such as spending more time indoors, changes in hygiene practices, and differences in seasonal food consumption. Contrary to our findings, other studies conducted in Türkiye have reported higher rates of parasitic infections during the spring and summer seasons (16,19). These differences highlight the potential impact of regional and environmental factors on the seasonality of parasitic infections.

CONCLUSION

The findings of this study provide current data on the prevalence and distribution of intestinal protozoan infections in the Kars region. In conclusion, the significant presence of protozoan parasites such as *E. histolytica* and *Blastocystis* spp. in Kars province highlights the ongoing public health issue posed by intestinal protozoan infections. This underscores the importance of continuous efforts to improve sanitation, water quality, and public awareness. The lower prevalence of *G. intestinalis* compared to previous studies may reflect improved public health measures and hygiene practices. Addressing these factors comprehensively is crucial for reducing the prevalence of *E. histolytica* infections, particularly among children, and for improving overall community health.

*Ethics

Ethics Committee Approval: This study was approved in Research Committee of Kafkas University approval no: 10, date: 28/12/2022.

Informed Consent: Retrospective study.

*Authorship Contributions

Concept: H.B., N.M., A.D., M.Ö.A., Design: H.B., N.M., A.D., M.Ö.A., Data Collection or Processing: H.B., N.M., A.D., M.Ö.A., Analysis or Interpretation: H.B., N.M., A.D., M.Ö.A., Literature Search: H.B., N.M., A.D., M.Ö.A., Writing: H.B., N.M., A.D., M.Ö.A.

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Molecular Diagnosis and Typing of *Cryptosporidium* spp. Species in Human Stools with Diarrhea

İshalli İnsan Dışkılarında Cryptosporidium spp. Türlerinin Moleküler Tanısı ve Tiplendirilmesi

Fatma Özkan[1], Anil İça[2]

[1]Private Denizli Tekden Hospital, Denizli, Türkiye

[2]Kütahya Dumlupınar University Faculty of Arts and Sciences, Department of Molecular Biology; Zoonoses Application and Research Center, Kütahya, Türkiye

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ABSTRACT

Objective: This study was conducted to molecularly identify and classify *Cryptosporidium* spp. in fecal samples (n=150) from patients with diarrhea received at the microbiology laboratory of a private hospital in Denizli.

Methods: In this study, the positivity of *Cryptosporidium* spp. in fecal samples was investigated using direct microscopy, Kinyoun's acid-fast staining method, and Nested polymerase chain reaction (PCR) techniques. Positive PCR products were sequenced.

Results: In the examined fecal samples of patients with diarrhea, no parasites were detected through direct microscopic examination. Using the Kinyoun acid-fast staining method, *Cryptosporidium* spp. was identified in 2.7% (n=4) of the samples, while Nested PCR detected it in 4.67% (n=7) of the samples. The four positive samples were sequenced using primers that amplify the *18S rRNA* gene region. The sequencing results identified the isolates as *C. parvum*.

Conclusion: Cryptosporidiosis is an important public health issue as it is a zoonotic disease caused by the *Cryptosporidium* parasite that can be transmitted from animals to humans. This study focuses on the molecular characterization of *Cryptosporidium* species detected in human fecal samples, which is significant for understanding which specific strains or species are involved in human infections. According to the findings, it is recommended that control measures be implemented to reduce the risk of exposure to *Cryptosporidium* in both humans and animals in Türkiye.

Keywords: *18s rRNA*, *Cryptosporidium*, Nested PCR, PCR, sequencing

ÖZ

Amaç: Bu çalışma, Denizli'deki bir özel hastanenin mikrobiyoloji laboratuvarına gelen ishalli hastalardan alınan dışkı örneklerinde (n=150) *Cryptosporidium* spp. türlerinin moleküler olarak tanımlanması ve tiplendirilmesi amacıyla yapılmıştır.

Yöntemler: Çalışma kapsamında dışkı örneklerinde *Cryptosporidium* spp. pozitifliği, direkt mikroskobik bakı, Kinyoun'un asit fast boyama yöntemi ve Nested-polimeraz zincir reaksiyon (PZR) yöntemleriyle araştırılmıştır. Pozitif PZR ürünleri sekanslanmıştır.

Bulgular: İshalli hastaların incelenen dışkı örneklerinde direkt mikroskobik inceleme ile parazit tespit edilmemiştir. Kinyoun'un asit fast boyama yöntemiyle %2,7 (n=4) oranında, Nested PZR ile %4,67 (n=7) oranında *Cryptosporidium* spp. tespit edilmiştir. Pozitif bulunan 4 örnek *18S rRNA* gen bölgesini amplifiye eden primerlerle sekanslanmıştır. Sekanslama sonucunda izolatlar *C. parvum* olarak tanımlanmıştır.

Sonuç: Cryptosporidiosis, *Cryptosporidium* parazitinin neden olduğu ve hayvanlardan insanlara bulaşabilen bir zoonotik hastalık olduğu için önemli bir halk sağlığı sorunudur. Bu çalışma, insan dışkı örneklerinde tespit edilen *Cryptosporidium* türlerinin moleküler karakterizasyonuna odaklanmakta olup, insan enfeksiyonlarında hangi özel suşların veya türlerin yer aldığını anlamak açısından önemlidir. Bulgulara göre, Türkiye'de hem insanlarda hem de hayvanlarda *Cryptosporidium*'a maruz kalma riskini azaltmak için kontrol önlemlerinin uygulanması önerilmektedir.

Anahtar Kelimeler: *18s rRNA*, *Cryptosporidium*, Nested PZR, PZR, sekanslama

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Address for Correspondence/Yazar Adresi: Fatma Özkan, Private Denizli Tekden Hospital, Denizli, Türkiye
Phone/Tel: +90 507 415 24 23 **E-mail/E-Posta:** fatmaarslan_43@hotmail.com **ORCID ID:** orcid.org/0000-0002-5394-2144

Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Cryptosporidiosis, is the name given to a wide range of diseases caused by *Cryptosporidium* spp. species, which have different genetic characteristics, from non-symptomatic infections to acute enteric diseases in animals and humans (1-3). *Cryptosporidium* spp. is widespread throughout the world, including in underdeveloped countries, rural areas of developed countries or urban centers (4-6). This prevalence, has been reported to be detected at rates ranging from 0.7 to 20% in more than 40 countries in the world; it has been observed that it is higher in developing or underdeveloped countries where sanitation measures are not taken and social and personal cleaning is not fully implemented (7-9).

By amplifying specific gene regions using molecular methods, more information was obtained about the transmission routes, species-specific data and biological characteristics of *Cryptosporidium* species (8).

Of the two different genotypes responsible for human cryptosporidiosis; it has been determined that the human genotype (I) is found only in humans while the bovine genotype (II) can cause infection in humans, sheep, cattle, deer, rarely pigs and mice. Genotype II has been reported to constitute the majority of human infections (3,10). The type I genotype responsible for human infection was determined to be *C. hominis* (previously called type I of *C. parvum* or the human genotype of *C. parvum*) and the type II genotype (previously named type II or bovine genotype) was *C. parvum*. It has been reported that *C. hominis* has very high host specificity and very low genetic variation while *C. parvum* has very low host specificity and very high genetic variation (1,5,8,11,12). The fact that *C. parvum* unlike *C. hominis* can infect not only humans, but also domestic and wild ruminants and rodents, proves that it is a zoonotic species (1). Although *C. parvum* with the zoonotic character and *C. hominis* with the anthroponotic character are the most common causes of cryptosporidiosis in humans (5), cases of *C. muris, C. meleagridis, C. suis, C. canis* and *C. felis* have also been reported to cause infections in humans (4,10,13). *Cryptosporidium parvum* species plays a dominant role especially in zoonotic cryptosporidiosis in humans (14).

The incubation period of cryptosporidiosis generally ranges from 5-28 days (7,15). Diarrhea is the most important clinical manifestation of the disease in both immunologically intact and suppressed individuals. Less frequently, patients also experience abdominal pain, fever, nausea-vomiting and weight loss (15). Resistant oocysts excreted in the feces of infective hosts are the route of transmission. Livestock especially calves and other domestic animals play a major role in human transmission (15). Human-to-human transmission is also possible. Human-to-human contamination can occur via fecal-oral route (15,16). Human-to-human infections were first seen in kindergartens (17). Epidemics, especially seen in child care homes and hospitals, are the biggest indicator that proves the importance of transmission between people. Today, *Cryptosporidium* is also known as the causative agent of tourist disease (15,18). Travels from developed countries to less developed countries also contribute to the spread of *Cryptosporidium*. It is known that cryptosporidiosis is transmitted homosexually among HIV-positive patients (17).

In epidemics, water is also shown as a source. The high rate of *Cryptosporidium* in spring and surface waters, the resistance of oocysts to disinfectants such as chlorine, the ability to pass through the filters of drinking water treatment networks and the very few oocysts that cause infection cause water-borne epidemics (1,14-16).

It is known that cryptosporidiosis, like all parasitic diseases, poses a major public health problem and causes economic losses. Due to the long duration of the treatment and the high cost of treatment, it is necessary to pay attention to the prevention of the disease (7). Since there is no effective treatment against this parasite yet, the infection in patients can become chronic and life-threatening. Since the basic biology of *Cryptosporidium* is not fully understood, an effective treatment method against the disease has not been determined (1,7).

Except for a few studies with small sample sizes, little is known about the molecular characterization of human *Cryptosporidium* species in Türkiye. Therefore, our study, aimed at molecular identification and characterization of the *Cryptosporidium* spp. species in diarrheal human stools.

METHODS

Sampling and Identification of *Cryptosporidium* Oocysts

A total of 150 stool samples (69/F, 81/M) collected from individuals aged 0-87 who presented to a private hospital in Denizli were examined for *Cryptosporidium*. In the analysis of the collected samples, only "age and sex" data were considered (Table 1).

All samples were stored at 4 °C until microscopic examination and DNA extraction. The samples were examined parasitologically by the native-Lugol direct examination method. Then, some feces were taken from the samples and subjected to formol-ethyl acetate precipitation and preparation processes. All samples were stained with Kinyoun's acid-fast staining method and examined microscopically.

The study was approved by the Ethical Committee of Kütahya Health Sciences University (09.02.2021/2021/02-16).

Genomic DNA Isolation

At this stage, 200 µL of each of the thoroughly homogenized stool samples was taken and DNA extraction was performed in accordance with the "Thermo Scientific GeneJET™" DNA Purification Kit procedure. The obtained DNAs were stored at -20 °C for use in the polymerase chain reaction (PCR) process.

SSU rRNA Nested PCR

Primer sets (Crypto F1 and Crypto R1) targeting a 1325bp region of the SSU-rRNA region and used in previous studies were preferred in PCR applications (19-21). In the first step of PCR, primers Crypto F1 (PCR Forward Primer) and Crypto R1 (PCR Reverse Primer) were used, which amplify a 1325bp DNA fragment from the SSU rRNA encoding DNA region of *Cryptosporidium*

Table 1. Gender-age distribution of sample owners

	Number of individuals	Age	(Range/average)
Male	81	0-86	24.3
Female	69	0-87	24.3

species. With the help of the methods used in previous studies, the Nested-PCR reaction was performed using Crypto F2 (Nested PCR Forward Primer) and Crypto R2 (Nested PCR Reverse Primer) primers, which amplify a region of 826-864bp (Table 2) (19-21). In order to detect amplicons obtained from genomic DNAs that underwent PCR and Nested PCR procedures, they were subjected to electrophoresis on a 1% agarose gel and visualized.

Sequence Analysis of SSU rRNA

Agarose gel electrophoresis was performed to detect the sequences obtained from genomic DNAs that underwent PCR and Nested PCR procedures. The specific bands obtained after the imaging process were cut from the gel and purified. During the purification process, the Gene Jet Purification Kit procedure was followed.

Sequence analysis was performed by a commercial firm. The data obtained as a result of the sequence analysis were compared with the data in GenBank (https://www.ncbi.nlm.nih.gov). Phylogenetic analyzes of the obtained sequences were performed using the MEGAX software (22,23).

Statistical Analysis

Relationships such as whether the positive results were related to age and gender, and whether there was a statistically significant difference, were examined using the chi-square test. The threshold level of statistical significance was taken as $p<0.05$ [SPSS version 20.0 (Statistical Package for the Social Sciences-SPSS, IBM, Chicago, USA) package software" chi-square test (X^2) (Two-Way Table in Worksheet)" test].

RESULTS

No oocysts could be detected in any of the samples examined by the native-lugol method to search for *Cryptosporidium* spp. oocysts in stool samples sent to the laboratory for different examinations. After the condensation process with formol-ethyl acetate, the preparations were prepared and examined by Kinyoun's acid-fast dying method. After the examination, positivity was detected in 4 samples (2.7%) (Figure 1).

As a result of molecular examinations, positivity (Table 3) was detected in 7 samples (4.67%). Four of them are samples that were found positive by microscopic examination (Figure 2). The other 3 had negative results in microscopic examination.

It was determined that 54% of all samples studied were men and 46% were women, and 4 of the positive samples were found in the feces of females and 3 of them were found in male feces. According to the data obtained, no significant relationship was found between gender and positivity (p=3.24).

In this study, 71.43% of the positives were isolated from children in the 0-10 age group (57% especially in the 0-5 age group) and 28.57% from adults aged 24-35, which we can define as the young age group. *Cryptosporidium* has not been found in any of the individuals over the age of 35. This situation was statistically significant ($p<0.05$). According to the data obtained, it was understood that the disease was associated with age (Figure 3).

Four of the positive samples were sequenced. As a result of sequencing, 685bp, 746bp, 832bp and 748bp sequences were obtained in the *18S rRNA* gene region. Table 4 was obtained as a result of comparing the sequences obtained in this study with other sequences known to be deposited in GenBank. As a result of the Blast and phylogenetic analyzes, the phylogenetic tree given below was formed (Figure 4). According to the data obtained, it was determined that 99.997-100% of the isolates were identical with *Cryptosporidium parvum*.

While examining the evolutionary history of the sequences we obtained, the "Neighbor-Joining" method was used (24). The optimal tree is shown with length=0.59990219. The percentage of replicated (1000 copies) trees associated with the clustered taxon is given together with tree branches in the bootstrap test (25). Evolutionary distance was calculated using the "Maximum Composite Likelihood" method (26) and units of base numbers per region. This analysis includes 28 nucleotide sequences. All ambiguous positions (with double delete option) have been removed for each row pair. A total of 1909 positions were found in the latest data set. Evolutionary analyzes were performed using MEGA X (22,23).

Sequence data of *Cryptosporidium* isolates obtained in the study are deposited in GenBank with accession numbers OL621907, OL689399-401.

DISCUSSION

Cryptosporidium species are coccidian protozoa (15) and their sizes vary according to the species and reproduction stages (21). *Cryptosporidium* species are monoxen parasites and transmission occurs by oral ingestion of fecal oocysts (2,12). People who live in the same house with infected people, people they have sexual contact with, health workers, veterinarians, people dealing with agriculture and animal husbandry, people traveling to endemic areas and nursery children are at risk. It can also be transmitted to humans from pets, laboratory animals and farm animals. Oocysts are seen in untreated wastewater, surface, and underground

Table 3. Investigation methods and positivity status

	Native-Lugol	Kinyoun's acid-fast	PCR/Nested PCR
Positive	0	4 (1F/3M)	7 (4F/3M)
Negative	0	146 (68F/78M)	143 (65F/78M)

F: Female, M: Male, PCR: Polymerase chain reaction

Table 2. Used primer sequences

PCR/Nested PCR primers	Sequences	Long (bp)
Crypto F1 (PCR forward primer)	5'-TTCTAGAGCTAATACATGCG-3'	1325
Crypto R1 (PCR reverse primer)	5'-CCCATTTCCTTCGAAACAGGA-3'	
Crypto F2 (Nested PCR forward primer)	5'-GGAAGGGTTGTATTTATTAGATAAAG-3'	826-864
Crypto R2 (Nested PCR reverse primer)	5'-AAGGAGTAAGGAACAACCTCCA-3'	

PCR: Polymerase chain reaction

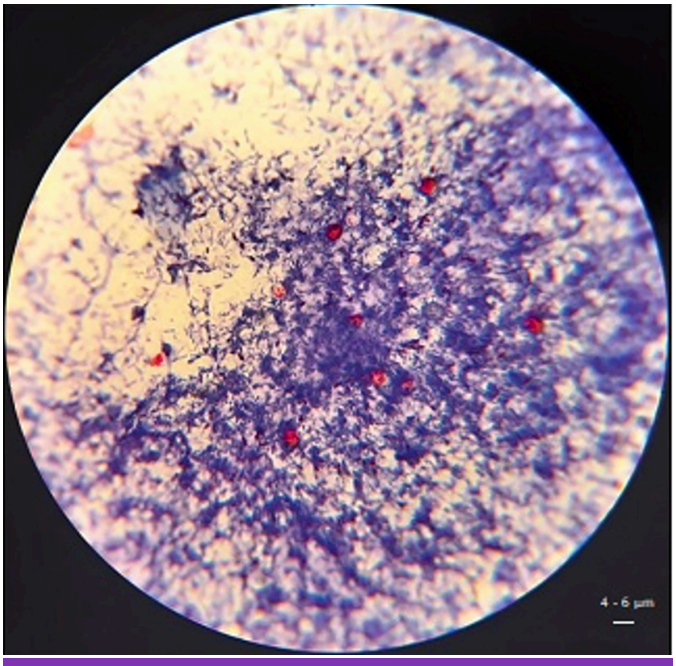
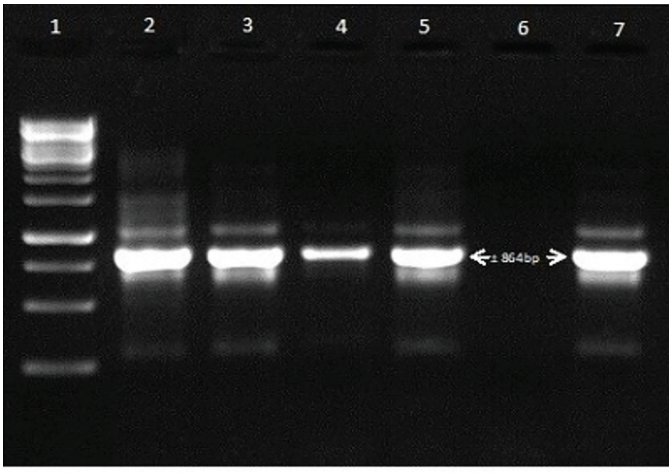


Figure 1. *Cryptosporidium* spp. oocysts



1: 1 kb ladder; 2-5: Nested 1-Nested 4 isolates; well 6. Nested negative control; well 7. Nested positive control.

Figure 2. Band images of isolates obtained in the Nested PCR
PCR: Polymerase chain reaction

waters, as well as in pool waters, especially as a result of fecal contamination of small children's pools. It has also been reported that contamination can be seen with certain foods consumed, such as sausages, offal, and raw milk, which are prepared by not paying attention to their cleanliness. Respiratory transmission occurs in immunocompromised patients and AIDS patients. In addition, it can be transmitted by carrier hosts such as soil, arthropods and birds (16,27). As with malaria, there are indications that the spread of cryptosporidiosis across geographic regions is likely related to modes of transmission. Reasons such as suppression of the host's immune system, low number of oocysts causing infection, the ability of oocysts to remain in the latent phase in the external environment for a long time (for months at 20 °C) and to be resistant to many disinfectants, the infectivity of oocysts when excreted from the host, animals as reservoirs for some genotypes are factors that determine the epidemiology of *Cryptosporidium*

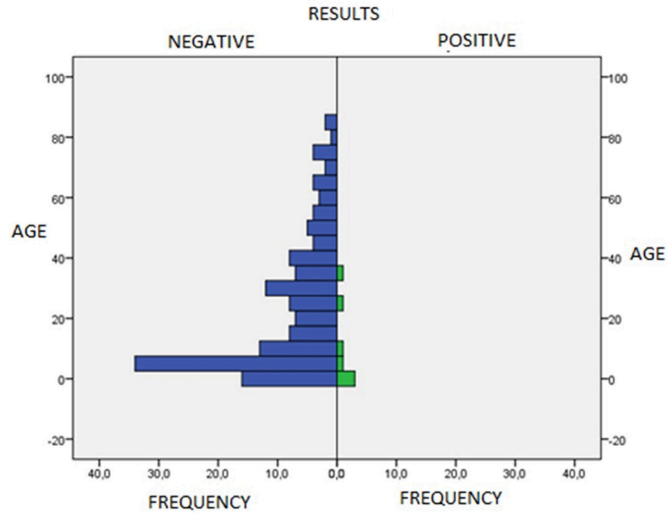


Figure 3. Frequency of positive and negative samples by age

(2,7,27). Currently, regional studies are carried out to detect cryptosporidiosis in healthy and immunocompromised individuals both in Türkiye and around the world.

When we look at the studies done in the world, it has been reported that cryptosporidiosis is a zoonotic disease seen in all regions with a hot climate except Antarctica (4,7,15). Recent studies have focused more on the molecular typing, genotypic properties, methods used for the detection of the parasite and the comparison of these methods, isolation from water resources and ecological environment, its effect on epidemics, sanitation or ways of protection etc. rather than the incidence of *Cryptosporidium*. In order to examine the zoonotic potential of *Cryptosporidium* in the Netherlands, 70% of *C. hominis*, 19% of *C. parvum*, 10% of both combinations and 1% of *C. felis* isolates were detected from humans and 100% *C. parvum* isolates from cattle by genotyping from infected human and bovine feces (28). In a study conducted in environmental water samples in Germany, *Cryptosporidium* was detected at a rate of 30.4% by IFA method, 41.9% by Nested PCR, and 43.6% by LAMP method (27).

There are many studies conducted in Türkiye on human feces with diarrhea. In the examinations made in the Mediterranean Region, *Cryptosporidium* positivity was reported at a rate of 5.3% by staining method, 10.6% with ELISA method in Adana (7), in another study, it was 1.3% by direct examination method, 5.2% by staining method and 24% by ELISA method (16).

In studies conducted in the Eastern Anatolia Region, one study reported a *Cryptosporidium* positivity rate of 2.2% (11), while another study determined it to be 20% (13). In patients with chronic renal failure, the positivity rate was 32%, and in healthy individuals, it was 3.3% (10). In a study conducted in Ağrı, *Cryptosporidium* spp. was positive in 7 cases (3.80%) and identified as *C. parvum*, belonging to the IId subtype family (9). In the researches carried out in the Aegean Region, *Cryptosporidium* positivity was reported rate of 3.6% with the direct view method, 4.4% with the painting method in Afyonkarahisar (18), 2.7% by staining method and 3.4% by ELISA method in Kütahya (12). In the studies carried out in the South East Anatolian Region, the rate was 3.2% with the modified acid-fast staining method and 5.8% with the ELISA method in Diyarbakır (15), in another study conducted in the same city, it was 3% with the

Table 4. *Cryptosporidium* isolates with phylogenetic comparisons around the world

Accession number	Isolation source	Species	Isolate	Origin
MN803326	Calf	*Cryptosporidium ryanae*	ERU-KyCrya1	Türkiye-Kayseri
MN803325	Calf	*Cryptosporidium parvum*	ERU-KyCpar1	Türkiye-Kayseri
MN803324	Heifer	*Cryptosporidium bovis*	ERU-KyCbov1	Türkiye-Kayseri
JX644908	Human	*Cryptosporidium viatorum*	31332	Chinese
KP730318	Brushtail rock kangaroo	*Cryptosporidium fayeri*	BW993	Australia
JQ029723	Human	*Cryptosporidium hominis*	27156	Chinese
KC305650	Horse	*Cryptosporidium* sp. hedgehog gnt.	M1047	Algeria
GQ983349	Human	*Cryptosporidium parvum*	W14595	United Kingdom
AF329187	Human	*Cryptosporidium meleagridis*	5095	Peru
AF108862	Cat	*Cryptosporidium felis*	C Horse 1 (131)	Australia
KP899827	Human	*Cryptosporidium ubiquitum*	VE20	Venezuelan
KJ790244	Pig	*Cryptosporidium suis*	CTC2	Chinese
MH807493	Human	*Cryptosporidium occultus*	GX996	Chinese
AB210854	Dog	*Cryptosporidium canis*	-	Japan
AF093495	Chicken	*Cryptosporidium baileyi*	CBA01	USA
FJ463193	Dairy cow	*Cryptosporidium ryanae*	23	Chinese
FJ896053	Sheep	*Cryptosporidium xiaoi*	191.1	USA
AY741305	Cattle	*Cryptosporidium bovis*	-	USA
AF151376	Snake	*Cryptosporidium serpentis*	-	US
HM116388	Eurasian silktail (bird)	*Cryptosporidium galli*	14	Chinese
EU162751	Javanese frog	*Cryptosporidium fragile*	Clone A	Malaysia
AY642591	Large Japanese dormouse	*Cryptosporidium muris*	Kaw Horseabi	Japan
FJ463171	Dairy cow	*Cryptosporidium andersoni*	1	Chinese

dyeing method and 4% with the ELISA method (2). The Central Anatolia, *Cryptosporidium* positivity was reported 5.6% in a study conducted in Ankara (20), in another study at the rate of 0% with the direct view method, 1% with the painting method and 4% with the DFA method in Sivas (3). Studies in the Marmara Region, *Cryptosporidium* positivity was reported 2.1% of dialysis patients with end stage renal disease (17).

In this study, diarrheal stool samples of 150 people (69F/81M) from different age groups (0-87) who applied to a private hospital in Denizli city center were examined. In the examined fecal samples of patients with diarrhea, no parasites were detected through direct microscopic examination. Using the Kinyoun acid-fast staining method, *Cryptosporidium* spp. was identified in 2.7% (n=4) of the samples, while Nested PCR detected it in 4.67% (n=7) of the samples. The four positive samples were sequenced using primers that amplify the 18*S rRNA* gene region. The sequencing results identified the isolates as *C. parvum*. These results show compatibility with previous studies in terms of prevalence values and differences in results between the applied methods and the detected species.

In a study, *Cryptosporidium* was detected in adults older than 20 years of age with an incidence of 1.25% and in those younger than 20 years of age with an incidence of 17.8%. Within these data, cryptosporidiosis was largely recorded as a pediatric disease (9,29). In another study, it was reported that the vast majority (80%) of human cases occurred in children aged 0-9 years (28).

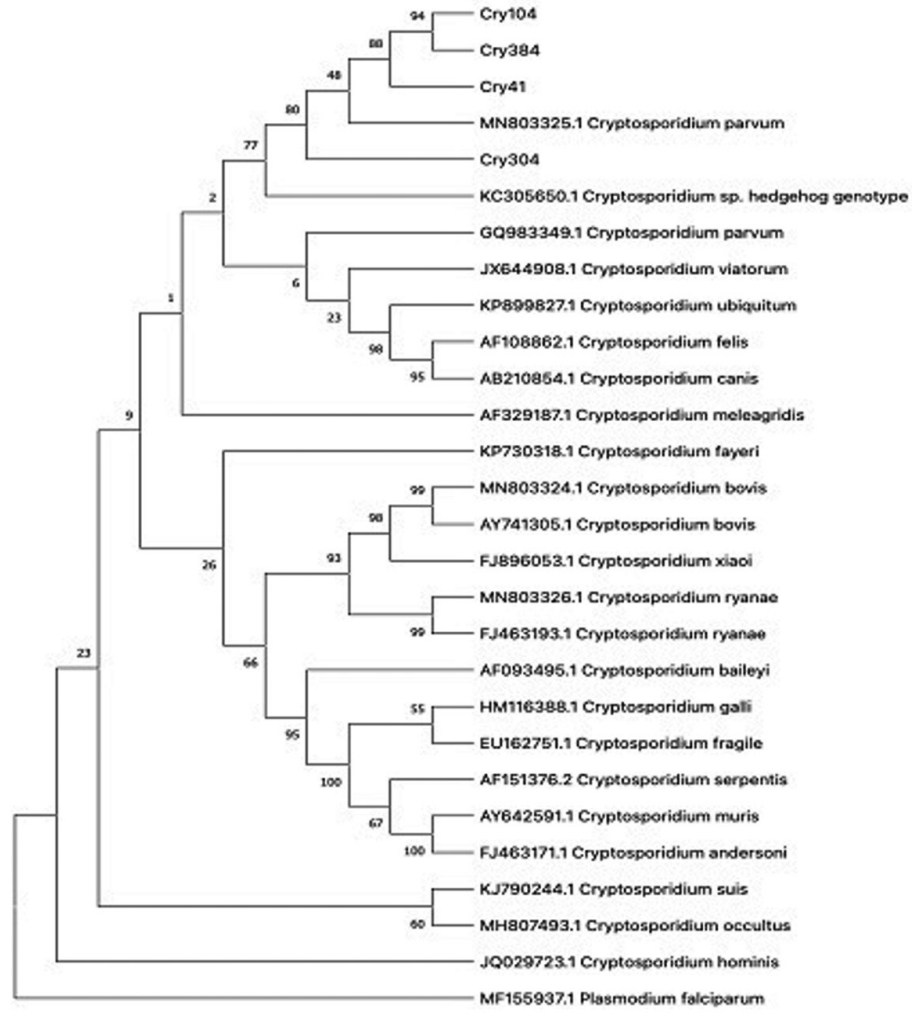
In this study, the majority of the samples detected with cryptosporidiosis belong to children in the 0-10 age group (57% especially in the 0-5 age group). This rate has an incidence of 71.43% among positives. Other positive samples belong to adults aged 24-35 with a rate of 28.57%. No oocyst was detected in any of the individuals over the age of 35. Of the studied samples, 54% belonged to male individuals and 46% to female individuals. These results are in agreement with other studies.

It has been observed that *Cryptosporidium* species have a very high incidence among parasite species isolated from large epidemics (29), environmental waters (14,27), and rural areas where well water is used as drinking water (5,6,9,13). It has been determined that *Cryptosporidium* species can easily pass through the filters of the water processed in the treatment plants before being used in the city network (14,27). It is stated that *Cryptosporidium* species are resistant to many disinfectants. In addition, the presence of heavy metals pumping carriers of the species supports its ability to survive (30).

The fact that the aforementioned protozoan, which threatens both human and environmental life, was identified as an infectious agent in our study, the inadequacy of drugs used in the treatment of other coccidian protozoa in the treatment of cryptosporidiosis, effective treatment protocols against *Cryptosporidium* infections have not yet been established (30) once again reveals the seriousness of the situation we are facing.

CONCLUSION

For this reason, *Cryptosporidium* species should be added to the fecal indicator organisms, which are among the water quality



While examining the evolutionary history of the sequences we obtained, the "Neighbor-Joining" method was used (Saitou et al., 1987). The optimal tree is shown with length=0.59990219. The percentage of replicated (1000 copies) trees associated with the clustered taxon is given together with tree branches in the bootstrap test (Felsenstein et al., 1985). Evolutionary distance was calculated using the "Maximum Composite Likelihood" method (Tamura et al., 2004) and units of base numbers per region. This analysis includes 28 nucleotide sequences. All ambiguous positions (with double delete option) have been removed for each row pair. A total of 1909 positions were found in the latest data set. Evolutionary analyzes were performed using MEGA X (Kumar et al., 2018; Stecher et al., 2020).

Figure 4. Phylogenetic tree

parameters applied in rural-urban basins. Considering the fecal-oral contamination, care should be taken not to irrigate agricultural products with unfiltered water and sewage mixing water. It is necessary to inform the treatment water facilities and municipal authorities on the subject and to support the studies on the eradication process. Sanitation should be given importance in order to contribute to the economy of both the country and the world, minimize health expenses, and most importantly, to protect public health. In order to ensure personal hygiene and protect against infections, individuals should be made aware, and health policies that improve environmental factors should be developed and implemented.

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This study was summarized from the same titled PhD thesis.

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*Ethics

Ethics Committee Approval: The study was approved by the Ethical Committee of Kütahya Health Sciences University (09.02.2021/2021/02-16).

Informed Consent: No personal data was used in the study because no samples were specifically collected from patients and the samples brought to the hospital for routine tests were analyzed a second time.

*Authorship Contributions

Design: F.Ö., A.İ., Data Collection or Processing: F.Ö., Analysis or Interpretation: F.Ö., A.İ., Literature Search: F.Ö., Writing: F.Ö., A.İ.

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

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Percutaneous Aspiration Injection and Re-aspiration as A Minimally Invasive Treatment for Spinal Cystic Echinococcosis: A Case Report

Spinal Kistik Ekinokokkozisde Minimal İnvaziv Tedavi Olarak Perkütan Aspirasyon İnjeksiyonu ve Reaspirasyon: Olgu Sunumu

Özge Metin Akcan[1], Kadir Yılmaz[2], Mustafa Gençeli[1], Süleyman Bakdık[3], Ülkü Kerimoğlu[3]
[1]Necmettin Erbakan University Faculty of Medicine, Departments of Pediatric Infectious Diseases, Konya, Türkiye
[2]Necmettin Erbakan University Faculty of Medicine, Departments of Pediatrics, Konya, Türkiye
[3]Necmettin Erbakan University Faculty of Medicine, Departments of Raiology, Konya, Türkiye

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ABSTRACT

Cystic echinococcosis is a parasitic disease with significant importance for public health in endemic regions. Spinal cystic echinococcosis, however, is a rare form that may lead to severe complications due to its localization. In this manuscript, we presented a 16-year-old male patient who admitted with abdominal and back edema for 2 months, evaluated with preliminary diagnoses of Pott's abscess and malignant mass, subsequently diagnosed with spinal cystic echinococcosis. It was concluded that cystic echinococcosis should be considered in differential diagnosis of large cystic masses and percutaneous aspiration, injection, reaspiration method might be a safe and effective treatment option particularly for cases of complicated spinal cystic echinococcosis.
Keywords: Cystic echinococcosis, spinal, child, percutaneous aspiration injection and re-aspiration

ÖZ

Kistik ekinokokkozis, endemik bölgelerde halk sağlığı açısından büyük öneme sahip bir paraziter hastalıktır. Spinal kistik ekinokokkozis ise çok nadir görülen ve yerleşim yeri sebebiyle ciddi komplikasyonlara yol açabilen bir formudur. Bu makalede 2 aydır karın ve sırtta yaygın şişlik şikayeti ile başvuran, Pott apsesi ve malign kitle ön tanılarıyla değerlendirilen spinal kistik ekinokokkozis tanısı alan 16 yaşında erkek hasta sunulmuştur. Sonuç olarak, büyük kistik lezyonların ayırıcı tanısında kistik ekinokokkozisin akla getirilmesi gerektiği ve perkütan aspirasyon, enjeksiyon, reaspirasyon yönteminin özellikle kompleks spinal kistik ekinokokkozis olgularında güvenli ve etkili bir tedavi seçeneği olabileceği kanısına varılmıştır.
Anahtar Kelimeler: Kistik ekinokokkozis, spinal, çocuk, perkütan aspirasyon enjeksiyon ve reaspirasyon

INTRODUCTION

Cystic echinococcosis (CE) is a serious disease caused by the *Echinococcus granulosus* tapeworm (1). Infections with *E. granulosus* complex parasites are frequently acquired during childhood, yet the cysts they generate might take several years to grow to a size detectable enough to cause symptoms. These distinct cystic lesions predominantly manifest in the liver (70%) and the lungs (20%), but other parts of the body can also be affected (2,3). Spinal CE, a rare and serious condition with a high risk of recurrence (1,4).

Herein we present a case of spinal CE who initially presented with a massive abdominal mass and was successfully treated with percutaneous aspiration, injection, and re-aspiration (PAIR).

CASE REPORT

A previously healthy 16-year-old male was referred to our clinic for evaluation of a painless, large mass covering the left abdomen and back. His family reported that the mass had been enlarging over the past two months. There was no history of animal

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Address for Correspondence/Yazar Adresi: Özge Metin Akcan, Necmettin Erbakan University Faculty of Medicine, Departments of Pediatric Infectious Diseases, Konya, Türkiye
Phone/Tel: +90 332 223 63 46 **E-mail/E-Posta:** drozgemetin@gmail.com **ORCID ID:** orcid.org/0000-0002-3465-6994

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contact, weight loss, or night sweats, and the patient had no known medical conditions or exposure to tuberculosis. The patient was residing in a town within Central Anatolia Region.

On admission, vital signs were normal. Physical examination revealed a 9x10 cm swelling in the left lower quadrant of the abdomen and a 12x10 cm mass on his back. Vertebral tenderness was noted upon deep palpation of the L2-L3 vertebrae.

Laboratory tests revealed a white blood cell count of 5,880/mm^3, hemoglobin level 13.7 g/dL, platelet count 291,000/mm^3. C-reactive protein of 3.7 mg/L (normal range <5 mg/L) and an erythrocyte sedimentation rate of 24 mm/h (normal range <20 mm/h). Serum transaminases, electrolytes, creatinine, immunoglobulins were normal and HIV serology was negative. Blood culture, brucella immunocapture test, tuberculin skin test and cyst hydatid indirect hemagglutination test were requested.

Abdominal ultrasonography revealed a large mass with dense cystic regions and thick walls. The mass originated from the lower vicinity of the left kidney, occupied the left lumbar lobe and lower quadrant, and extended towards the proximity of the bladder. Subsequent abdominal magnetic resonance imaging (MRI) showed cystic lesions that were multilobulated and septated, originating from the paravertebral region, causing displacement of the left kidney towards the anterosuperior direction. The MRI further revealed the mass extending into the iliopsoas muscle and the subcutaneous tissue of the lumbar region, measuring 12x12x23 cm (Figure 1a). There were T2 hyperintense lesions in the medullary cavity of lumbar 2nd and 3rd vertebra consistent with bone involvement. These findings were compatible with Pott's disease. Tuberculin skin test and brucella immunocapture tests were negative.

For diagnostic and therapeutic purposes, the interventional radiology department performed several procedures, including a planned biopsy for differential diagnosis. During the biopsy, it was noted that the mass lacked solid characteristics. Instead, purulent to clear and colorless to pale yellow fluid was observed. A catheterization drain was then inserted, and daily irrigation was carried out via 1000 cc of normal saline. By the 6th day of treatment, the drained fluid transformed into a rock-colored water with white membranes floating in it (Figure 2). Hydatid cyst was diagnosed with histopathological examination. An indirect hemagglutination test revealed the presence of anti-hydatid cyst antibodies at a titer of 1/640. Other organs were screened and no cysts were detected.

Albendazole therapy was initiated. During the last visit, the patient was asymptomatic and he was discharged with a plan for albendazole therapy lasting a minimum of six months, along with regular follow-up visits. The control MRI at the six-month mark indicated the absence of any residual cystic lesions, with the observations interpreted as residual inactive areas (Figure 1b).

DISCUSSION

Spinal CE is a rare manifestation of hydatid disease, accounting for less than 1% of all cases. It can be difficult to diagnose without typical clinical, laboratory, and imaging findings because it can mimic other conditions, such as tuberculosis, bone tumors and metastatic

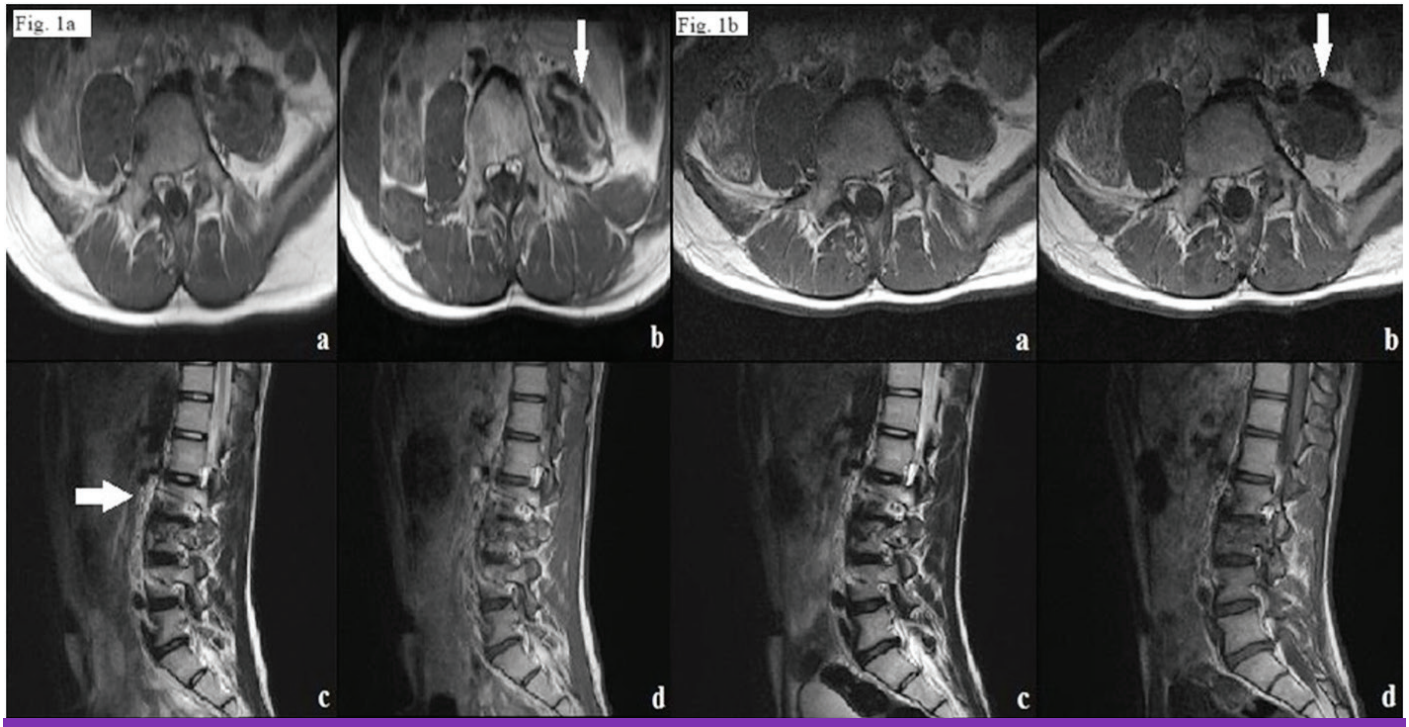

Figure 1a. Abdominal MRI reveals a multiloculated, septated cystic lesion (arrow) originating from the paravertebral region, extending into the medullary cavity of lumbar vertebrae and displacing the left kidney anterosuperiorly. (a) T1-weighted axial MRI (b) T1-weighted axial MRI with contrast (c) T2-weighted sagittal MRI (d) T1-weighted sagittal MRI with contrast; **Figure 1b**. There are several residual, inactive foci (arrow) in the paravertebral area, at the site of the previous hydatid cyst as revealed by the control MRI at month 6 after treatment. The bone involvement was stabil (a) T1-weighted axial MRI image (b) T1-weighted axial MRI with contrast (c) T2-weighted sagittal MRI (d) T1-weighted sagittal MRI with contrast

MRI: Magnetic resonance imaging

Figure 2. An image showing a container of drainage fluid, with white membranes floating in a rock-colored water

diseases. Upon reviewing radiological findings indicating a paravertebral cystic lesion, Pott's disease (PD) emerged as a potential differential diagnosis for our patient. PD, also known as tuberculous spondylodiscitis, is a type of osseous spinal tuberculosis (4,5). Back pain is the most common presenting symptom, while constitutional symptoms such as fever or weight loss may not be present (6). Computed tomography scans can show bone hydatid disease, which can sometimes be mistaken for tuberculosis, metastases, giant cell tumors, or bone cysts. However, MRI findings are more distinctive, especially in distinguishing spinal CE from other conditions (3,4,7). MRI shows well-circumscribed, cystic lesions, with CSF-like signal intensities, hypointense on T1-weighted imaging, and hyperintense on T2-weighted imaging. T2-weighted images show a low-intensity rim surrounding the homogeneous hyperintense cyst contents. The cyst wall may be thin and regular, isointense, or demonstrate a slightly lower signal than its contents. A markedly hypointense cyst wall on T1- and T2-weighted MRIs is characteristic of hydatid disease (8).

Treatment for vertebral CE combines surgery and antiparasitic therapy. Removing intact cysts is essential, as cyst perforation can cause systemic spread and chronic recurrence (4). Decompression surgery is vital in managing these patients due to the increased risk of spinal cord compression (2). There is a case report in the literature detailing successful treatment of intra and para-sacral cysts using rigid endoscopy (9). Although a limited number of patients have been treated with PAIR combined with albendazole, one patient with a cervical vertebral hydatid cyst and severe clinical symptoms achieved complete resolution, while another patient with recurrent disease following complex surgery was successfully treated, resulting in the complete resolution of two vertebral cysts. The authors recommended the PAIR for extensive disease where radical removal is not feasible and where previous surgical interventions may exacerbate re-operation (10,11). We successfully treated our patient with PAIR procedure combined with albendazole, with no complications. Further research is needed to evaluate the long-term efficacy and safety of the PAIR for spinal CE. In an another study in which a total of 50 pediatric CE patients were evaluated, spinal CE was detected in two of them and they underwent surgical excision. They were treated with albendazole. No recurrence was observed (12). An 11-year-old male patient diagnosed with multiple CE in the liver and lungs presented with complaints of difficulty in walking and leg pain one year after discontinuing albendazole treatment and then he diagnosed with spinal CE. The patient underwent surgery and was then treated with albendazole for another 4 years. No recurrence was observed in the subsequent one-year follow-up. It was emphasized that recurrences and systemic spread should always be kept in mind (13). Albendazole or mebendazole treatment is essential to prevent recurrence. The optimal duration of treatment remains uncertain, and treatment should be individualized (14).

While serological tests serve as valuable tools in differential diagnosis, they can yield a high rate of false-negative results. The immune response tends to be higher in ruptured hydatid cysts. Serological tests often produce negative results when a cyst is aging, calcified, or no longer viable (3). Our patient exhibited a titer of 1/640 on the indirect hemagglutination test.

CONCLUSION

Hydatid cysts can manifest in diverse clinical presentations, and spinal hydatid cyst, although rare, represents a serious condition with significant morbidity and a complex treatment course. Patients with an abdominal mass of spinal origin should prompt consideration for hydatid cysts in the differential diagnosis, and the PAIR procedure appears to be a promising treatment option.

*Ethics

Informed Consent: Written informed consent was obtained from the parents. Approval was obtained from our patient's parents for the data to be published in the journal.

*Authorship Contributions

Surgical and Medical Practices: Ö.M.A., S.B., Concept: K.Y., Design: M.G., Analysis or Interpretation: Ü.K., Literature Search: M.G., Ü.K., Writing: Ö.M.A., K.Y., S.B.

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Mysterious Allergy Caused by Tick Bite: Alpha-Gal Syndrome

Kene Isırmasının Neden Olduğu Gizemli Alerji: Alpha-Gal Sendromu

Muhammed Nalçacı
Ege University, Graduate School of Natural and Applied Sciences, Department of Biology, İzmir, Türkiye
Present adress: Postdoc, Ege University Faculty of Medicine Department of Parasitology, İzmir, Türkiye

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ABSTRACT

Alpha-Gal syndrome (AGS) manifests as an intricate allergic response characterised by the formation of specific immunoglobulin E (IgE) antibodies targeting a carbohydrate termed galactose-α-1.3-galactose (α-Gal). Alpha-Gal antigens, which play a role in AGS, have been detected in the salivary glands and saliva of various tick species, especially *Amblyomma americanum*. Identifying these antigens in tick saliva underlines the potential role of tick bites in sensitising individuals to α-Gal and contributes to the complex immunological processes associated with AGS. When people with α-Gal allergy eat beef, pork, lamb, or the flesh of other mammals, they experience an allergic reaction that causes various symptoms, including rash, nausea, vomiting, and diarrhoea. In some cases, AGS can be life-threatening requiring emergency medical attention. Moreover, these reactions do not occur only due to red meat; intake of medical drugs, vaccines, and antidotes containing α-Gal epitopes can also trigger allergies. The fact that the symptoms causing IgE antibodies are directed against a carbohydrate moiety the unusual delay between food consumption and the onset of symptoms, and the differences in the reactions shown by α-Gal allergy make α-Gal syndrome an unprecedented allergic disease and distinguish it from other food allergies. Interestingly, α-Gal antigens involved in the development of AGS have been discovered in salivary secretions of different tick species in several continents. However, the underlying causes of α-Gal-specific IgE production and immune responses to tick bites are not fully understood. This complex system is crucial for identifying and developing new therapies for the disease. This article reviews the evolution of α-Gal, the current understanding of AGS and its relationship to tick species.

Keywords: Tick, red meat, Alpha-Gal, Alpha-Gal syndrome, allergy, IgE

ÖZ

Alpha-Gal sendromu (AGS), primat olmayan memelilerin hücrelerinde ve dokularında bulunan, galaktoz-α-1,3-galaktoz (α-Gal) olarak bilinen bir karbonhidrata karşı spesifik immünoglobulin E (IgE) antikorları geliştiğinde ortaya çıkan karmaşık bir alerjik reaksiyondur. AGS'nin gelişiminde rol oynayan α-Gal antijenleri, başta *Amblyomma americanum* olmak üzere çeşitli kene türlerinin tükürük bezlerinde ve tükürüklerinde tespit edilmiştir. Kene tükürüğünde bu antijenlerin tanımlanması, kene ısırıklarının bireyleri α-Gal'e karşı duyarlı hale getirmedeki potansiyel rolünün altını çizmekte ve AGS ile ilişkili karmaşık immünolojik süreçlere katkıda bulunmaktadır. Alpha-Gal alerjisi olan kişiler sığır eti, domuz eti, kuzu eti veya diğer memelilerin etini yediğinde döküntü, mide bulantısı, kusma ve ishal gibi çeşitli semptomlara neden olan alerjik reaksiyonla karşılaşırlar. Bazı olgularda AGS, acil tıbbi müdahale gerektirecek şekilde hayatı tehdit edici olabilir. Üstelik bu reaksiyonlar sadece kırmızı ete bağlı olarak ortaya çıkmaz; α-Gal epitopları içeren tıbbi ilaçların, aşıların ve panzehirlerin alımı da alerjileri tetikleyebilir. Semptomlara neden olan IgE antikorlarının bir karbonhidrat parçasına karşı yönlendirilmiş olması, gıda tüketimi ile semptomların başlangıcı arasındaki olağan dışı gecikme ve α-Gal alerjisinin gösterdiği reaksiyonlardaki farklılıklar, α-Gal sendromunu benzeri görülmemiş bir alerjik hastalık haline getirmekte ve diğer gıda alerjilerinden ayırmaktadır. İlginç bir şekilde, AGS gelişiminde rol oynayan α-Gal antijenleri çeşitli kıtalarda farklı kene türlerinin tükürük salgılarında keşfedilmiştir. Bununla birlikte, α-Gal'e özgü IgE üretiminin ve kene ısırıklarına karşı bağışıklık tepkilerinin altında yatan nedenler tam olarak anlaşılamamıştır. Bu karmaşık sistem, hastalığa yönelik yeni tedavilerin tanımlanması ve geliştirilmesi için çok önemlidir. Bu derleme α-Gal'in evrim sürecini, AGS'nin mevcut anlayışını ve bunun kene türleriyle ilişkisini gözden geçirmektedir.

Anahtar Kelimeler: Kene, kırmızı et, Alpha-Gal, Alpha-Gal sendromu, alerji, IgE

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Address for Correspondence/Yazar Adresi: Muhammed Nalçacı, Ege University, Graduate School of Natural and Applied Sciences, Department of Biology, İzmir, Türkiye
Present adress: Postdoc, Ege University Faculty of Medicine Department of Parasitology, İzmir, Türkiye
Phone/Tel: +90 555 677 48 39 **E-mail/E-Posta:** muhammednalcaci@gmail.com **ORCID ID:** orcid.org/0000-0002-9265-2887

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INTRODUCTION

Ticks are obligate ectoparasites and must feed on blood to complete their development at all life cycle stages (larva, nymph, adult). Ticks, as significant vectors of viruses, bacteria, and protozoan pathogens, hold paramount global public health importance (1). It is currently recognised as the second most important vector of infectious diseases in humans globally after mosquitoes. Their role in disease transmission is multifaceted, involving the transmission of various pathogens, including bacteria, viruses, protozoa, and helminths. The growing recognition of ticks as vectors underscores the imperative for comprehensive research initiatives to better understand their ecology, host interactions, and the diverse array of pathogens they harbour, with implications for global public health strategies and disease prevention (2).

The hematophagy nature and host specificity of ixodid ticks may influence their capacity to acquire, maintain and transmit various pathogens and thus contribute to tick bite-associated conditions, including Alpha-Gal syndrome (AGS), tick paralysis and babesiosis (2). While feeding on their hosts, ticks secrete a multi-component saliva that modulates host immune responses and contributes to the establishment of tick-borne viral, protozoal and bacterial pathogens in the host (3-8). In the United States of America (USA), a surveillance study by the Centers for Disease Control and Prevention between 2004 and 2016 revealed that ticks were responsible for 77% of reported cases of vector-borne diseases. (4,9). Recent research predicts an increase in tick-borne diseases, including AGS, due to the geographical expansion of several tick species (10,11).

Alpha-Gal syndrome, also called α-Gal allergy, red meat allergy or mammalian meat allergy, develops when the immune system reacts to the carbohydrate α-Gal, leading to hypersensitivity reactions (10,12). Alpha-Gal is found in most mammals, including farm animals, but is absent in humans and some primates (13). Individuals with AGS develop hypersensitivity to α-Gal and manifest as a delayed allergic reaction to mammalian meat and products containing α-Gal (e.g., dairy products, gelatin-containing colloids and pharmaceuticals) (10,14-16). Unlike traditional food allergies, allergic reactions in AGS appear late and are multifaceted. Reactions typically begin a few hours after consuming mammalian meat or other animal-based food products (Figure 1) (13). Symptoms range from severe anaphylaxis to angioedema, diarrhoea, shortness of breath, urticaria, vomiting and itching (13,17,18). The AGS first reported when specific IgE antibodies targeting α-Gal were identified in patients who developed an allergic reaction to the cancer treatment drug cetuximab and then were subsequently identified in people with hypersensitivity to mammalian meat and products (15,17).

In 2009, Australian researchers were the first to describe the relationship between AGS and tick bites. Subsequently, US researchers studying patients with anaphylactic reactions to cetuximab, a cancer drug, established that the *Amblyomma americanum* tick, the so-called "Lone Star Tick", was linked to the allergy (19,20). Since then, AGS has been reported in several world

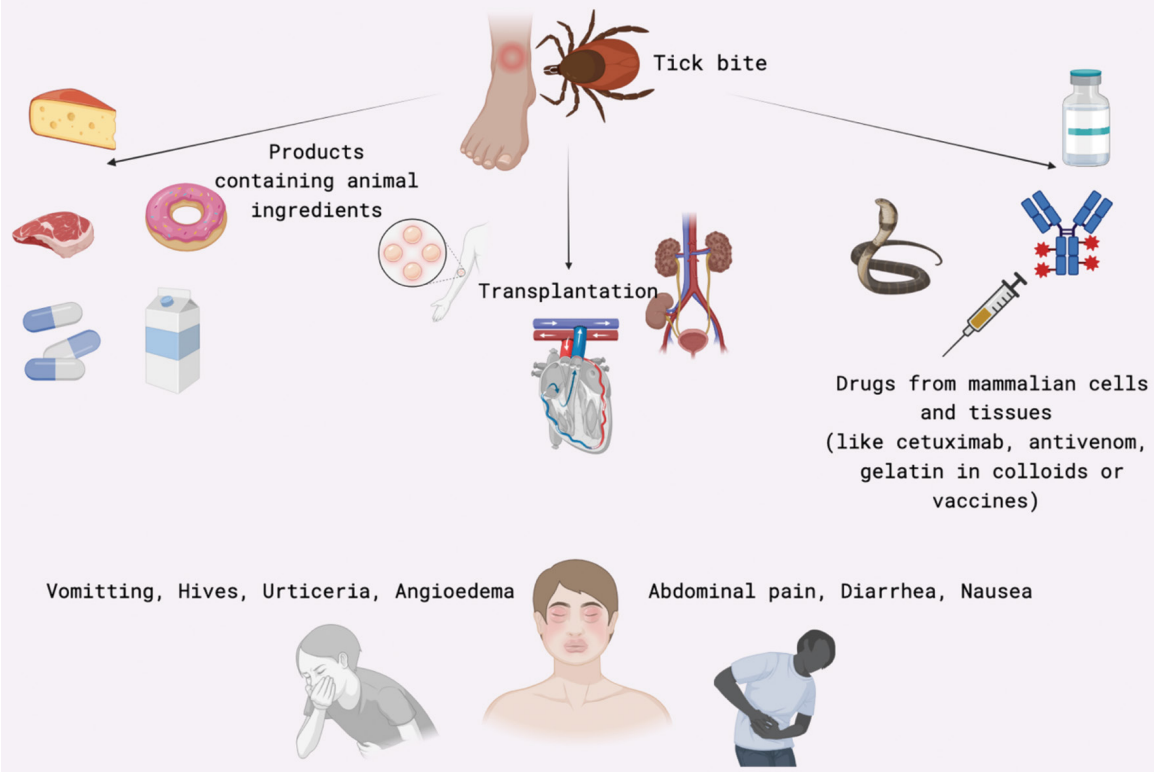

Figure 1. Tick bites are AGS's most common and important cause. Drugs derived from mammalian tissues (e.g., cetuximab, antivenoms, gelatine in some medical suspensions and vaccines) can trigger this allergy. Because of the tendency of AGS to cause a severe allergic reaction, organ transplants from animals to humans are unsuccessful. Consumption of mammalian meat or offal, dairy products, dairy products used in desserts, etc., may cause an allergic reaction

AGS: Alpha-Gal syndrome

regions, including the USA, Europe, Australia, Japan, and South Africa (21-24). In the USA, tick bites from *A. americanum* are thought to be the primary cause of AGS. However, the clustering of cases in areas outside the range of this tick suggests that other tick species or vectors may also contribute to α-Gal susceptibility (15,25,26). Globally, other tick species that cause AGS include *I. ricinus*, *I. holocyclus* and *H. longicornis* (20,27).

Although the processes that cause sensitisation to α-Gal in humans have not been fully resolved, it is thought to be linked to the α-Gal antigen present in the saliva of some tick species (25,26). Several recent studies have shown a strong association between AGS and tick bites (19,28). This phenomenon has been observed in diverse geographical locations, indicating its ubiquity. Additionally, it has been noted that patients who actively avoid recurrent tick exposures often experience a decline in blood levels of α-Gal IgE. However, the pace and extent of this decrease exhibit variability among individual patients, highlighting the intricate and potentially distinctive nature of the relationship between tick bites and the immunological response associated with AGS (19). The amount of meat consumed and the presence of cofactors (alcohol, activity, use of spices, menstrual cycles) affect the delay before the reaction and the subsequent clinical signs. Still, there may not be a correlation between the severity of the response and the IgE titre specific to α-Gal (29). Specific to AGS, recent tick bites sensitise patients to previously tolerated exposures and even lower the reactivity threshold (30).

Allergen avoidance is one of the main steps in the management of AGS (31). Although AGS is similar to other food allergies, mammalian-derived products are more difficult to avoid due to inadequate labelling and common ingredients such as "natural" sweeteners in many foods. For <10% of patients, the allergen avoidance diet also includes the elimination of gelatine as well as dairy products and derivatives (32). Furthermore, numerous pharmaceuticals are derived from mammalian sources, and specific tissues from mammals are incorporated into medical devices. Items like heart valves, plasma expanders containing gelatin, and pancreatic enzymes are potential sources of exposure to α-Gal (33,34). Due to the ubiquity of mammalian-derived products in food and healthcare, avoiding allergens can present particular challenges for patients with AGS (32).

Alpha-Gal Epitope and Generation of Human Anti-α-Gal Response

The *α-1.3-galactosyltransferase* gene (*α1.3 GT* or *GGTA1*), which has distinctive evolutionary features, has played a crucial role in the evolutionary process of mammalian species. This gene arose early in mammalian evolution and is absent from other vertebrate taxa. Its activity can be observed in various mammalian lineages, including marsupials, non-primate placental mammals, prosimians, and new world monkeys. The *α-1.3-GT* gene encodes the α-1.3-GT enzyme, synthesising a carbohydrate antigen recognised as the "α-Gal epitope". The unique distribution and functionality of the *α-1.3 GT* gene across various mammalian taxa underscore its evolutionary significance and potential implications for understanding immunological responses to the α-Gal epitope in the context of AGS. The α-Gal epitope is abundant in glycolipids and glycoproteins in cell membranes (35). The gene for the enzyme α-1.3-galactosyltransferase, which is necessary for α-Gal synthesis, was inactivated due to a frameshift mutation in the ancestors of old world monkeys (Cercopithecids) and great apes. Hence, α-Gal expression is lacking in humans and old world primates, rendering this molecular construct highly immunogenic in these species. As a result of this gene inactivation, these species lack α-Gal epitopes and naturally produce an antibody known as "anti-α-Gal antibody", which binds specifically to α-Gal epitopes and is most prevalent in humans. It is estimated that approximately 1% of circulating antibodies in healthy individuals are against α-Gal. Approximately 1% of healthy individuals' circulating antibodies are considered anti-α-Gal. When these antibodies interact with the α-Gal epitope found in mammalian organs (e.g., porcine organs), they can activate the complement system, which could result in hyperacute reactions during the transplantation (35).

Studies examining anti-α-Gal antibody classes have revealed several immunoglobulin types in human serum, including IgG, IgM, and IgA. Of note, the IgA isotype is the predominant class in human secretions, including saliva, tears, respiratory and intestinal secretions, colostrum, milk, bile, and vaginal fluid. The predominance of anti-α-Gal IgA antibodies in these secretions highlights their importance as a major component of total secretory immunoglobulins (36).

The structural similarity between the chemical composition of the α-Gal antigenic determinant and the blood group B antigen is striking. Both antigens share the configuration of two terminal galactoses connected by an α-1.3 bond. The hallmark of the blood group B antigen is the presence of a fucose molecule linked to one of the terminal galactoses via an α-1.2-glycosidic bond. This chemical parallelism underscores potential immunological cross-reactivity and further investigates the intricate relationship between anti-α-Gal antibodies and blood group B antigens, contributing to our understanding of immune responses and possible implications in health and disease (37). Galili et al. (37) findings, demonstrating the capacity of specific anti-α-Gal IgG antibodies to recognise blood group B antigens, underscore the intricate interplay between anti-α-Gal immune responses and blood group specificity. McMorrow et al. (38) research revealed a noteworthy correlation wherein individuals expressing the blood group B antigen (encompassing blood groups B and AB) exhibited reduced levels of α-Gal IgG antibody reactivity compared to those not expressing the B antigen (including blood groups O and A) (37-39). This correlation adds a layer of complexity to the understanding of immune responses to α-Gal, suggesting potential interactions with blood group determinants that warrant further exploration and elucidation. Beyond the association with B antigen, the antibody response to α-Gal exhibits significant interindividual variability and contributes to the complexity of the immune response (37-39).

Reports indicate a discernible pattern showing a strong correlation between the IgE and IgG antibody responses to α-Gal. This finding underscores the complex and interconnected relationship between various classes of immunoglobulins in the context of α-Gal immunity. Furthermore, it is worth noting that individuals with α-Gal allergy and IgE antibodies against α-Gal demonstrate significantly higher levels of anti-α-Gal IgG1 antibodies compared to healthy individuals (40,41). IgE antibodies to α-Gal are associated with allergic reactions to mammalian meat, mammalian-derived products, and α-Gal-containing drugs. However, generating an antibody response against α-Gal may benefit the organisms that produce this response. Anti-α-Gal IgM and IgG antibodies have been correlated with diminished susceptibility to *Plasmodium*

infection, the etiological agent of malaria. In areas where malaria is endemic, IgM antibody responses to α-Gal have been shown to prevent malaria infection caused by *P. falciparum* (42,43). A study demonstrated an inverse association between high titers of anti-α-Gal IgM antibodies and malaria parasite transmission (43,44). In neonates, anti-α-Gal IgG antibodies exhibit low levels for the first six months of life, followed by a gradual rise over 2-4 years until reaching adult equivalence (44). Therefore, it has been suggested that the higher risk of malaria in young children than in adults is because their immune systems have not yet produced enough natural antibodies that recognise the α-Gal carbohydrate structure. In contrast, those with high levels of these antibodies have been found to have a lower risk of contracting malaria (45). Anti-α-Gal antibodies target *Plasmodium* sporozoites and promote the death of sporozoites on the skin by blocking the sporozoites' ability to migrate from the skin to the liver. However, if erythrocytes enter the bloodstream after the parasite's mosquito bite, these antibodies do not alleviate the severity of the disease (46).

Numerous research papers have addressed the practicalities of developing anti-α-Gal antibodies in humans, highlighting their potential to induce immunogenic responses against parasites with α-Gal epitopes, such as *Trypanosoma* and *Leishmania* species (47,48). In Chagas (48) and leishmaniasis (49), anti-α-Gal antibodies protect against parasite infections. In a study in which anti-α-Gal antibodies were raised in a mouse model in which the *α-1.3 GT* gene was silenced (GGTA1- KO or α -1.3 GTKO), it was observed that the severity of *Leishmania* infection decreased (49,50). The presence of the α-Gal epitope on *Leishmania* parasites suggests it could be a vaccine candidate for blocking human cutaneous and visceral leishmaniasis (50). In addition, α-Gal antibodies have also been reported to provide protection against malaria infection, promote the healing of burn wounds and tissue repair, increase the immunogenicity of HIV and cancer vaccinations, and exhibit lytic activity against *T. cruzi* parasites (50).

Alpha-Gal expression extends beyond ticks and mammalian tissues to include various bacteria such as *Escherichia*, *Klebsiella* and *Salmonella*. Many bacteria are integral to the human intestinal microbiome. This broad distribution suggests that producing anti-α-Gal antibodies could potentially serve as a mechanism to resist microbial proliferation or mitigate the adverse effects of pathogen colonisation within the human body. The interplay between α-Gal and the gut microbiome raises intriguing questions about the immunomodulatory functions of anti-α-Gal antibodies and their role in shaping host-microbe interactions in the intricate ecosystem of the human body. Further research is essential to elucidate the complexities of these relationships and their implications for human health and immune homeostasis (51,52). Glycans play an essential role in the interaction between hosts and pathogens (53,54). The view that bacteria in the gut microbiome act as a stimulus for the continuous production of anti-α-Gal antibodies is supported by the fact that some *E. coli* and *Klebsiella* strains have been obtained from human faecal samples (51). The possible protective function of anti-α-Gal antibodies might have a broader scope, containing not only against vector-borne pathogens but also infections caused by non-vector-borne pathogens such as *Mycobacterium* spp., which are accountable for different types of tuberculosis and mycobacteriosis. In fact, anti-α-Gal antibodies may reduce mycobacteria's ability to bind to galactose-containing antigens, thus preventing their entry into host cells (51).

Additionally, they may also be effective against mycobacterium-induced inflammation. Notably, all pathogens associated with these diseases exhibit the α-Gal epitope on their surfaces (55). This broader spectrum of pathogenic targets suggests that the immune response elicited by anti-α-Gal antibodies could play a role in conferring resistance or mitigating the severity of infections caused by diverse pathogens, shedding light on the intricate interactions between α-Gal epitopes and the immune system's defence against a range of infectious agents. Studies conducted on this subject matter have indicated that the presence of anti-α-Gal immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies in the human body can effectively protect against a wide range of pathogens that possess α-Gal antigens on their outer surfaces (50,56). An experimental study on α-Gal knockout mice effectively validated the emergence of IgM antibodies targeting *E. coli* O86:B7, an important bacterium in the human intestinal tract exhibiting α-Gal expression. Significantly, these antibodies exhibited a noteworthy defensive effect in the mice, protecting them against malaria transmission (43)

The ability of anti-α-Gal IgA antibodies derived from human colostrum to effectively hinder the attachment of *Neisseria meningitidis* to human buccal cells has been observed, as these antibodies have displayed a remarkable propensity to bind to a diverse range of Gram-negative commensal bacteria (57). These findings propose a potential protective role of secreted anti-α-Gal IgA antibodies on mucosal surfaces, suggesting a broader impact beyond their role in allergic responses. Studies conducted on Türkiye's have demonstrated that the presence of gut microbiota in bacteria expressing high levels of α-Gal can effectively shield against clinical aspergillosis and impede the formation of lung granulomas. Interestingly, the oral administration of *E. coli* O86:B7, can significantly diminish the incidence of granulomas in the lungs. This protective mechanism serves as a safeguard for Türkiye's, effectively preventing the onset of acute aspergillosis. These multifaceted interactions underscore the potential immunomodulatory functions of α-Gal epitopes in diverse biological contexts (46).

Allergy Understanding Differentiated by AGS

Alpha-Gal syndrome, recognised as a novel food allergy syndrome, is distinguished by intense allergic responses after ingesting certain red meat types, namely beef, lamb, or pork. In contrast to the typical pattern in which allergic reactions to food are primarily directed against protein epitopes and occur immediately after ingesting the allergens, allergic reactions to red meat are specifically directed against the carbohydrate epitope α-Gal. Noteworthy is the distinctive temporal aspect of these reactions, with manifestations occurring several hours after the allergen intake. This unusual feature sets AGS apart from conventional food allergies and underscores the need for comprehensive understanding and tailored management strategies (58). Unlike protein antigens, α-Gal stands out as one of the two carbohydrates implicated in life-threatening allergic reactions, exhibiting a remarkable resistance to denaturation even at elevated temperatures (58,59). Recent studies have drawn more attention to proteins glycosylated by α-Gal and thus become responsible for red meat allergic reactions. Researchers have identified transmembrane proteins in pork, which they have

named AP-N and ACE-1. These proteins are the major IgE-binding molecules in pig kidneys. AP-N and ACE-1 proteins were involved in the primary mechanism of red meat allergy. The symptoms have been observed to cause a shorter delay (<2 hours) and more consistent reactions, especially after the consumption of pork kidneys, where these proteins are abundant (13,58).

Since the elucidation of α-Gal in 2007, numerous investigations have been undertaken to elucidate this emerging food allergy (14,60,61). Typically, food allergies are categorised into two main types: IgE-mediated and cell-mediated; the latter is also called non-IgE-mediated. IgE-mediated allergies manifest with rapid onset of clinical symptoms occurring within 30 minutes following exposure to the antigen (62,63). In the case of α-Gal syndrome, reactions tend to be severe, occasionally resulting in fatality. Moreover, the onset of clinical symptoms associated with AGS may vary, ranging from 2 to 10 hours post-exposure, depending on factors such as the antigen's route, source, and nature (17,32,64).

Delayed responses following eating red meat have been seen in individuals with α-Gal syndrome, marking a distinct clinical characteristic. It is unclear exactly how people with this disease experience a delayed sensitivity to red meat. Still, several processes are considered involved, including meat's digestion, absorption, transportation, and subsequent presentation to the host immune system. Studies also describe the effect of age and atopy on AGS. In German, Italian and Spanish patients, investigations reported no correlation between age and sensitisation to α-Gal (64,65).

A cohort of researchers presented findings indicating that the elderly population was more likely to acquire α-Gal sensitisation. These individuals were documented to exhibit a diverse assortment of clinical manifestations, encompassing urticaria, angioedema, pruritus, and systemic anaphylaxis. Before the onset of this syndrome, specific individuals reported experiencing additional symptoms such as nausea, indigestion, diarrhoea and abdominal discomfort (66,67). However, none of the above symptoms have been reported to occur in some patients after exposure to α-Gal, emphasising the unusual nature of AGS. Differences in the host's lipid or fatty acid metabolism, one of the most important of these extraordinary circumstances, may delay the detection of α-Gal in the bloodstream and the onset of AGS symptoms (22,68).

Investigating the influence of allergen dose on AGS patients, a study observed a correlation between meat source and the incidence of delayed anaphylactic reactions to α-Gal (69,70). Beef consumption elicited the highest reaction rate (53%), followed by pork (47%). Lamb and venison demonstrated a significantly lower prevalence of reactions (9.1% and 7.3%, respectively). Notably, some patients exhibited no response to the tested meats but experienced anaphylaxis after consuming offal containing indeterminate amounts of α-Gal (71). Exogenous and endogenous factors influence the digestive process quantitatively and qualitatively. Physical exercise, alcohol consumption, non-steroidal anti-inflammatory drugs, infections, and menstruation can affect the intestinal absorption of food allergens. When allergen concentrations exceed a critical threshold, an immune response is triggered, manifesting as an allergic reaction (70,72).

Eating meat and organs from a much more comprehensive range of mammals in Europe and Türkiye is normal. This includes cloven hoof and soliped animals' liver, lung, heart, tripe (intestine) or kidney. Clinical symptoms within two hours of ingesting mammalian viscera are typically more severe and progress rapidly (15,69,73). Studies have revealed that pig kidney harbours significantly higher amounts of α-Gal epitopes than muscle meat. This observation suggests a potential link between the severity and temporal heterogeneity of anaphylaxis in AGS patients and the amount of bioavailable α-Gal in ingested meat sources (69,74).

Epidemiology of Alpha-Gal

All continents except Antarctica have reported cases of AGS following tick bites. Studies have found that the highest incidence rates are in the United States of America, Canada, and Australia (75,76). The prevalence of α-Gal sensitisation exhibits variability contingent upon geographic regions, the demographic composition under examination, and the designated threshold for defining a positive α-Gal IgE level (22).

Since 2007, researchers, including Commins and Platts-Mills, have identified the epitope in red meat that triggers the specific IgE antibody (20). They have also gathered evidence supporting van Nunen's observation that tick bites can lead to mammalian meat allergy (17). The global identification of AGS has resulted in establishing a connection between AGS and tick bites, offering valuable insight into the mechanisms through which various tick species can trigger IgE sensitisation in humans (Table 1). In 2007, a significant milestone in medical research was achieved with the emergence of the inaugural report, which shed light upon the remarkable ability of ticks to instigate the development of a perplexing condition known as red meat allergy. During this time, van Nunen et al. (77), a distinguished expert in his field, conducted an extensive investigation, meticulously examining the reactions of a considerable cohort of 25 patients after they consumed red meat. Astonishingly, the results of this groundbreaking study revealed that a staggering 92% of the participants, a total of 23 individuals, exhibited unmistakable signs of allergic responses. These cases occurred in the southern parts of Australia and the Sydney coast, endemic areas inhabited by the *I. holocyclus* tick. Thus, the first hypothesis was confirmed and paved the way for further research in this fascinating field (28,78).

In 2008, instances of hypersensitivity reactions to a pharmaceutical formulation containing the monoclonal antibody cetuximab, which is employed in cancer treatment, were identified in specific regions within the borders of the USA (79,80). It was determined that individuals who experienced hypersensitivity reactions to this drug possessed IgE antibodies specifically targeting cetuximab within their serum, implying that these antibodies play a potential role in the progression of anaphylaxis (81). Subsequent investigations unveiled a direct correlation between tick bites and the emergence of IgE antibodies directed against red meat. It has also been reported that the incidence rate of AGS has increased in regions of the United States where the *A. americanum* tick has a significant presence. In addition, climatic factors contribute to the possibility of ticks being seen in different regions. Consequently, it is thought that the incidence of AGS will continue to increase (11,17,18,42). The preliminary records of the AGS in the United States in 2009 accounted for a mere 24 officially reported cases. However, a subsequent study has since revealed a significant escalation in the prevalence, updating the documented instances to 34,000 (82). Again, in the United States, 295,400 people were tested as part of a comprehensive study covering the years 2017-2022. As a result of this rigorous research, 90,018 people, approximately 30.5 percent of the study sample, tested positive. The study also documented a significant

Table 1. Tick species associated with Alpha-Gal sensitisation (71)

Scientific name	Commonly used name	Geographical range
Amblyomma americanum	Lone Star Tick	North America (Southeastern USA, Canada, Mexico)
Amblyomma cajennense	Cayenne Ticks	North and Central America
Amblyomma hebraeum?	South African Bont Tick	South Africa
Amblyomma sculptum	N/A	South America
Amblyomma testudinarium	N/A	South Asia (India, Sri Lanka) and East Asia
Amblyomma variegatum?	Tropical Bont Kenes,	Southeast Asia, Africa
Haemaphysalis longicornis	Asian Longhorned Tick, Bush Tick	Japan
Ixodes australiensis	N/A	Australia
Ixodes holocyclus	Paralysis Tick	Australia, South Asia
Ixodes nipponensis?	Cattle Tick	Asia (including Korea and Japan)
Ixodes ricinus	Sheep Tick	North America, Europe and North Asia, Africa
Ixodes scapularis	Deer Tick	Central America, North America
Rhipicephalus spp.	Asian Blue Tick, Australian Cattle Tick	South Asia, South America, North America

A question mark after the name of the tick concerned means that the tick species listed are suggested but not definitively linked to the development of α syndrome.
N/A: There is no commonly used name

increase in the prevalence of positive test results, from just 13,371 cases in 2017 to 18,885 cases in 2021 (83).

Relying on the identification of α-Gal within salivary glands, the *H. longicornis* tick has been hypothesised as a potential causative agent for AGS. Reports of AGS cases in Japan further corroborate the association with bites from the *H. longicornis* tick. At the termination of the investigation, it was documented that sure tick bites in Japan resulted in the production of IgE antibodies against α-Gal, which is present in the salivary glands of the *H. longicornis* tick (21). Furthermore, it was found that the salivary gland proteins of the *H. longicornis* tick were detected in the sera of most patients who exhibited symptoms of red meat allergy (21,82). Similarly, Hamsten et al. (84) conducted a study which uncovered traces of α-Gal in the midgut of the *I. ricinus* tick, thereby formulating a hypothesis that this particular carbohydrate may contribute to the development of red meat allergy in Sweden. Subsequently, researchers undertook a comparative analysis of α-Gal epitopes derived from *A. americanum* and *I. ricinus* ticks. These ultimately disclosed specific distinctive characteristics shared by both species, albeit with some variations (84,85). This significant finding implies the possible existence of a correlation between *I. ricinus* ticks and red meat allergy. In addition to the countries above, Spain, Türkiye, Germany, and Switzerland have also reported cases of the AGS (86).

In seroprevalence studies in various South African countries, individuals exhibited IgE antibodies targetting explicitly towards the α-Gal antigen. It is essential to highlight that, despite the existence of these antibodies, no evident allergic responses was documented after ingesting red meat (10). It is pertinent to note that comprehensive information regarding AGS cases in Central America remains unavailable, emphasising the need for further research and surveillance in this region. Several other tick species that fall under the taxonomic classification of the genera *Amblyomma* and *Ixodes*, which have been identified in various South and Central American geographical areas, can feed on human blood (28). Nevertheless, research has demonstrated that the saliva of *A. sculptum* obtained from its natural habitat in Brazil harbours α-Gal containing epitopes, which can stimulate an immune reaction and may play a role in the emergence of red meat allergy in Brazil. In Türkiye, instances of α-gal allergy have been documented in regions where *I. ricinus* species are prevalent and hazelnuts are cultivated (87). In a study conducted in 2021, IgE ratios were examined in the blood of 18 patients and anti-α-Gal specific antibody ratios were found to be high in 14 of them. In addition, it was reported that 16.7% of the patients with positive results had similar allergy symptoms in their family members after red meat consumption (88).

How Do Tick Bites Induce An IgE Response?

It is of utmost significance to make a discerning observation that the mechanisms behind the manifestation of an IgE response as a result of tick bites are subject to no less than three distinct theories: Firstly, the induction of said response may be attributed to the ordinary components of saliva that are inherent to ticks. Secondly, mammalian-derived glycoproteins or glycolipids in a tick acquired during a previous blood meal may be essential in triggering the α-Gal response. Lastly, it is plausible that the initiation of the reaction may be attributed to the presence of another organism within the tick (42,89).

Recent studies have provided robust evidence suggesting the possibility of an anti-α-Gal IgE response being primarily linked to ticks. In their enlightening research, Hamsten et al. (84) successfully conducted immunolocalisation experiments, enabling them to observe the α-Gal epitope within the gastrointestinal tract of the *I. ricinus* ticks. Building upon this groundbreaking discovery, Araujo et al. (90) further strengthened the argument by employing ELISA and immunoblotting techniques to identify this epitope's presence in *Amblyomma sculptum* ticks' saliva. The researchers additionally made a significant observation, noting that the α-Gal epitope derived from tick saliva had the uncanny ability to elicit an immune response, thereby stimulating the production of anti-α-Gal IgE antibodies in α-galactosyltransferase knockout mice following the administration of tick saliva via both injections and bites. To delve deeper into the molecular intricacies underpinning the endogenous synthesis of α-Gal in ticks, the researchers successfully identified three α-galactosyltransferase

genes within the tick species *I. scapularis* genome. Each noteworthy finding collectively provides substantial evidence to support the notion that tick-borne α-Gal is a potent trigger for allergy development, thus further solidifying claims (91).

Here add a topic sentence. The tick-borne pathogen *A. phagocytophilum* has been found to induce an increase in α-Gal levels within the cells of infected ticks, as reported in various studies. Furthermore, certain bacteria belonging to the *Enterobacteriaceae*, *Rizobiaceae*, and *Caulobacteriaceae* families possess the α-1.3-GT enzyme (92,93). Interestingly, similar bacteria from these families and groups have also been identified within the tick salivary microbiome. Thus, exploring the potential impact of bacterial presence within ticks and its association with α-Gal would be highly intriguing (94).

Host and Tick Factors in the Development of AGS

Information regarding the host factors contributing to AGS development is limited in scope. Although there have been studies documenting the presence of high levels of anti-α-Gal IgE antibodies, it has been observed that some individuals fail to manifest AGS symptoms (95,96). Based on the available body of evidence, two primary categories of factors have been implicated in accounting for this variability within the host population: a) genetic factors associated with the host, such as blood type and atopy, and b) associated factors encompassing the host's microbiome, dietary patterns, and medication usage. These multifactorial elements could contribute to the observed variation in AGS manifestation. Additionally, individuals who exhibit hypersensitivity to specific chemotherapeutic agents like cetuximab and drugs containing gelatin, as well as those with a history of idiopathic anaphylaxis and systemic mastocytosis, have been identified as being more prone to developing mammalian meat allergy after a tick bite (58,76). In addition, individuals who have previously undergone procedures involving organ and tissue transplantation, such as bovine or porcine bioprosthetic heart valves, may also be at increased risk of developing AGS (97). Apart from the factors mentioned, individuals in certain occupational groups, such as forestry workers, rural workers and individuals whose working life is in open areas, were associated with a high rate of α-Gal IgE sensitisation (98).

A comprehensive investigation in Spain unveiled that the titres of α-Gal IgE among individuals engaged in forestry-related activities and those employed in the forestry sector were notably greater when compared to the general population serving as the control group. It has been established that individuals with occupational exposure to outdoor environments or those residing in rural regions face an elevated vulnerability towards acquiring sensitisation to α-Gal, primarily due to the heightened probability of being subjected to tick bites originating from ticks closely associated with their habitats (98,99).

Several investigations have documented variations in the immune response against α-Gal among individuals with different blood types. For instance, a study conducted in Sweden revealed that individuals with B-negative blood type exhibited a higher prevalence of α-Gal allergy than those with other blood types (84). Interestingly, B-positive individuals demonstrated the presence of a-Gal-specific antibodies, whereas B-negative individuals show cased antibodies that exhibited cross-reactivity with the B antigen. Some scientific studies have indicated that genetic predisposition or atopy may play an essential role in the development of food allergies (17). Individuals with atopy typically demonstrate a pronounced Type I hypersensitivity in their immune responses, characterised by excessive production of IgE in response to common allergens such as mites and food (100). Several studies have indicated a potential connection between atopy and the presence of anti-α-Gal IgE antibodies (68,79). The levels of anti-α-Gal IgE are elevated in individuals with increased total IgE, suggesting that atopy may be a significant predisposing factor in AGS development (22). However, another study found no correlation between AGS and previous atopic tendencies (101). In addition, the presence of a prior occurrence of atopic disease is inadequate to establish AGS (102). Patients diagnosed with AGS may be evaluated to determine their likelihood of developing additional allergic conditions, such as conventional food protein allergies (89).

By utilising a wide range of research studies conducted within the discipline, one can identify numerous intricate factors that are inherently connected with ticks and have the potential to impact the development of AGS. These factors can be neatly classified into two distinct groups, namely, intrinsic factors and extrinsic factors. Intrinsic factors, which are fundamental components existing inside ticks, involve the intricate interaction between the tick microbiome, denoting the varied population of microorganisms residing in the tick, and the tick glycosylation mechanism, relating to the mechanism through which glycosylation, or the attachment of sugar molecules, that takes place within the tick's organism. The tick microbiome plays a crucial role in various physiological processes within the tick, influencing its overall physiology and ability to transmit pathogens. In contrast, the tick glycosylation mechanism modifies proteins and other molecules essential for tick survival and reproduction. The significance of tick intrinsic factors holds paramount importance in understanding AGS development. Current research suggests that extrinsic and intrinsic factors contribute to the distinct N-glycan patterns observed in *Ixodes scapularis* and *Amblyomma americanum* ticks (91,103). Intrinsic factors within the tick are thought to be responsible for synthesising or recycling α-Gal, potentially sensitising the host to this antigen during blood feeding (104).

The feeding process begins with the tick penetrating the host's skin using its barbed mouthparts, followed by attachment and continuous secretion of saliva rich in antigens (105). Blood acquisition by the tick's mouthparts disrupts the integrity of the skin barrier, causing trauma and potentially facilitating the introduction of tick-borne microbes. The composition of the tick microbiota is believed to play a critical role in the context of AGS (106). The microbiota within ticks plays a crucial role in the context of AGS. Microbiota-derived galactose is an essential energy molecule and a pivotal component for synthesising glycosylated exopolysaccharides or lipopolysaccharides (LPS), which may act as α-gal antigens. These findings underscore the significance of investigating the involvement of tick microbiota in AGS, as they may play a role in modulating ticks' metabolic activities and glycosylation mechanisms (104).

Human Immune System and AGS

Ticks pose a growing threat to human and animal health globally with the many organisms they carry. Notably, some animal species exhibit acquired tick resistance (ATR) following exposure to tick infestations. This resistance has been associated with a tick-specific IgE response. For instance, ATR is related

to allergic density, which impedes tick feeding and potentially confers resistance to tick-borne tularemia. Moreover, it is worth noting that human tick infestations have been closely associated with AGS, characterised by an IgE-mediated allergic reaction to the α-Gal carbohydrate. This particular glycan can be found in tick salivary proteins and on the surface of tick-borne pathogens responsible for causing Lyme disease and granulocytic anaplasmosis. It is essential to highlight that although most individuals sensitised to α-Gal develop specific IgE antibodies, only a subset of these individuals progress to AGS, indicating the complexity and variability of the immune response to this particular allergen (107).

The tick-host interface is a complex battlefield. A host-directed hemostatic response is initiated when the tick damages the host's skin with its spiny hypostome, disrupting the epithelial barrier (108) Haemostasis is the host's natural protective mechanism triggered in response to physical harm. It encompasses blood coagulation, platelet aggregation, and vasoconstriction (105,109). During the initial stage of tick attachment to the skin, the humoral and cellular components of the host's natural immune system react by activating the complement system, inducing inflammation, and facilitating the infiltration of leukocytes into the area of the tick bite (110). Following a tick bite, keratinocytes, endothelial cells and leucocytes are triggered by tick saliva and hypostome exposure (111). These cells unleash the secretion of antimicrobial peptides, pro-inflammatory chemokines, and cytokines, such as interleukin-8 (IL-8), interleukin-1b (IL-1b), and tumour necrosis factor (TNF), to facilitate the recruitment of an assortment of inflammatory cells, including neutrophils. Consequently, the adaptive immune system undergoes a division, with activated T and B-cells (in the event of secondary invasion) intensifying the inflammatory response via cytokine release and generating targeted antibodies against the tick. This, in turn, induces the further activation of the complement system and sensitises mast and basophil cells (109,111).

Choudhary and colleagues conducted research utilising the α-Gal knockout mouse model to investigate how tick bites elicit an immune response targeting anti-α-Gal IgE antibodies (112). Their analysis of these genetically modified mice showed that exposure to tick saliva led to the generation of IgE antibodies directed explicitly against α-Gal, consequently leading to hypersensitivity reactions upon consumption of mammalian meat (56,110,112). These studies reveal the critical role of tick saliva in developing α-Gal allergy. Tick saliva encompasses a multifaceted array of compounds, a considerable proportion possessing immunomodulatory characteristics capable of dampening host immune responses. This initiates wound-healing mechanisms in the host (109). The components present in insect saliva have been shown to stimulate the activation of T-cells towards the Th2 phenotype, which causes reactions (94). While some studies propose that tick saliva possesses immunomodulatory properties that promote Th2 polarisation, most individuals exposed to bites from blood-feeding insects experience only transient, localised IgE-mediated hypersensitivity reactions (95).

In order to ensure a constant blood flow without causing an immune reaction in the host, the tick secretes a complex combination of substances that relieve pain and itching in the host during the feeding process. This mixture includes agents that inhibit vasodilation, platelet aggregation and molecules that inhibit the cascade process of blood clotting (113,114). Additionally, ticks secrete diverse salivary compounds that reduce pro-inflammatory cytokine production, including TNF-α, interleukin-12, and IL-1β. Concomitant with activating immune cells, the tick bite also induces the synthesis of anti-inflammatory molecules, such as interleukin-10 (IL-10) and transforming growth factor-beta (TGF-β). This shift towards a Th2-dominant immune response is believed to be critical for developing AGS. Disruption of the skin epithelium by the tick bite triggers wound healing processes, where M2 macrophages play a crucial role. These macrophages suppress inflammation and potentially attenuate the excessive Th1 cell response by upregulating cytokines that reduce inflammation and oedema, such as IL-10 and TGF-β (111). Furthermore, various constituents within tick saliva, including prostaglandins, sphingomyelinase, and cysteine protease inhibitors, have been documented as crucial elements in modulating the innate immune response by promoting the induction of a TH2 profile (115-118).

Tick saliva is enriched with prostaglandin E2 (PGE2), and this molecule contributes to various mechanisms of AGS development. Prostaglandin E2 promotes vasodilatation, facilitates blood flow during feeding, and reduces inflammation at the bite site (119). However, this anti-inflammatory effect could help healing by hindering fibroblast migration. Additionally, PGE2 stimulates the recruitment and activation of macrophages, which can further amplify PGE2 production, creating a positive feedback loop. Studies show that this PGE2-mediated modulation of the immune response shifts it towards a Th2 phenotype characterised by B-cell proliferation and increased antibody production (120).

Cabezas-Cruz et al. (56) documented that tick saliva elicits reactions resembling those of a venom antigen, engaging with the immune system and instigating immune sensitisation. The first encounter between the salivary antigen secreted by the tick and host immune cells occurs in the skin epithelium during a tick bite. Following tick bite exposure, antigen-presenting cells (APCs) within the skin, such as Langerhans cells (LCs) and dendritic cells (DCs), play a critical role in initiating the immune response to α-Gal. These APCs recognise, capture, and process α-Gal antigens from tick saliva. Subsequently, they migrate to the lymph nodes, where they participate in the sensitisation of B-cells. These sensitised B-cells then present processed α-Gal antigens to T-cells, releasing pro-inflammatory cytokines and eventually activating mast cells and basophils, critical effector cells in allergic reactions (Figure 2) (112,121).

Various human cells are involved during the development of AGS and the allergic response. In the early sensitisation phase, skin-resident antigen-presenting cells are of vital importance. DCs connect the innate and adaptive immune systems. The cells possess unique receptors for the innate immune response and act as cells that present antigens, facilitating the initiation of the adaptive immune response. In the skin and mucosal tissues, immature DCs detect antigens (122). Dendritic cells undergo maturation and subsequently migrate to the draining regional lymph nodes by simultaneously activating receptors that recognise patterns. In the given context, dendritic cells play a critical role in presenting processed antigens to T-cells, which occurs within the groove of major histocompatibility complex (MHC) I or MHC II molecules. This process initiates a subsequent adaptive immune response (122). The primary function of these cells revolves around the processing of antigens bound to α-Gal and introduced into the body through tick injections (123).

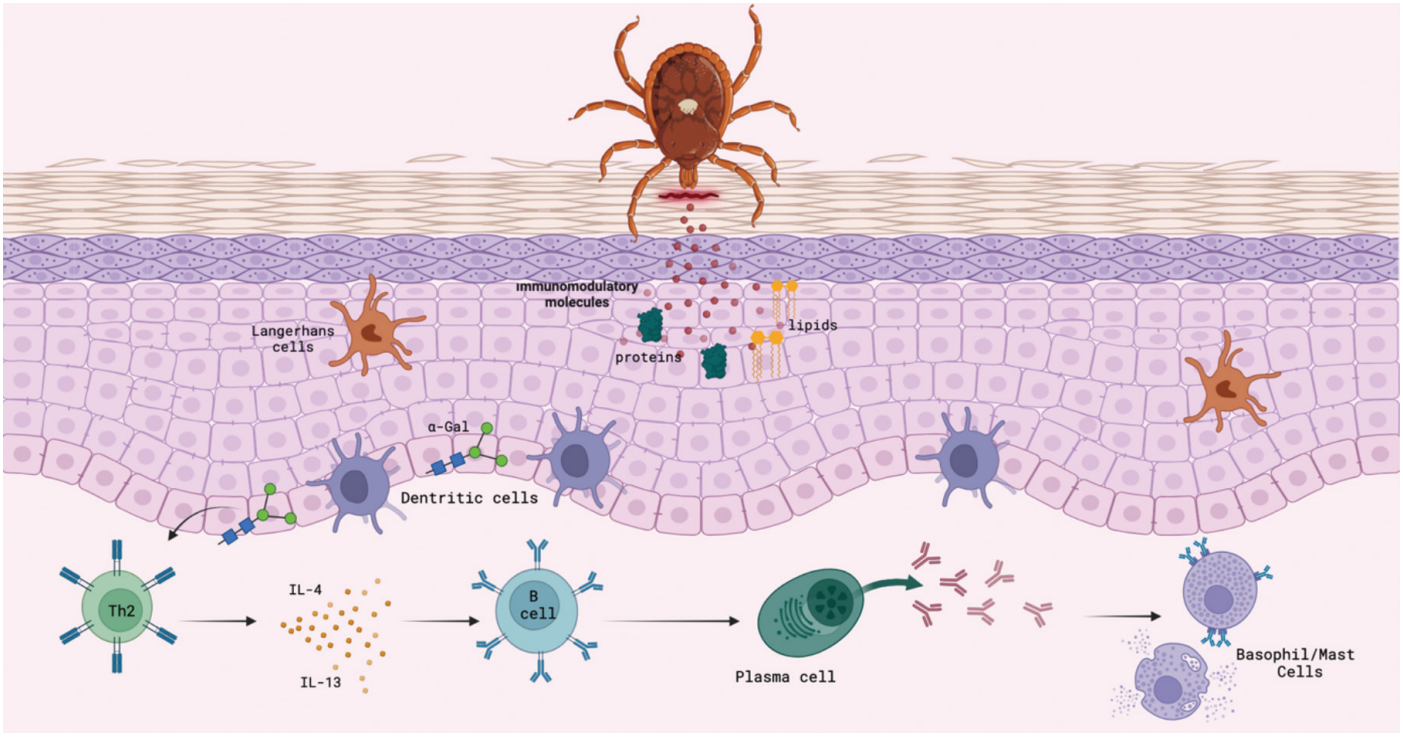

Figure 2. Sensitisation phase of AGS. During feeding, tick mouthparts cause physical trauma to the skin epithelial barrier and introduce α-Gal, potentially pathogenic bacteria and adjuvants in tick saliva. Antigen-presenting cells (APCs), specifically Langerhans cells located in the epidermis and dermal dendritic cells residing in the dermis, exhibit reactivity towards antigens secreted by ticks. These antigens encompass glycoproteins, glycolipids, and tick cement containing α-Gal moieties. In a pro-inflammatory Th2 microenvironment, skin-resident APCs internalise α-Gal and present it to naïve CD4+ T-cells, prompting their differentiation into Th2. The Th2 subset, specific to α-Gal, induces B-cell activation, facilitating their class switch to produce anti-α-Gal-specific immunoglobulin E (IgE). This immunoglobulin variant contributes to the generation of plasma cells. After synthesis, anti-α-Gal IgE binds to high-affinity IgE receptors (FcεRI), expressed on mast cells and basophils

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The saliva of the *I. ricinus* tick, which has been linked to the development of α-Gal syndrome, can impede the maturation and movement of specialised APCs, as indicated by recent research (124). These dendritic cells play a crucial role in initiating allergic reactions to other protein allergens, as they are responsible for presenting the allergens to T-cells and creating an environment that promotes the activation of pro-allergic Th2 cells. Therefore, *I. ricinus* tick saliva may disrupt this process and hinder the progression of allergic reactions (124). Dendritic cells exposed to the saliva of the *I. ricinus* tick species effectively inhibit their ability to elicit pro-inflammatory Th1 or Th17 responses, instead favouring the promotion of Th2 pro-allergic responses. Additionally, it is noteworthy that the presence of α-Gal on the glycoprotein may enhance the efficiency of antigen internalisation by dendritic cells (125).

Basophils, categorised as granulocytes circulating in the bloodstream, are similar to mast cells expressing the IgE receptor FceRI. When activated, these cells degranulate, leading to the release of histamine and various other mediators. It should be noted that basophils are important in chronic allergic inflammation and contribute to the development of protective immunity against parasites, as proven in the scientific literature (126). Within the specific immune response to tick infestation, it has been conclusively established that basophils mobilise to the tick-feeding site during subsequent infestations. Basophils accumulate in the skin and play an essential role as tick rejection factors in tick infestation. Given these findings, it is hypothesised that basophils may trigger the allergic response following exposure to α-Gal, an allergen of particular interest (127). Basophils secreting interleukin-4 (IL-4) are essential in allergic sensitisation and initiating Th2 immune responses. Moreover, they enable the differentiation of CD4+ T-cells into Th2 cells. This ability of basophils suggests that they could potentially serve as key players in the complex network of events that result in the development of an allergic reaction and subsequent activation of Th2 cells (128).

Given that the α-Gal epitope might also be present in glycolipids, it is conceivable that lipids containing α-Gal could also exist in tick saliva. In such a scenario, natural killer T-cells (iNKT), a subset of T-cells, contribute to the sensitisation process to α-Gal. iNKT cells can recognise lipids and generate IL-4 (129). In one study, patients with α-Gal allergy exhibited a 2.5-fold increase in circulating CD69+ iNKT cells. Consequently, it was observed that circulating iNKT cells displayed heightened cellular proliferation in individuals with α-Gal allergy (130).

AGS Diagnosis and Prevention

The diagnosis of AGS is often distinguished from typical food allergies by the delay in the onset of symptoms after mammalian meat is consumed. Nonetheless, the time at which symptoms

commence heavily relies on the source of the allergen (offals have a greater potency than muscle meat) and numerous modifying factors (e.g., alcohol and exercise) that abbreviate the time preceding reactions. The allergic reactions triggered by tick bites and the distinct manifestation of AGS render the diagnosis intricate and demanding. Consequently, a comprehensive patient history encompassing all clinical facets should be considered before conducting laboratory tests (71,131).

Determining the preliminary diagnosis entails conducting skin prick tests (SPT) and ascertaining the presence of serum-specific IgE antibodies (132). Employing α-Gal containing extracts to expose the patient's skin is a frequently utilised diagnostic method; however, significant variations in the sensitivity of skin tests have been documented. SPTs, particularly those employing commercially available meat extracts, are unreliable, often yielding feeble or false-negative outcomes, thus potentially misleading patients (17). Furthermore, SPT with local meat and meat products frequently gives false negative or only weak skin reactions. The subcutaneous injection of freshly prepared pig or bovine kidney extracts, specifically through intradermal testing, has demonstrated heightened sensitivity compared to consuming cooked or raw muscle meat derived from the same animal species (17,71).

The gold standard for the diagnosis of food allergies is still recognised as food testing. However, the delayed allergic reaction seen in AGS has rendered this test inadvisable. The use of food testing to diagnose AGS carries the risk of causing severe and potentially fatal anaphylactic reactions. Instead, the only recommended strategy to prevent recurrent episodes of allergic reactions in patients with AGS is to avoid foods, supplements and medications containing α-Gal. By strictly adhering to this avoidance strategy, patients can significantly reduce their risk of experiencing allergic reactions associated with AGS (133,134)

CONCLUSION

The hypersensitivity responses mediated by IgE antibodies against the glycan α-Gal, as opposed to specific food proteins, present numerous challenges and are currently reshaping our understanding of the underlying mechanisms that govern the pathogenesis of food allergies. The intricate and particular mechanisms through which tick bites sensitise individuals to α-Gal, ultimately leading to the onset of AGS, remains insufficiently elucidated, thus necessitating further investigation and research. The triggering response that leads to AGS is the IgE antibody response to α-Gal; however, the specific molecules and immune mechanisms that orchestrate this phenomenon have yet to be fully identified. Comprehensive and detailed characterisation of these molecules and mechanisms is crucial. It may improve the accuracy and efficiency of AGS diagnosis and enable the development of preventive and therapeutic strategies to manage and control this disease effectively.

The confirmation of the α-Gal epitope's presence in various species of ticks has provided valuable insights into the molecular nature of these organisms. However, much is still to be discovered about the intricate processes involved in these molecules' synthesis, origin, and transduction at the tick-host interface, which warrants further investigation. Moreover, a significant knowledge gap persists in understanding how the tick microbiome influences AGS development. In order to bridge this gap, it is imperative to conduct a comparative analysis of the microbiomes found in different tick species and explore their underlying genetic mechanisms using genomic and transcriptomic approaches. Extensive research using omics technologies could potentially uncover novel genes that play an essential role in synthesising the α-Gal epitope, thus improving our understanding of AGS and its consequences.

The occurrence of AGS has become increasingly common in various regions across the globe, such as America, Asia, Europe, and Australia, where ticks are abundant. It is worth noting that the spread of ticks in these areas is greatly influenced by climate change. Furthermore, the accelerated expansion of tick populations due to climate change is expected to contribute to a rapid escalation in the prevalence of AGS. Additional research is essential to a comprehensive understanding of the epidemiology, incidence, geographical distribution, and risk factors associated with AGS. These investigations should focus on examining the population and cohorts frequently exposed to tick environments to shed light on various aspects of this syndrome.

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