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# Türkiye PARAZİTOLOJİ Dergisi

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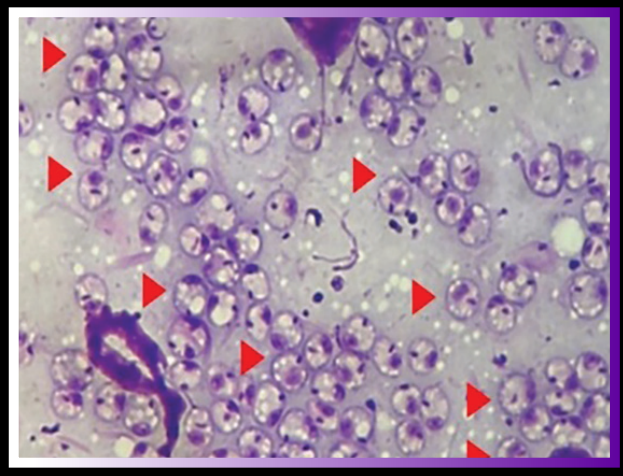

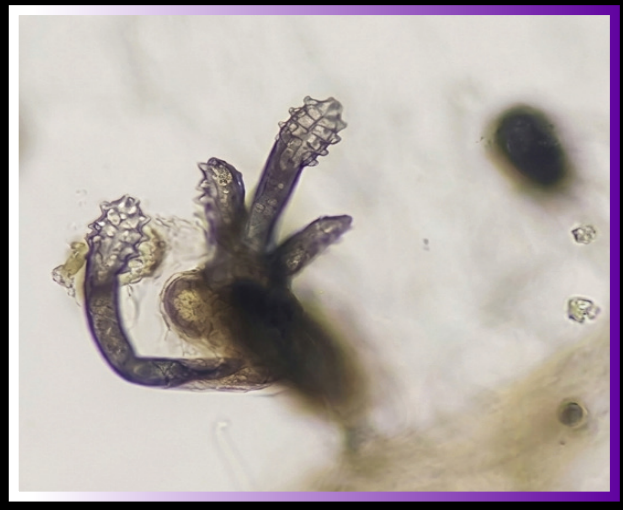

### Original Investigations / Özgün Araştırmalar



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## EDİTÖRDEN

2026 yılının üçüncü sayısını biri yurtdışından olmak üzere 5 özgün araştırma makalesi ile çıkarmaktayız. Özgün çalışmalar arasında, ülkemizdeki en önemli şark çıbanı etkeni *Leishmania tropica*'nın faredeki enfeksiyon dinamikleri ve farklı kültürlerdeki üretilmelerinin enfektivite üzerine etkilerinin incelendiği bir çalışma ile yine aynı parazite ait kurtarma test protokolünün optimizasyonunu içeren ikinci bir çalışma yer almaktadır. Farklı bir grupta, beta talassemi majör hastası çocuklarda toxoplasmosis seroprevalansının incelendiği bir çalışma, maske kullanan kişilerdeki *Demodex* sıklık durumunun ortaya konulduğu bir çalışma ve son yıllarda artan vaka sayıları ile kamuoyunda da dile getirilen uyuz hastalığı için cildiye uzmanlarının görüşlerinin yer aldığı bir çalışma da bu sayımızda yer almaktadır.

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

SCI/SCI-Expanded kapsamında olan dergilerde yapacağınız yayınlarda dergimizde yer alan makalelere atıf yapılmasının, dergimizin bu endekse başvuru/kabul sürecinde büyük önem taşıdığını yeniden belirtmek isterim. Bilim alanımızın en önemli unsurlarından ve bizleri güçlendiren araçlarından biri olan "Türkiye Parazitoloji Dergisi"nin bu sayısının da bilimsel çalışmalarınıza ve birikimlerinize yararlı olmasını umuyorum.

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Türkiye PARAZİTOLOJİ Dergisi

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# Infection Dynamics of a *Leishmania tropica* Isolate in the BALB/c Mouse Model: Effects of Pre-infection Culture Conditions on Experimental Infectivity

## *Leishmania tropica İzolatının BALB/c Fare Modelinde Enfeksiyon Dinamikleri: Enfeksiyon Öncesi Kültür Koşullarının Deneysel Enfektivite Üzerine Etkisi*

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## ABSTRACT

**Objective:** To evaluate the effects of distinct pre-infection culture conditions [RPMI-1640 and Schneider's insect medium (SIM)] on the experimental infectivity of a clinical *Leishmania tropica* isolate and to determine how culture-associated differences are reflected in infection dynamics in the BALB/c mouse model.

**Methods:** A clinical *Leishmania* isolate, obtained from a patient with cutaneous leishmaniasis and cryopreserved in liquid nitrogen, was recovered in NNN medium. Species identification was performed using an internal transcribed spacer 1-targeted probe-based real-time polymerase chain reaction followed by melting-curve analysis. Following post-thaw recovery, logarithmic-phase promastigotes were transferred to RPMI-1640 or SIM and maintained for five days without serial subpassaging before experimental infection. BALB/c mice (n=5 per group) were subcutaneously inoculated in the right hind footpad with $1\times10^7$ promastigotes. Infection dynamics were evaluated over six weeks by monitoring footpad diameter, amastigote burden, and promastigote recovery.

**Results:** Molecular analysis identified the clinical isolate as *L. tropica* (MHOM/TR/2025/CBU152). Mice infected with SIM-derived promastigotes developed visible lesions from week 2. At the same time, longitudinal footpad measurements differed significantly between the SIM and RPMI groups from week 1 onward (Sidak-adjusted $p<0.0001$ at each time point). By week 6, the mean absolute footpad diameter reached 4.22 mm in the SIM group, compared with 3.52 mm in the RPMI group. Amastigote burden was significantly higher in the SIM group than in the RPMI group [median (interquartile range [IQR]): 6 (5-6) vs. 2 (2-2); p=0.0079]. Promastigote recovery on day 7 was also significantly higher in the SIM group [median (IQR): 1.15 (1.12-1.18) vs. 0.15 (0.14-0.16)$\times10^6$/mL; p=0.0079]. No evidence of infection was observed in the control group.

**Conclusion:** Pre-infection culture conditions significantly influence the experimental infectivity of an *L. tropica* isolate in BALB/c mice. SIM-derived promastigotes exhibited a more infectious phenotype than RPMI-derived promastigotes, supporting SIM preconditioning as a useful approach for establishing experimental *L. tropica* infection in BALB/c mice.

**Keywords:** BALB/c mouse model, cutaneous leishmaniasis; *Leishmania tropica*, experimental infectivity, Schneider's insect medium

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## ÖZ

**Amaç:** Bu çalışmada, farklı enfeksiyon öncesi kültür koşullarının [RPMI-1640 ve Schneider's insect medium (SIM)] klinik bir *Leishmania tropica* izolatının deneysel enfektivitesi üzerindeki etkisinin değerlendirilmesi ve kültür koşullarıyla ilişkili farklılıkların BALB/c fare modelindeki enfeksiyon dinamiklerine nasıl yansıdığının belirlenmesi amaçlanmıştır.

**Yöntemler:** Kutanöz leishmaniasisli bir hastadan elde edilerek sıvı azotta kriyoprezerve edilen klinik *Leishmania* izolatı NNN besiyerinde canlandırılmıştır. Tür tanımlaması, transkripsiyonlar arası bölge 1 bölgesini hedefleyen prob tabanlı gerçek zamanlı polimeraz zincir reaksiyonu ve ardından erime eğrisi analizi kullanılarak gerçekleştirilmiştir. Kriyoprezervasyon sonrası canlandırılan logaritmik fazdaki promastigotlar RPMI-1640 veya SIM besiyerine aktarılmış ve deneysel enfeksiyon öncesinde seri alt pasaj yapılmaksızın beş gün süreyle kültüre edilmiştir. BALB/c farelere (her grupta n=5) sağ arka ayak tabanından subkütan yolla $1\times10^7$ promastigot inoküle edilmiştir. Enfeksiyon dinamikleri; ayak tabanı çapı, amastigot yükü ve promastigot geri kazanımı değerlendirilerek altı hafta boyunca izlenmiştir.

**Bulgular:** Moleküler analiz sonucunda klinik izolat *L. tropica* (MHOM/TR/2025/CBU152) olarak tanımlanmıştır. SIM kaynaklı promastigotlarla enfekte edilen farelerde gözle görülür lezyon gelişiminin 2. haftadan itibaren ortaya çıktığı belirlenmiştir. Longitudinal ayak tabanı ölçümlerinin ise SIM ve RPMI grupları arasında 1. haftadan itibaren anlamlı farklılık gösterdiği saptanmıştır (her bir zaman noktası için Sidak-düzeltilmiş p<0,0001). Altıncı haftada ortalama mutlak ayak tabanı çapı SIM grubunda 4,22 mm, RPMI grubunda ise 3,52 mm olarak belirlenmiştir. Amastigot yükünün SIM grubunda RPMI grubuna kıyasla anlamlı düzeyde daha yüksek olduğu saptanmıştır [medyan (çeyrekler arası aralık [ÇAA]): 6 (5-6) ve 2 (2-2); p=0,0079]. Yedinci gündeki promastigot geri kazanımının da SIM grubunda anlamlı düzeyde daha yüksek olduğu belirlenmiştir [medyan (ÇAA): 1,15 (1,12-1,18) ve 0,15 (0,14-0,16) $\times10^6$/mL; p=0,0079]. Kontrol grubunda enfeksiyon bulgusuna rastlanmamıştır.

**Sonuç:** Enfeksiyon öncesi kültür koşulları, bir *L. tropica* izolatının BALB/c farelerindeki deneysel enfektivitesini anlamlı düzeyde etkilemektedir. SIM kaynaklı promastigotlar, RPMI kaynaklı promastigotlara kıyasla daha enfektif bir fenotip sergilemiş ve bu bulgular, SIM'de enfeksiyon öncesi kültürlemenin BALB/c farelerinde deneysel *L. tropica* enfeksiyonunun oluşturulmasında yararlı bir yaklaşım olduğunu desteklemiştir.


**Anahtar Kelimeler:** BALB/c fare modeli, kutanöz leishmaniasis, *Leishmania tropica*, deneysel enfektivite, Schneider's insect medium

## INTRODUCTION

Cutaneous leishmaniasis (CL) is a major vector-borne disease caused by protozoan parasites of the genus *Leishmania* and transmitted by infected female sand flies (*Phlebotomus* spp. in the Old World) (1). Among the Old World species, *Leishmania tropica* is the principal cause of anthroponotic CL across the Middle East, North Africa, Central Asia, and the Mediterranean basin. Its persistent and often disfiguring skin lesions contribute substantially to chronic morbidity, while current treatments remain limited by toxicity, variable efficacy, cost, and emerging drug resistance (2).

The infectivity of *Leishmania* is determined largely by metacyclogenesis, the differentiation of non-infective procyclic promastigotes into infective metacyclic forms within the sand fly vector. This process is accompanied by morphological, biochemical, and molecular changes that enhance parasite survival and establishment in the mammalian host (3). Under *in vitro* conditions, promastigote differentiation is influenced by multiple culture-associated factors, including medium composition, pH, nutrient availability, serum supplementation, parasite density, growth phase, and passage history (4-6). Consequently, the biological state of culture-derived promastigotes represents an important experimental variable in animal infection studies.

This concept is particularly relevant for *L. tropica,* for which reproducible infection in conventional murine models has historically been more difficult to achieve than in the classical *L. major*-BALB/c model. Rather than producing rapidly progressive lesions, experimental *L. tropica* infection typically follows a chronic, slowly developing, and predominantly non-ulcerative course. Disease outcome is strongly influenced by parasite strain, inoculum dose, developmental stage, route of inoculation, and host-related factors (7,8). Consistent with this variability, a previous study using a human-derived *L. tropica* isolate from Türkiye reported footpad lesions in only four of ten BALB/c mice following subcutaneous inoculation of $2\times10^7$ culture-derived promastigotes, with lesions appearing approximately 45 days after infection (9).

Pre-infection culture conditions may contribute to this variability by altering the developmental composition and infectivity-related phenotype of promastigote populations. RPMI-1640 is widely used for routine cultivation of *Leishmania* promastigotes, whereas Schneider's insect medium (SIM) provides a physicochemically distinct environment, originally developed for insect cell culture. Experimental studies have shown that manipulation of culture medium and extracellular pH can enhance metacyclic differentiation and modify infectivity-related phenotypes in several *Leishmania* species, although these effects remain species- and condition-dependent (4-6). More recently, optimized acidic SIM was shown to increase metacyclic promastigote production and enhance complement resistance and macrophage infectivity in *Leishmania (Mundinia) orientalis* (4). However, whether similar culture-dependent effects influence the experimental infectivity of clinical *L. tropica* isolates has not been established.

This study aimed to compare the experimental infectivity of a clinical *L. tropica* isolate after pre-infection cultivation in RPMI-1640 versus SIM, and to determine whether these culture-associated differences are reflected in infection dynamics in BALB/c mice, as assessed by longitudinal footpad changes, tissue amastigote burden, and promastigote recovery.

## METHODS

### *Ethical Approval*

This study was approved by the Local Ethics Committee for Animal Experiments, Faculty of Medicine, Manisa Celal Bayar University (date: 29.07.2025, decision no: 77.637.435/330). Written informed consent was obtained from the patient at the time of the original clinical sample collection. The archived parasite isolate used in the present study was de-identified, and no identifiable patient information was accessed.

### *Preparation of Culture Media*

**(I) NNN Medium:** To prepare NNN medium, 5 g of agar, 2 g of peptone, and 1 g of NaCl were dissolved in 200 mL of distilled water, sterilized by autoclaving at 121 °C for 20 minutes, and

subsequently cooled to room temperature. The solidified base was enriched with 1% gentamicin, 1% penicillin/streptomycin, and 30 mL of defibrinated rabbit blood, then dispensed into sterile slanted tubes and stored at 4 °C for at least 48 hours to allow proper conditioning.

**(II) RPMI-1640 Medium:** RPMI-1640 was supplemented with 10% fetal calf serum (FCS), 1% penicillin/streptomycin, and 1% gentamicin. The pH was monitored using a calibrated pH meter and adjusted to 7.2 by the gradual addition of 1 M HCl or 1 M NaOH, as required. The medium was stored at 4 °C and brought to room temperature before use.

**(III) SIM:** SIM was was prepared according to the manufacturer's instructions and supplemented with 20% FCS, 1% L-glutamine, and 1% penicillin/streptomycin. The pH was monitored using a calibrated pH meter and adjusted to 5.5 by gradual addition of 1 M HCl or 1 M NaOH, as required. The medium was subsequently sterilized through a 0.22-µm membrane filter, stored at 4 °C, and equilibrated to room temperature before inoculation.

### *In vitro Maintenance of the Clinical Leishmania Isolate*

The clinical *Leishmania* isolate, originally obtained from a cutaneous lesion on the right cheek of a patient with CL in Türkiye and cryopreserved in the Parasite Bank of the Faculty of Medicine at Manisa Celal Bayar University, was retrieved from liquid nitrogen and rapidly thawed in a 37 °C water bath. Following thawing, the promastigotes were immediately transferred onto freshly prepared NNN medium and cultured at 26 °C to allow initial recovery and growth. Promastigote growth was assessed by routine light microscopy, and motile promastigotes in the logarithmic growth phase were confirmed through evaluations performed every 2-3 days (10). No serial passaging was performed in NNN medium. Logarithmic-phase promastigotes obtained from this single post-thaw recovery culture were used directly in the subsequent experimental preconditioning procedure.

### *Molecular Identification of the Clinical Leishmania Isolate*

Molecular species identification of the clinical *Leishmania* isolate was performed using internal transcribed spacer 1 (ITS1) -targeted probe-based real-time polymerase chain reaction (PCR) followed by melting-curve analysis. Briefly, genomic DNA was prepared using the Template Preparation Kit (Roche, Germany) according to the manufacturer's instructions. Molecular identification was performed using a probe-based real-time PCR assay targeting the ITS1 region located between the genes encoding SSU rRNA and 5.8S rRNA, using specific primers and probes as previously described (11). Melting-curve analysis was performed using a Rotor-Gene real-time PCR system. Four reference strains, *L. tropica* (MHOM/AZ/1974/SAF-K27), *L. major* (MHOM/SU/1973/5ASKH), *L. infantum* (MHOM/TN/1980/IPT1), and *L. donovani* (MHOM/IN/1980/DD8), were included as positive controls for species identification.

### *In vitro Preconditioning of Promastigotes for Experimental Infection*

To evaluate whether pre-infection culture conditions influence subsequent experimental infectivity, logarithmic-phase promastigotes of the clinical *Leishmania* isolate, which was initially recovered in NNN medium, were transferred to either RPMI-1640 supplemented with 10% FCS or SIM supplemented with 20% FCS. Cultures were incubated at 26 °C for five days, during which parasite proliferation, motility, and morphological integrity were evaluated daily by light microscopy (12). During this five-day preconditioning period, no further subpassaging was performed in either SIM or RPMI-1640. Following the five-day culture period, stationary-phase promastigotes from the SIM and RPMI-1640 cultures were harvested separately and washed twice with sterile phosphate-buffered saline (PBS) by centrifugation at 1,000× g for 10 min. After each centrifugation step, the supernatant was carefully removed. Following the final wash, the parasite pellets were resuspended in sterile physiological saline. Promastigotes were counted using a hemocytometer, and the parasite suspensions were adjusted to a final concentration of $1\times10^8$ promastigotes/mL. The same harvesting, washing, counting, and resuspension procedure was applied to both experimental groups to standardize inoculum preparation and minimize carryover of residual culture medium and serum components.

### *Experimental Infection of BALB/c Mice with the Clinical Leishmania Isolate*

Male BALB/c mice (6-8 weeks old; n=15) were randomly allocated to three experimental groups (SIM, RPMI, and control; n=5 per group) using a simple lottery method based on coded lots assigned to individually identified animals. The SIM and RPMI groups received promastigotes of the clinical *Leishmania* isolate that had been previously cultured in SIM (pH 5.5; 20% FCS) and RPMI-1640 (pH 7.2; 10% FCS), respectively. These media represented two complete pre-infection culture conditions. The individual effects of pH, serum concentration, and basal medium composition were not evaluated independently. Before inoculation, promastigotes from both cultures were washed twice with sterile PBS and resuspended in sterile physiological saline. Each mouse was inoculated subcutaneously in the plantar surface of the right hind footpad with 100 µL of the standardized suspension ($1\times10^8$ promastigotes/mL), corresponding to $1\times10^7$ promastigotes per mouse. Control mice received 100 µL of sterile physiological saline. Animals were monitored for six weeks post-infection under standardized housing and welfare conditions (13).

### *Evaluation of Experimental Infection Parameters*

### *Footpad Diameter Measurement*

The absolute diameter of the inoculated right hind footpad was measured weekly with a digital caliper and recorded in millimeters (mm) to monitor changes over time. Measurements represent the absolute footpad diameter and were not calculated by subtracting the contralateral footpad measurement (14). Each mouse was treated as an independent biological replicate, and group means and standard deviations (SD) were calculated directly from the individual animal-level measurements (n=5 per group) at each time point. No technical replicate measurements were treated as independent observations. To minimize measurement bias, weekly footpad diameter measurements were performed using a digital caliper by an observer blinded to the experimental group allocation.

### Assessment of Amastigote Burden in Touch Preparations

At week six, animals were euthanized and infected tissue samples were collected. Touch preparations were obtained by gently pressing tissue sections onto clean glass slides, followed by air-drying and staining with 10% Giemsa (pH 7.2) for 30 min. Amastigote burden was assessed using a semi-quantitative scoring system. Smears were examined under oil immersion (1000× magnification), and parasite burden was classified according to the following criteria: 6+ (>100 parasites per field), 5+ (10-100 parasites per field), 4+ (1-10 parasites per field), 3+ (1-10 parasites per 10 fields), 2+ (1-10 parasites per 100 fields), 1+ (1-10 parasites per 1000 fields), and 0 (no parasites observed in 1000 fields) (15). To minimize observer bias, microscopic evaluation and semi-quantitative amastigote scoring were performed by an observer blinded to the experimental group allocation.

### Assessment of Promastigote Recovery in NNN Medium

Additional tissue fragments were aseptically minced and inoculated into NNN medium. Cultures were incubated at 26±1 °C, and promastigote recovery was quantitatively assessed on day 7 post-culture. Approximately 0.5 mL of the liquid phase was withdrawn from each NNN culture. To enhance detection sensitivity, the collected samples were centrifuged, and the resulting pellet was resuspended in 0.2 mL RPMI to concentrate the parasites. Promastigotes were counted using a Thoma hemocytometer after fixation with 10% formaldehyde. Viability was assessed by trypan blue exclusion, and only viable promastigotes were included in the quantitative analysis (16).

### Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 9 (GraphPad Software, USA). Weekly footpad measurements were analysed using two-way repeated-measures analysis of variance (ANOVA), with experimental group as the between-subject factor and time as the within-subject repeated-measures factor, followed by Sidak's multiple-comparisons test for comparisons between SIM and RPMI at individual time points. Amastigote burden scores were treated as ordinal data and were presented as median [interquartile range (IQR)]. Promastigote recovery data are also presented as median (IQR). For amastigote burden and promastigote recovery, inferential comparisons were restricted to the two experimentally infected groups (SIM and RPMI) and performed using two-tailed Mann-Whitney U tests. The saline-inoculated group served as a negative control for these outcomes, and the outcomes were summarized descriptively. All statistical tests were two-sided, and $p<0.05$ was considered statistically significant.

## RESULTS

### Molecular Identification of the Clinical Isolate

ITS1-targeted probe-based real-time PCR followed by melting-curve analysis identified the clinical isolate MHOM/TR/2025/CBU152 as *Leishmania tropica*, based on its molecular profile in comparison with the reference strains.

### Lesion Development in BALB/c Mice

Longitudinal analysis of weekly footpad measurements, using a two-way repeated-measures ANOVA, demonstrated significant effects of experimental group, time, and the group-by-time interaction (all $p<0.0001$), indicating distinct lesion progression trajectories between the SIM and RPMI groups over the six-week observation period. Post-hoc multiple comparisons showed no significant difference between the groups at baseline (week 0); however, a significant difference was detected at week 1 and persisted through weeks 2-6 (adjusted $p<0.0001$ for each comparison). Despite this early quantitative difference, visible footpad swelling and lesion development were first observed in the SIM group at approximately week 2, whereas in the RPMI group these findings appeared later and remained less pronounced throughout follow-up. By week 6, the mean ± SD absolute footpad diameter was 4.22±0.01 mm in the SIM group and 3.52±0.01 mm in the RPMI group. No visible inflammatory response or lesion formation was observed in the saline control group. Under the conditions tested, SIM-derived promastigotes were associated with greater longitudinal footpad enlargement, higher amastigote burden, and greater parasite recovery than RPMI-derived promastigotes (Figures 1A-C and 2; Table 1).

### Amastigote Burden in Touch Preparations

At week six post-infection, necropsy was performed and infected footpad tissues were collected for microscopic examination. Touch preparations were made on clean glass slides, air-dried, and stained with 10% Giemsa solution (pH 7.2) for 30 min, following standard protocols. Slides were examined under oil immersion at 1000× magnification using a bright-field light microscope. Amastigote burden was significantly higher in the SIM group than in the RPMI group [median (IQR): 6 (5-6) vs. 2 (2-2); Mann-Whitney U=0, exact two-tailed p=0.0079]. No amastigotes were detected in tissue samples from control mice [0 (0-0)] (Figure 1D-F; Table 1).

### Promastigote Recovery in NNN Cultures

Following necropsy at week 6 post-infection, fragments of infected plantar tissue were aseptically inoculated into NNN medium and monitored for promastigote proliferation. Promastigote recovery on day 7 was significantly greater in cultures established from the SIM group than in those established from the RPMI group [median (IQR): 1.15 (1.12-1.18)×$10^6$/mL vs. 0.15 (0.14-0.16)×$10^6$/mL; Mann-Whitney U=0, exact two-tailed p=0.0079]. No promastigotes were recovered from the saline-inoculated control group (Figure 1G-I; Table 1).

## DISCUSSION

CL is primarily shaped by cell-mediated immune mechanisms, and its clinical spectrum varies markedly across *Leishmania* species—including *L. major, L. tropica, L. mexicana,* and *L. amazonensis*—as well as by host genetic background and qualitative differences in immune responses (17). This diversity, ranging from self-resolving lesions to chronic non-healing disease, underscores the importance of reliable animal models for dissecting CL immunopathology and evaluating candidate interventions (18). BALB/c and C57BL/6 mice remain central to this work, as their distinct immunological profiles—Th2-driven susceptibility in BALB/c and Th1-mediated resistance in C57BL/6—provide

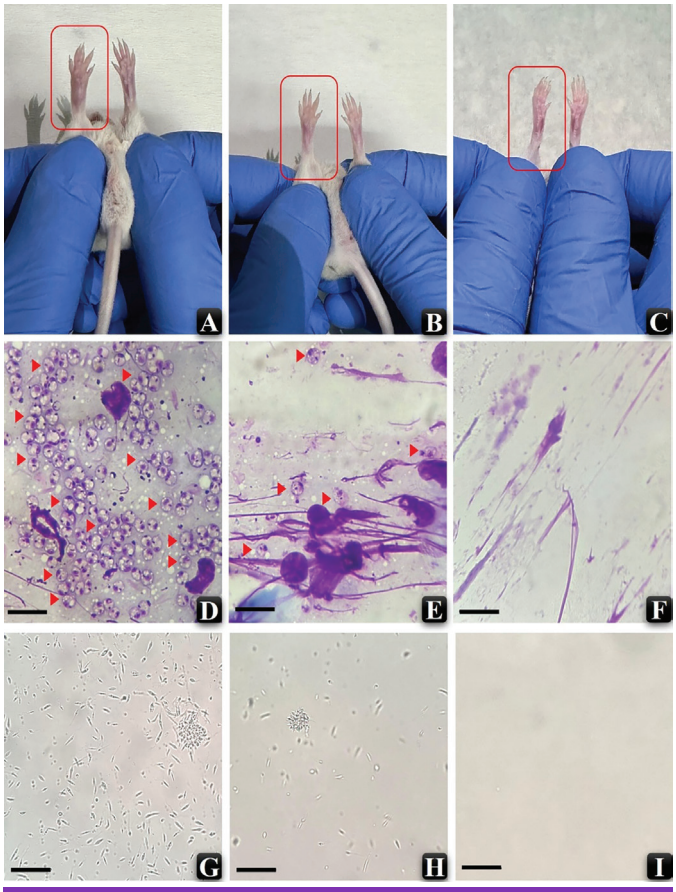


**Figure 1.** Macroscopic and light microscopic findings from footpad lesions of BALB/c mice six weeks after experimental *Leishmania tropica* infection

**(A-C)** Absolute footpad diameter: promastigotes derived from SIM cultures induced greater footpad swelling with well-defined edema and erythema **(A)**, whereas RPMI-derived parasites produced only mild to moderate enlargement **(B)**. Saline-injected controls exhibited no visible pathology **(C)**. **(D-F)** Amastigote burden: Giemsa-stained impression smears revealed a dense intracellular amastigote burden in the SIM group **(D)**, a lower burden in the RPMI group **(E)**, and no detectable parasites in the control group **(F)**. Arrowheads denote intracellular amastigotes. **(G-I)** Promastigote recovery: representative microscopic fields from NNN cultures established from footpad tissues of the SIM **(G)**, RPMI **(H)**, and control **(I)** groups on day 7. Promastigotes were observed in cultures from both infected groups, whereas none were detected in control cultures. Scale bar, D-F: 10 µm; G-I: 10 µm

*SIM: Schneider's insect medium*

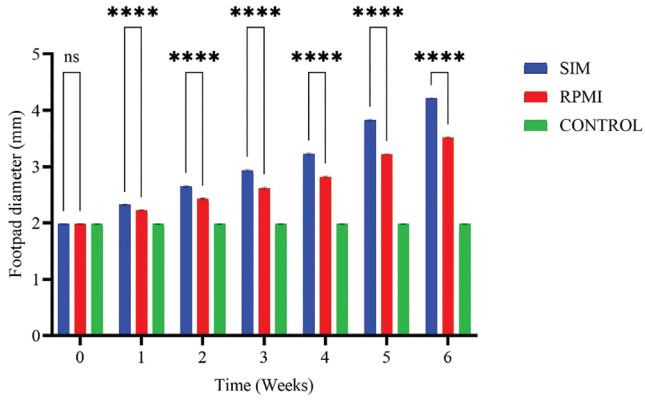


**Figure 2.** Longitudinal changes in absolute footpad diameter in BALB/c mice infected with *Leishmania tropica* promastigotes cultured under different *in vitro* conditions. Footpad diameter was measured weekly using a digital caliper in mice inoculated with SIM- or RPMI-derived promastigotes and in uninfected controls. Data are presented as mean ± SD (n=5 per group). Statistical analysis was performed using two-way repeated-measures ANOVA followed by Sidak's multiple-comparisons test for prespecified SIM-RPMI comparisons at each time point. No significant differences were detected at week 0, whereas significant differences were observed from week 1 through week 6

*****: Adjusted p<0.0001, ns: Not significant, SIM: Schneider's insect medium, SD: Standard deviation, ANOVA: Analysis of variance*

valuable contrasts for interrogating cellular processes including Th1/Th2 polarization, macrophage activation, cytokine dynamics, and parasite dissemination (19). Within this broader framework, *L. tropica*, the principal agent of anthroponotic CL in the Old World, presents particular experimental challenges because it typically produces only small, non-ulcerative nodules and modest delayed-type hypersensitivity responses in mice, limiting its capacity to generate progressive disease in standard murine models (20). This has historically hampered detailed pathogenicity studies. In the present study, we addressed this limitation by testing whether pre-infection culture conditions could enhance the infectivity of a clinical *L. tropica* isolate in highly susceptible BALB/c mice.

In the sand fly vector, *Leishmania* parasites undergo a series of differentiation steps—including the procyclic, nectomonad, leptomonad, haptomonad, and metacyclic stages—culminating in the production of metacyclic promastigotes, the infective form transmitted to mammals (21). Following inoculation, these metacyclics rapidly enter macrophages and transform into intracellular amastigotes, establishing infection (22). Metacyclogenesis is therefore a key determinant of virulence, as metacyclic promastigotes possess characteristic adaptations including elongated flagella, a dense LPG coat, and upregulated gp63, all of which enhance complement resistance and early survival within host phagocytes (23). The extent to which metacyclics are represented in an inoculum directly influences infection outcomes. While metacyclogenesis occurs physiologically in the sand fly gut, it can also be induced *in vitro* by environmental cues, particularly nutrient limitation and pH reduction—stressors that mimic those encountered during blood meal digestion in the vector (24). Under axenic conditions, promastigotes similarly undergo partial metacyclogenesis as cultures reach the stationary phase. However, the efficiency of differentiation varies widely among species and strains, with some producing only limited numbers of metacyclics and others exhibiting strong stress-induced differentiation responses (12).

To investigate whether pre-infection culture conditions could alter subsequent experimental infectivity, promastigotes of the clinical *L. tropica* isolate were maintained in SIM (pH 5.5, 20% FCS) or

**Table 1.** Quantitative assessment of footpad measurements, amastigote burden, and promastigote recovery in BALB/c mice infected with *Leishmania tropica* promastigotes cultured under different *in vitro* conditions (SIM and RPMI)

| Group | Footpad diameter[a] mm, (mean ± SD) | Amastigote burden[b] median (IQR) | Promastigote recovery[c] x10$^6$/mL median (IQR) | n[d] |
|---|---|---|---|---|
| SIM | 4.22±0.01 | 6 (5-6) | 1.15 (1.12-1.18) | 5 |
| RPMI | 3.52±0.01 | 2 (2-2) | 0.15 (0.14-0.16) | 5 |
| Control | 1.98±0.01 | 0 (0-0) | 0 (0-0) | 5 |

[a]: Absolute footpad diameter was measured at the inoculated right hind footpad using a digital caliper. Each mouse represented one independent biological replicate, and values are presented as mean ± SD calculated from the five individual animal-level measurements in each group (n=5)
[b]: Amastigote burden was assessed in Giemsa-stained touch preparations using the following ordinal semi-quantitative scoring system: 6+ (>100 parasites/field), 5+ (10-100 parasites/field), 4+ (1-10 parasites/field), 3+ (1-10 parasites/10 fields), 2+ (1-10 parasites/100 fields),1+ (1-10 parasites/1000 fields), and 0 (no parasites detected in 1000 fields). Because amastigote burden represents ordinal data, values are presented as median (IQR). The prespecified comparison between the SIM and RPMI groups was performed using a two-tailed Mann-Whitney U test.
[c]: Promastigote densities (×10$^6$/mL) were quantified on day 7 following incubation in NNN medium and presented as median (IQR). The prespecified comparison between the SIM and RPMI groups was performed using a two-tailed Mann-Whitney U test.
n[d]: Number of animals included in each experimental group
mm: Millimeters, IQR: Interquartile range, SD: Standard deviation, SIM: Schneider's insect medium

RPMI-1640 (pH 7.2, 10% FCS) before inoculation into BALB/c mice. These conditions were selected because previous studies in other *Leishmania* species have shown that environmental factors, including acidic pH and nutrient composition, can influence promastigote differentiation and infectivity. Under the conditions tested, the *in vivo* results demonstrated that SIM-derived promastigotes exhibited greater experimental infectivity in BALB/c mice than RPMI-derived promastigotes. SIM-cultured promastigotes induced earlier lesion development (detectable by week 2) and greater absolute footpad diameter by week 6 (4.22±0.01 mm vs. 3.52±0.01 mm; Sidak-adjusted $p<0.0001$), and a significantly higher amastigote burden in the SIM group than in the RPMI group [median (IQR): 6 (5-6) vs. 2 (2-2); $p=0.0079$]. Promastigote recovery on day 7 was also significantly greater in cultures established from the SIM group than in those established from the RPMI group [median (IQR): 1.15 (1.12-1.18)×10$^6$/mL vs. 0.15 (0.14-0.16)×10$^6$/mL; $p=0.0079$]. Collectively, in the SIM group, the earlier lesion onset, greater lesion development, higher amastigote burden, and enhanced parasite recovery provide consistent evidence that SIM-derived promastigotes have greater experimental infectivity than RPMI-derived promastigotes.

These findings are consistent with previous studies demonstrating that acidic culture conditions can promote metacyclic differentiation and increase infectivity in several *Leishmania* species. Bates and Tetley (25) showed that *L. mexicana* cultured in SIM at pH 5.5 developed a predominantly metacyclic-like population characterized by increased macrophage infectivity and complement resistance (25). Similarly, Zakai et al. (26) reported increased proportions of metacyclic promastigotes and enhanced infectivity under acidic culture conditions in several *Leishmania* species. These observations provide a biologically plausible framework for interpreting the greater experimental infectivity observed in our SIM-derived *L. tropica* promastigotes. In the present study, metacyclic promastigotes were not directly quantified; therefore, the increased infectivity cannot be specifically attributed to enhanced metacyclogenesis. Instead, our findings demonstrate that SIM preconditioning is associated with a more infectious phenotype in this clinical *L. tropica* isolate under the experimental conditions tested.

The mechanisms underlying the greater infectivity of SIM-derived promastigotes cannot be determined from the present experimental design. SIM and RPMI differed in pH, serum concentration, and basal nutrient composition, all of which may influence parasite physiology and infectivity. Nutrient availability and metabolic stress have been implicated in promastigote differentiation in *Leishmania* spp. (27) while previous studies also support an important role for acidic culture conditions in modulating parasite differentiation and infectivity (25). However, because these variables were not independently controlled in the present study, the greater infectivity observed after SIM preconditioning cannot be attributed specifically to acidic pH or to any single medium component. Rather, our findings indicate that the overall SIM culture environment was associated with a more infective phenotype in this clinical *L. tropica* isolate.

### *Study Limitations*

The present study has several limitations. Metacyclic promastigotes were not directly identified or quantified; therefore, the greater infectivity observed after SIM preconditioning cannot be attributed specifically to enhanced metacyclogenesis, and this requires confirmation using stage-specific approaches. In addition, SIM and RPMI-1640 differed in pH, FCS concentration, and basal medium composition, preventing determination of the individual contributions of culture-associated factors. The exploratory design included a relatively small sample size (n=5 animals per group), lacked a formal a priori power calculation, and evaluated only a single clinical *L. tropica* isolate; therefore, the findings should be considered isolate-specific and not generalized to other isolates without further validation. Future studies using larger cohorts, additional clinical isolates, controlled culture conditions, and complementary stage-specific, histopathological, and immunological analyses are warranted to confirm and extend these findings.

## CONCLUSION

Our findings demonstrate that pre-infection culture conditions significantly influence the experimental infectivity of a clinical *L. tropica* isolate in BALB/c mice. SIM-derived promastigotes exhibited a more infective phenotype than RPMI-derived promastigotes, as evidenced by earlier lesion development, greater tissue amastigote burden, and enhanced parasite recovery. Although the underlying mechanisms remain to be elucidated, these findings support SIM preconditioning as a useful approach for establishing robust experimental *L. tropica* infection and provide a basis for further studies investigating culture-associated promastigote differentiation and infectivity.

### **Ethics*

**Ethics Committee Approval:** This study was approved by the Local Ethics Committee for Animal Experiments, Faculty of Medicine, Manisa Celal Bayar University (date: 29.07.2025, decision no: 77.637.435/330).

**Informed Consent:** Written informed consent was obtained from the patient at the time of the original clinical sample collection. The archived parasite isolate used in the present study was de-identified, and no identifiable patient information was accessed.

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### *Footnotes*

### **Authorship Contributions*

Surgical and Medical Practices: A.Ö., A.Y., Concept: A.Y, T.A., İ.Ç.Ş., Design: A.Y., T.A., Data Collection or Processing: A.Ö., A.Y., İ.Ç.Ş., Analysis or Interpretation: A.Ö., A.Y., T.A., Literature Search: A.Y., T.A., İ.Ç.Ş., Writing: A.Y., T.A.

**Conflict of Interest:**

One of the authors of this article (A.E.) is a member of the Editorial Board of this journal. He was completely blinded to the peer review process of the article.

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## REFERENCES

1. Blaizot R, Pasquier G, Kone AK, Duvignaud A, Demar M. Cutaneous leishmaniasis in sub-Saharan Africa: a systematic review of *Leishmania* species, vectors and reservoirs. Parasit Vectors. 2024; 17: 318.
2. Knight CA, Harris DR, Alshammari SO, Gugssa A, Young T, Lee CM. Leishmaniasis: recent epidemiological studies in the Middle East. Front Microbiol. 2023; 13: 1052478.
3. Mendes BP, da Silva IA, DaMata JP, Castro-Gomes T, Vieira LQ, Ribeiro-Dias F, et al. Metacyclogenesis of *Leishmania (Viannia) guyanensis*: a comprehensive study of the main transformation features in axenic culture and purification of metacyclic promastigotes by negative selection with *Bauhinia purpurea* lectin. Parasitology. 2019; 146: 716-27.
4. Chanmol W, Jariyapan N, Preativatanyou K, Mano C, Tippawangkosol P, Somboon P, et al. Stimulation of metacyclogenesis in *Leishmania (Mundinia) orientalis* for mass production of metacyclic promastigotes. Front Cell Infect Microbiol. 2022; 12: 992741.
5. Siripattanapipong S, Boontanom P, Leelayoova S, Mungthin M, Tan-Ariya P. *In vitro* growth characteristics and morphological differentiation of *Leishmania martiniquensis* promastigotes in different culture media. Acta Trop. 2019; 197: 105039.
6. Hendricks L, Wright N. Diagnosis of cutaneous leishmaniasis by *in vitro* cultivation of saline aspirates in Schneider's Drosophila medium. Am J Trop Med Hyg. 1979; 28: 962-4.
7. Rostamian M, Niknam HM. *Leishmania tropica*: what we know from its experimental models. Adv Parasitol. 2019; 104: 1-38.
8. Lira R, Méndez S, Carrera L, Jaffe C, Neva F, Sacks D. *Leishmania tropica*: the identification and purification of metacyclic promastigotes and use in establishing mouse and hamster models of cutaneous and visceral disease. Exp Parasitol. 1998; 89: 331-42.
9. Girginkardeşler N, Balcioğlu IC, Yereli K, Ozbilgin A, Ozbel Y. Cutaneous leishmaniasis infection in Balb/c mice using a *Leishmania tropica* strain isolated from Turkey. J Parasitol. 2001; 87: 1177-8.
10. Yıldırım A, Özbilgin A, Yereli K. *Trypanosoma cruzi*, *Leishmania tropica* ve *Toxoplasma gondii* Parazitlerinin J774, Vero ve HeLa hücre hatlarında *ex vivo* kültivasyon potansiyellerinin değerlendirilmesi [Evaluation of *ex vivo* cultivation potentials of *Trypanosoma cruzi*, *Leishmania tropica* ve *Toxoplasma gondii* parasites in J774, Vero and HeLa Cell Lines]. Mikrobiyol Bul. 2023; 57: 71-82.
11. Toz SO, Nasereddin A, Ozbel Y, Ertabaklar H, Culha G, Sevil N, et al. Leishmaniasis in Turkey: molecular characterization of *Leishmania* from human and canine clinical samples. Trop Med Int Health. 2009; 14: 1401-6.
12. da Luz RI, Vermeersch M, Dujardin JC, Cos P, Maes L. In vitro sensitivity testing of *Leishmania* clinical field isolates: preconditioning of promastigotes enhances infectivity for macrophage host cells. Antimicrob Agents Chemother. 2009; 53: 5197-203.
13. Yardley V, Croft SL. Animal models of cutaneous leishmaniasis. Handbook of Animal Models of Infection. 1999;775-81.
14. Attia H, Bali A, Sghaier RM, Martinez PAL, Mkannez G, Atri C, et al. Comparison between plethysmometer and caliper methods to monitor lesion-size induced by *Leishmania major* infection in BALB/c mouse experimental model. J Antivir Antiretrovir. 2015; 3: 179-85.
15. Ramírez JR, Agudelo S, Muskus C, Alzate JF, Berberich C, Barker D, et al. Diagnosis of cutaneous leishmaniasis in Colombia: the sampling site within lesions influences the sensitivity of parasitologic diagnosis. J Clin Microbiol. 2000; 38: 3768-73.
16. Ladopoulos T, Ntais P, Tsirigotakis N, Dokianakis E, Antoniou M. The proliferation potential of promastigotes of the main *Leishmania* species of the old world in NNN culture medium prepared using blood of four different mammals. Exp Parasitol. 2015; 157: 124-7.
17. Nateghi-Rostami M, Lipoldová M, Sohrabi Y. Improving reproducibility and translational potential of mouse models: lessons from studying leishmaniasis. Front Immunol. 2025; 16: 1559907.
18. Muñoz-Durango N, Gómez A, García-Valencia N, Roldán M, Ochoa M, Bautista-Erazo DE, et al. A mouse model of ulcerative cutaneous leishmaniasis by *Leishmania (Viannia) panamensis* to investigate infection, pathogenesis, immunity, and therapeutics. Front Microbiol. 2022; 13: 907631.
19. Restrepo CM, Llanes A, Herrera L, Ellis E, Lleonart R, Fernández PL. Gene expression patterns associated with *Leishmania panamensis* infection in macrophages from BALB/c and C57BL/6 mice. PLoS Negl Trop Dis. 2021; 15: e0009225.
20. Mears ER, Modabber F, Don R, Johnson GE. A review: the current *in vivo* models for the discovery and utility of new anti-leishmanial drugs targeting cutaneous leishmaniasis. PLoS Negl Trop Dis. 2015; 9: e0003889.
21. Becvar T, Vojtkova B, Siriyasatien P, Votypka J, Modry D, Jahn P, et al. Experimental transmission of *Leishmania (Mundinia)* parasites by biting midges (Diptera: Ceratopogonidae). PLoS Pathog. 2021; 17: e1009654.
22. Liévin-Le Moal V, Loiseau PM. *Leishmania* hijacking of the macrophage intracellular compartments. FEBS J. 2016; 283: 598-607.
23. da Silva IA Jr, Morato CI, Quixabeira VB, Pereira LI, Dorta ML, de Oliveira MA, et al. *In vitro* metacyclogenesis of *Leishmania (Viannia)* braziliensis and *Leishmania (Leishmania) amazonensis* clinical field isolates, as evaluated by morphology, complement resistance, and infectivity to human macrophages. Biomed Res Int. 2015; 2015: 393049.
24. Serafim TD, Figueiredo AB, Costa PA, Marques-da-Silva EA, Gonçalves R, de Moura SA, et al. *Leishmania* metacyclogenesis is promoted in the absence of purines. PLoS Negl Trop Dis. 2012; 6:e1833.
25. Bates PA, Tetley L. *Leishmania mexicana*: induction of metacyclogenesis by cultivation of promastigotes at acidic pH. Exp Parasitol. 1993; 76: 412-23.
26. Zakai HA, Chance ML, Bates PA. *In vitro* stimulation of metacyclogenesis in *Leishmania braziliensis*, *L. donovani, L. major* and *L. mexicana*. Parasitology. 1998; 116: 305-9.
27. Abdi Ghavidel A, Aghamiri S, Jajarmi V, Bandehpour M, Kazemi B. The influence of different culture media on the growth and recombinant protein production of iranian lizard *Leishmania* promastigote. Iran J Parasitol. 2022; 17: 543-53.

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# Optimization of the Parasite Rescue and Transformation Assay for *Leishmania tropica*: Analysis of Preconditioning and Back Transformation Dynamics

## *Leishmania tropica için Parazit Kurtarma ve Dönüştürme Test Protokolünün Optimizasyonu: Ön Koşullandırma ve Geri Dönüşüm Dinamiklerinin Analizi*

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## ABSTRACT

**Objective:** This study aimed to compare the effects of three culture media [RPMI-1640, Schneider's insect medium (SIM), and M199] on *Leishmania tropica* promastigote propagation, preconditioning, and back-transformation of intracellular amastigotes, with the aim of establishing a standardized parasite rescue and transformation assay (PRTA) capable of consistently generating infective metacyclic forms.
**Methods:** Nine experimental groups were designed by combining the three media during the preconditioning and back-transformation phases. Promastigotes were expanded in the test media and then used to infect differentiated THP-1 macrophages to obtain intracellular amastigotes. Back-transformation was monitored morphologically under an inverted phase-contrast microscope, and metabolic activity was quantified using the ATP-based CellTiter-Glo assay.
**Results:** The highest back-transformation efficiency was observed in test 5 (SIM→SIM) [relative luminescence units (RLU): 49.567±9.099, $p<0.05$], with microscopic analysis revealing abundant viable transformed promastigotes. In contrast, other medium combinations produced either limited or no transformation. Test 9 (M199→M199; RLU: 0.089±0.071) and test 1 (RPMI→RPMI; RLU: 0.118±0.080) showed minimal back-transformation without statistical significance ($p>0.05$).
**Conclusion:** These findings demonstrate that culture medium composition is a key determinant of *L. tropica* promastigote differentiation. SIM medium supported the most efficient generation of metacyclic promastigotes, likely because it approximates vector-like conditions. The PRTA model described here offers a reproducible *in vitro* system for studying parasite differentiation and provides a practical framework to support infection biology and antileishmanial drug development.
**Keywords:** Back-transformation, culture medium, *Leishmania tropica*, parasite rescue, preconditioning, Schneider's insect medium

## ÖZ

**Amaç:** Bu çalışmada, *Leishmania tropica* izolatı kullanılarak farklı kültür ortamlarının [RPMI-1640, SIM (Schneider's insect medium], M199) promastigot çoğaltımı, ön koşullandırma ve amastigotlardan promastigotlara dönüşüm süreçleri üzerindeki etkileri karşılaştırmalı olarak değerlendirilerek; enfektif metasiklik formların yüksek verimlilikle elde edilebildiği, biyolojik açıdan güvenilir ve metodolojik bakımdan standardize edilebilir bir parazit kurtarma ve dönüştürme test (PRTA) protokolünün geliştirilmesi amaçlanmıştır.
**Yöntemler:** *L. tropica* hücre içi amastigotlarının promastigot forma dönüşümünü değerlendirmek amacıyla, RPMI-1640, SIM ve M199 besiyerlerinin ön koşullandırma ve geri dönüşüm aşamalarında farklı kombinasyonlarla kullanıldığı dokuz test grubu

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oluşturulmuştur. NNN besiyerinden elde edilen promastigotlar üç farklı besiyerinde çoğaltılmış, farklılaşmış THP-1 makrofaj hücrelerinde enfeksiyon modeli oluşturulmuştur. Elde edilen hücre içi amastigotlar, PRTA protokolü kapsamında kültüre alınarak dönüşüm süreçleri morfolojik olarak invert mikroskopla izlenmiş; metabolik aktiviteleri ATP temelli CellTiter-Glo testi ile kantitatif olarak değerlendirilmiştir.

**Bulgular:** En yüksek dönüşüm verimi test 5 (SIM→SIM); grubunda elde edilmiştir [göreli lüminesans birimleri (RLU): 49,567±9,099, p<0,05]. Bu grupta mikroskobik incelemelerde yüksek yoğunlukta ve canlı dönüşen promastigotlar gözlemlenmiştir. Diğer kültür ortamı kombinasyonlarında ise dönüşüm ya düşük düzeyde kalmış ya da hiç gerçekleşmemiştir. Özellikle test 9 (M199→M199; RLU: 0,089±0,071) ve test 1 (RPMI→RPMI; RLU: 0,118±0,080) gruplarında dönüşüm başarısı düşük kalmıştır (p>0,05).

**Sonuç:** Kültür ortamı bileşiminin *L. tropica*'nın promastigot dönüşümünde belirleyici bir faktör olduğu gösterilmiştir. SIM besiyeri, vektör benzeri koşulları taklit etme kapasitesi sayesinde metasiklik promastigot üretiminde en yüksek verimliliği sağlamıştır. Bu çalışma, enfeksiyon yeteneği yüksek promastigotların elde edilmesini mümkün kılan güvenilir bir *in vitro* model sunarak enfeksiyon biyolojisi ve antileishmanial ilaç araştırmaları için güçlü bir deneysel altyapı sağlamaktadır.

**Anahtar Kelimeler:** Geri dönüşüm, kültür ortamı, *Leishmania tropica*, parazit kurtarma , ön koşullandırma, Schneider's insect medium

## INTRODUCTION

Leishmaniasis, a vector-borne disease caused by obligate intracellular protozoa of the genus *Leishmania,* remains a major public health concern in tropical and subtropical regions. According to the World Health Organization, nearly one million new cases are reported annually, and the disease continues to impose a substantial global burden due to high morbidity, permanent sequelae, and significant socioeconomic impact (1). Cutaneous leishmaniasis (CL) is the most common clinical form; if left untreated, it may lead to ulcerative skin lesions and disfiguring scars. *Leishmania tropica*, a causative agent of CL in the Mediterranean basin, the Middle East, and South Asia, represents a notable regional threat (2). Disease control is further complicated by limited therapeutic options, increasing drug resistance, and the growing number of unresponsive field isolates (3).

The life cycle of *Leishmania* involves morphological transitions between flagellated promastigotes in the sand fly vector and non-flagellated amastigotes within mammalian phagocytic cells. These stage conversions are regulated by environmental cues such as temperature, pH, oxygen availability, and nutrient content (4). *In vitro* modeling of these developmental transitions not only provides critical insights into parasite biology but also permits the isolation of biologically competent, infective forms. Nevertheless, propagation of promastigotes in culture is strongly influenced by the composition of the growth medium and surrounding environmental parameters, and *in vitro*-derived forms do not invariably exhibit infectivity *in vivo* (5). The induction of the metacyclic promastigote stage—the developmental form that initiates infection in mammalian hosts—necessitates culture conditions that accurately recapitulate the physiological transition from vector to host (6). Preconditioning, defined as the gradual exposure of parasites to host-like conditions prior to infection, has therefore been recognized as a critical step in enhancing infectivity (7).

To overcome inconsistencies in promastigote recovery and differentiation, the parasite rescue and transformation assay (PRTA) has been established as a robust analytical platform for quantitative assessment of parasite viability, infectivity, and the efficiency of metacyclic differentiation. Nonetheless, the assay's analytical sensitivity and inter-assay reproducibility remain critically dependent on the physicochemical properties of the culture medium and on the precision and efficacy of both preconditioning and back-transformation protocols (8). Previous studies have highlighted substantial interspecies and inter-isolate variation in culture adaptation, with delays in transformation and reduced differentiation rates frequently reported (6,9,10). These limitations raise concerns regarding the biological relevance of *in vitro*-derived promastigotes and underscore the need for standardized protocols that yield infective and functionally valid forms.

In this study, we investigated the effects of three culture media (RPMI, SIM, and M199) on the propagation, preconditioning, and back-transformation of *L. tropica* promastigotes from intracellular amastigotes. The aim was to develop a reproducible and standardized PRTA protocol capable of reliably generating metacyclic promastigotes, thereby providing a robust platform for infection biology research and antileishmanial drug development.

## METHODS

### *Ethical Approval*

This study was conducted with the approval of the Health Sciences Ethics Committee of the Manisa Celal Bayar University Faculty of Medicine (date: 07.05.2025, decision no: 20.478.486/3098). Patient consent was not required, as the study was performed using *L. tropica* strains and THP-1 cells obtained from a parasite bank, without direct use of human clinical specimens.

### *Media and Solutions*

To support the optimal *in vitro* growth of *L. tropica* promastigotes and THP-1 macrophages, all culture media and buffer solutions were prepared under aseptic conditions and stored in accordance with international laboratory standards.

**NNN Medium:** NNN medium was prepared by dissolving 5g of agar, 2g of peptone, and 1g of NaCl in 200mL of distilled water, followed by autoclaving at 121 °C for 20 minutes. After cooling to room temperature, the medium was supplemented with 1% gentamicin, 1% penicillin/streptomycin, and 30 mL of defibrinated rabbit blood. Aliquots were dispensed into sterile slant tubes and stored at 4 °C for a minimum of 48 hours to allow stabilization before use.

**RPMI-1640 Medium:** RPMI-1640 was supplemented with 10% fetal calf serum (FCS), 1% penicillin/streptomycin, and 1% gentamicin. The pH was adjusted to 7.2. The medium was stored at 4 °C and equilibrated to room temperature before use in cell cultures and promastigote cultures.

**Schneider's insect medium (SIM):** SIM was prepared according to the manufacturer's protocol and supplemented with 20% FCS, 1% L-glutamine, and 1% penicillin/streptomycin. The pH was adjusted to 5.5 to mimic insect midgut conditions. The medium was sterilized using a 0.22 µm filter, stored at 4 °C, and equilibrated to room temperature before use.

**M199 Medium:** M199 was enriched with 10% FCS, 1% penicillin/streptomycin, and 1% gentamicin to support the proliferation of host cells and promastigotes. The pH was adjusted to 7.4. Following sterile preparation, the medium was stored at 4 °C and equilibrated to room temperature prior to application.

**Sterile 0.05% Sodium Dodecyl Sulfate (SDS):** For controlled lysis in PRTAs, a 0.05% SDS solution was prepared by dissolving 0.05 g of SDS in 100 mL of sterile distilled water. The solution was mixed until fully dissolved, sterilized through a 0.22 µm membrane filter, and stored at 4 °C until use.

**Sterile 1xPBS:** 1xPBS was prepared by dissolving 8.0 g NaCl, 0.2 g KCl, 1.44 g $Na_2HPO_4 \cdot 2H_2O$, and 0.24 g $KH_2PO_4$ in approximately 800 mL of distilled water. The pH was adjusted to 7.2-7.4, and the final volume was brought to 1 L. The solution was sterilized by filtration through a 0.22 µm membrane filter and stored at 4 °C until use.

### *In vitro culture of L. tropica*

*L. tropica* (MHOM/TR/2022/CBU121), isolated from the lesion on the right cheek of a CL patient in Türkiye and subsequently maintained in the Parasite Bank of the Faculty of Medicine, Manisa Celal Bayar University, was rapidly thawed in a 37 °C water bath after removal from liquid nitrogen. The thawed suspension was inoculated into NNN medium for initial expansion and incubated at 26 °C. Parasite proliferation was monitored regularly under a light microscope, and viable promastigotes in the logarithmic growth phase were obtained through periodic observations every 2-3 days (11).

### *THP-1 Macrophage Cell Culture*

The THP-1 monocytic cell line was removed from liquid nitrogen and rapidly thawed in a 37 °C water bath. Cells were centrifuged at 1500 rpm for 10 minutes to obtain a pellet, which was then resuspended in 4 mL of RPMI-1640 medium. A 1 mL aliquot of the suspension was transferred into culture flasks containing 9 mL of fresh RPMI-1640. Cells were incubated at 37 °C in a humidified atmosphere with 5% $CO_2$. Cell morphology and viability were monitored regularly under an inverted microscope, with viability assessed using trypan blue exclusion. Cell counts were performed before experimental use, using a Thoma chamber, after a 1:3 dilution. Cultures were passaged regularly, and the medium was replaced every other day. The medium was supplemented with 100 ng/mL phorbol-12-myristate-13-acetate for differentiation into macrophage-like cells and incubated for 24 hours (12).

### *Preconditioning of Promastigotes*

To enhance the infectivity of *L. tropica* promastigotes, a preconditioning protocol was implemented. This process involved controlled exposure to environmental stressors that mimic the transition from the sandfly vector to the mammalian host. Promastigotes in the logarithmic phase, initially cultured in NNN medium, were transferred to RPMI-1640 (10% FCS), SIM (20% FCS), and M199 (10% FCS) and incubated at 26 °C for five days. Throughout the incubation period, cultures were monitored daily by light microscopy to assess cell morphology and viability. This approach aimed to obtain biologically active and infective promastigotes prior to host-cell infection. The impact of each medium on the preconditioning process was comparatively evaluated (7).

### *Infection of THP-1 Macrophages with L. tropica*

Differentiated THP-1 macrophages were harvested using a cell scraper upon reaching confluency in the culture flasks, and then centrifuged at 1500 rpm for 10 minutes. The resulting cell suspension was counted and adjusted to a concentration of $5\times10^5$ cells/mL, and then seeded into fresh RPMI-1640-containing flasks. Cells were incubated at 37 °C in a humidified atmosphere with 5% $CO_2$ for 24 hours. Subsequently, infection was carried out using *L. tropica* promastigotes at a 1:10 (cell:parasite) ratio (13). The promastigotes had reached the logarithmic growth phase in RPMI-1640, SIM, or M199. After infection, non-internalized promastigotes were removed by washing with sterile 1xPBS, and fresh RPMI-1640 medium was added. Infected cultures were then incubated for 48 hours at 37 °C under 5% $CO_2$.

### *Back-transformation of Amastigotes to Promastigotes*

The back-transformation capacity of *L. tropica* amastigotes developed within macrophages was systematically evaluated under defined *in vitro* conditions. This process serves as a functional indicator of the parasite's adaptability to extracellular environments and enables a comparative analysis of how culture media modulate promastigote re-differentiation efficiency.

### *Controlled Lysis and Rescue of L. tropica Amastigotes*

Infected macrophage cultures in 96-well plates were washed three times with sterile 1× PBS and then 20 µL of 0.05% SDS was added to each well to induce controlled host-cell lysis. Plates were gently agitated for 30 seconds to ensure efficient lysis while preserving the viability of released amastigotes. Subsequently, 180 µL of complete medium (RPMI-1640, SIM, or M199) was added to each well. Plates were incubated at 26 °C for 96 hours to evaluate the transformation of intracellular amastigotes into promastigotes.

### *Quantitative ATP Assay for Back-transformed L. tropica Promastigotes*

Following 96 hours of incubation at 26 °C, viable amastigotes successfully differentiated into promastigote forms. Subsequently, 100 µL of CellTiter-Glo® reagent was added to each well, and the plates were incubated at room temperature for 10 minutes. Luminescence signals were recorded using a luminometer and expressed as relative luminescence units (RLU). This ATP-based assay provides a quantitative measure of promastigote metabolic activity, offering insight into their energy production capacity and proliferative potential under specific culture conditions.

### *Morphological Evaluation of Back-transformed L. tropica Promastigotes*

For morphological evaluation of the transformation into promastigote forms, cultured amastigotes were examined at the end of the incubation period using an inverted phase-contrast microscope. Cultures were divided into nine test groups (tests 1-9) based on different medium combinations used during the preconditioning and recovery phases (Table 1). After 96 hours of incubation, promastigotes in each group were examined microscopically, and the images were organized accordingly. Observations focused on assessing promastigote morphological integrity, cell density, and motility.

**Table 1.** Distribution of experimental groups (test 1-9) according to the culture media used in the preconditioning and back transformation phases for the morphological evaluation of *Leishmania tropica* promastigote transformation

| Test | Preconditioning | Back transformation |
|---|---|---|
| Test 1 | RPMI | RPMI |
| Test 2 | RPMI | SIM |
| Test 3 | RPMI | M199 |
| Test 4 | SIM | RPMI |
| Test 5 | SIM | SIM |
| Test 6 | SIM | M199 |
| Test 7 | M199 | RPMI |
| Test 8 | M199 | SIM |
| Test 9 | M199 | M199 |

### *Statistical Analysis*

Statistical analyses were performed to evaluate differences in transformation efficiency among nine test groups, each defined by distinct combinations of preconditioning and back-transformation media (RPMI-1640, SIM, and M199). RLU values obtained from the CellTiter-Glo® assay were expressed as mean ± standard deviation. One-way analysis of variance followed by Tukey's post-hoc test was used to identify statistically significant differences between groups, with significance set at $p<0.05$. All analyses were conducted using GraphPad Prism version 9. Additionally, morphological evaluations were performed using inverted phase-contrast microscopy to assess promastigote integrity, density, and motility after a 96-hour incubation.

## RESULTS

The back-transformation capacity of *L. tropica* amastigotes was assessed across nine experimental groups that combined different culture media during the preconditioning and back-transformation phases (Figure 1). Quantitative analysis demonstrated the highest efficiency in test 5 (SIM→SIM), which yielded a significantly elevated RLU value (49.567±9.099; $p<0.05$) compared with all other groups. Microscopic examination confirmed these findings, revealing dense cultures of motile promastigotes with characteristic morphology (Figure 2E).

When RPMI was used for preconditioning, notable transformation was observed only when SIM served as the recovery medium (test 2), with moderate RLU levels and corresponding microscopic evidence of viable promastigotes (Figure 2B). In contrast, test 1 (RPMI→RPMI) and test 3 (RPMI→M199) failed to support transformation, with negligible RLU values and no detectable promastigotes under microscopy (Figures 2A, 2C) (Table 2).

SIM-based preconditioning combined with RPMI recovery (test 4) resulted in partial back-transformation, as evidenced by moderate RLU values and visible promastigotes (Figure 2D). However, test 6 (SIM→M199) yielded markedly reduced transformation (RLU: 0.196±0.219), consistent with the presence of only a few motile promastigotes (Figure 2F; Table 2).

Overall transformation success was lowest in groups preconditioned with M199. Test 9 (M199→M199) yielded only minimal RLU values (0.089±0.071) and displayed no microscopically appreciable promastigote proliferation (Figure 2I). Similarly, test 7 (M199→RPMI) remained at very low levels (Figure 2G), while test 8 (M199→SIM) displayed slightly improved transformation (RLU: 1.018±0.547) with low-to-moderate promastigote density (Figure 2H; Table 2).

Taken together, quantitative and microscopic data indicate that SIM, when applied during the preconditioning and back-transformation phases, most efficiently supports the back-transformation of *L. tropica*. By contrast, RPMI and M199 alone were insufficient, with M199 showing the poorest performance across test groups.

## DISCUSSION

*Leishmania* spp. are digenetic parasites that alternate between the insect vector and the mammalian host, undergoing distinct morphological transitions during their life cycle. Modeling these developmental stages under laboratory conditions contributes substantially to understanding infection biology and to advancing therapeutic strategies. However, the biological relevance of such models is largely determined by the influence of the culture medium on parasite differentiation and viability (14,15). In clinical diagnosis, microscopic examination and *in vitro* culture are among the most frequently employed direct approaches. However, their diagnostic sensitivity may exhibit substantial variability, largely influenced by sample quality, the experience of the operator, and the degree of optimization of the culture systems used (16). Although culture isolation is theoretically considered the diagnostic gold standard, its practical yield is influenced by multiple biological and technical factors. These include the composition of the culture medium, the virulence characteristics of the isolate, and the initial parasite burden in

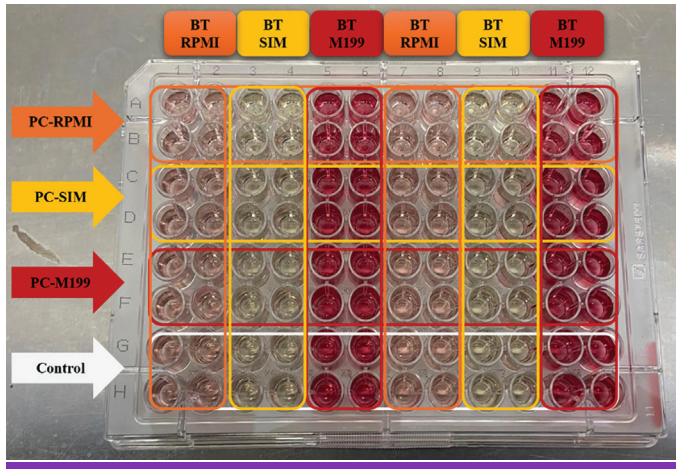


**Figure 1.** Layout of a 96-well microtiter plate used to assess the back-transformation of *Leishmania tropica* amastigotes into promastigote forms. [The plate was organized into nine experimental groups representing distinct combinations of culture media applied during the preconditioning (PC) and back-transformation (BT) phases. PC conditions (left axis) included RPMI (orange), SIM (yellow), and M199 (red), while BT conditions (top axis) consisted of RPMI (orange), SIM (yellow), and M199 (red). The white row served as the negative control]. Each condition was tested in triplicate technical replicates

*SIM: Schneider's insect medium*

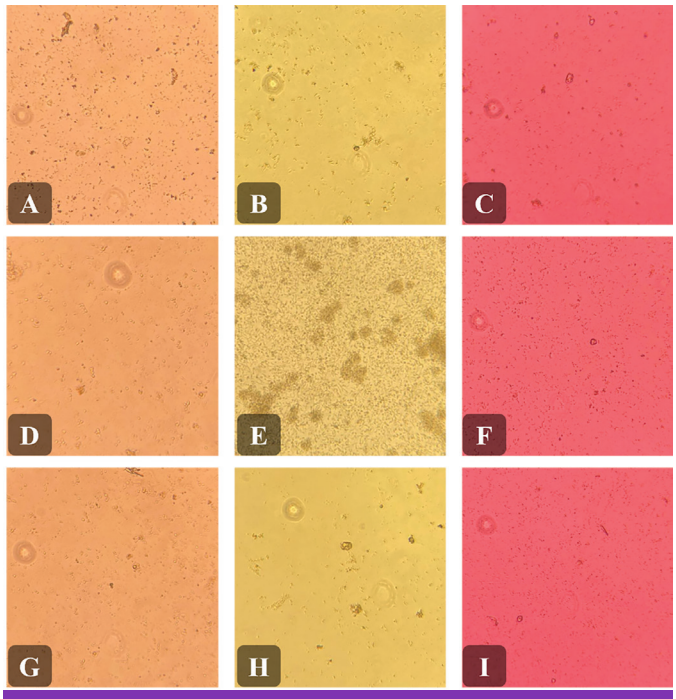


**Figure 2.** Representative inverted phase-contrast micrographs showing *Leishmania tropica* growth following back-transformation of intracellular amastigotes into promastigotes under different culture conditions. [Test groups were defined by the culture media used during the preconditioning and back-transformation phases: (A) test 1: RPMI→RPMI, (B) test 2: RPMI→SIM, (C) test 3: RPMI→M199, (D) test 4: SIM→RPMI, (E) test 5: SIM→SIM, (F) test 6: SIM→M199, (G) test 7: M199→RPMI, (H) test 8: M199→SIM, (I) test 9: M199→M199]

*SIM: Schneider's insect medium*

**Table 2.** Mean relative luminescence unit (RLU ± SD) values reflecting the back-transformation efficiency of *Leishmania tropica* amastigotes into promastigotes under different preconditioning and back-transformation medium combinations. [Test groups are defined as follows: test 1 (RPMI→RPMI), test 2 (RPMI→SIM), test 3 (RPMI→M199), test 4 (SIM→RPMI), test 5 (SIM→SIM), test 6 (SIM→M199), test 7 (M199→RPMI), test 8 (M199→SIM), and test 9 (M199→M199)]

| Test | RLU (mean ± SD) |
|---|---|
| Test 1 | 0.118±0.080 |
| Test 2 | 2.671±2.636 |
| Test 3 | 0.010±0.008 |
| Test 4 | 2.404±1.015 |
| Test 5 | 49.567±9.099 |
| Test 6 | 0.196±0.219 |
| Test 7 | 0.107±0.051 |
| Test 8 | 1.018±0.547 |
| Test 9 | 0.089±0.071 |

SD: Standard deviation, RLU: Relative luminescence units

the sample (17). Therefore, culture systems should be optimized not only for diagnostic purposes but also to enhance their physiological relevance and standardization for translational applications, including vaccine development, drug susceptibility testing, and experimental infection modeling (18). In this context, our study compared culture conditions that influence the production of infective metacyclic promastigotes of *L. tropica* using RPMI-1640, SIM, and M199 media. The findings suggest that culture medium composition is a critical determinant, affecting not only the parasite's capacity for *in vitro* proliferation but also the differentiation processes governing the transition from promastigote to infective phenotypes.

In *Leishmania* spp., the process of metacyclogenesis is largely regulated by the specific environmental conditions of the thoracic midgut of the vector. In permissive vectors such as *Lutzomyia longipalpis*, the acidic pH environment of the thoracic midgut has been suggested to facilitate the development of infective metacyclic forms (19). In our study, a notable increase was observed in the proportion of promastigotes exhibiting morphological traits consistent with metacyclogenesis when cultured in SIM medium at pH 5.5, despite the relatively low overall parasite density. This observation suggests a possible association between acidic conditions and the *in vitro* differentiation process of *L. tropica*. These findings are consistent with the report by Siripattanapipong et al. (15), who demonstrated a high rate of metacyclic differentiation of *L. martiniquensis* in SIM medium. Both studies indicate that SIM medium, through its ability to mimic environmental cues that trigger the transition to the metacyclic phenotype, appears to exert a pronounced inductive effect on differentiation. Furthermore, this observation is consistent with reports describing natural mechanisms of metacyclogenesis in *L. mexicana*, *L. braziliensis*, *L. donovani*, and *L. major*, where spontaneous acidification of the medium during the stationary phase has been shown to promote differentiation (6). In our study, the markedly elevated RLU observed in test 5 (SIM→SIM) (49.567±9.099; $p<0.05$) suggests that SIM medium exerts a stimulatory effect on metacyclogenesis. This finding is consistent with the notion that low pH and the compositional factors of SIM may collectively generate favorable signals that promote differentiation. Specific components within SIM may further enhance this effect by more effectively mimicking the environmental cues that drive the transition to the metacyclic phenotype. By contrast, culture media commonly used, such as RPMI-1640 (pH 7.2) and M199 (pH 7.4), exhibited markedly lower back-transformation efficiencies. For instance, test 1 (RPMI→RPMI), test 3 (RPMI→M199), test 6 (SIM→M199), test 7 (M199→RPMI), and test 9 (M199→M199) all yielded very low RLU values, suggesting that these media do not adequately reproduce the environmental stress conditions required for successful differentiation of field isolates. Although such media are frequently employed, their use may contribute to reduced infectivity potential during laboratory adaptation, particularly given the heightened sensitivity of field isolates to environmental stress.

The declining response rates to first-line therapies across all clinical forms of leishmaniasis represent a growing public health concern that threatens the effectiveness of current standard treatment regimens. This trend underscores the critical need for systematic monitoring of drug resistance and the assessment of resistance rates, particularly in endemic regions (20). This

underscores the need to standardize and strengthen *in vitro* drug susceptibility assays based on field isolates. As molecular markers of resistance are not yet fully defined or clinically validated, assays employing viable *Leishmania* stages remain the primary tool for the epidemiological surveillance of drug resistance. However, the reliable assessment of drug susceptibility in patient-derived field isolates continues to face major methodological limitations due to the lack of standardized, reproducible, and physiologically relevant test systems. These shortcomings hinder the ability to predict treatment outcomes and pose significant challenges for the early detection of therapeutic failure. Consequently, there remains an urgent need for more robust and standardized *in vitro* systems that can provide biologically meaningful and reliable evaluations of clinical *Leishmania* isolates (7). In this study, the PRTA was employed as an *in vitro* system to evaluate the controlled back-transformation of amastigotes isolated from infected host cells into promastigotes. The assay represents a platform with potential utility in both diagnostic and translational research contexts. The differentiated THP-1 macrophage model used herein demonstrates a high degree of biological relevance. Specifically, motile promastigotes were observed microscopically within 24 hours after transferring infected host cells from 37 °C to 26 °C, and by 96 hours, a high density of promastigotes was confirmed, both freely proliferating in extracellular culture and still residing intracellularly (21). These findings suggest that the PRTA protocol can model the transition of *Leishmania* to the infective stage under laboratory conditions in a biologically relevant manner and may serve as a suitable platform for drug susceptibility testing.

PRTA is an *in vitro* technique used to evaluate the reversion of parasites isolated from infected host cells to proliferative forms. The reliability of PRTA depends on the careful execution of several key procedural steps. One critical step is the effective removal of non-internalized *Leishmania* promastigotes from the culture after infection. Infected THP-1 macrophage cultures were washed multiple times with PBS, ensuring that only intracellular parasites remained in the assay system. This washing step minimizes the risk of false-positive results and enables the generation of more reliable data in subsequent analyses (8-21). The second critical step is the lysis stage, which aims to release intracellular amastigotes. This is achieved by subjecting infected THP-1 cells to a brief lysis with a buffer containing 0.05% SDS, allowing controlled disruption of host cell membranes. Such controlled lysis facilitates the release of intact amastigotes and is intended to enhance the overall sensitivity of the assay (8,22). In this study, the transformation of viable amastigotes into promastigotes progressed gradually following the transfer of infected macrophage cultures from 37 °C to 26 °C. Motile promastigotes of *L. tropica* were microscopically detected as early as 24 hours after incubation at 26 °C, both within the cytoplasm of host macrophages and in the extracellular culture medium. Over time, parasites entered an active proliferation phase; by 96 hours, large numbers of extracellular promastigotes were observed, either freely motile in suspension or clustered in colony-like aggregates. Notably, a subset of promastigotes remained within macrophages even at 96 hours, as confirmed by microscopic examination. These findings demonstrate that the back-transformation from intracellular amastigotes to promastigotes can be reliably monitored under controlled culture conditions and further support the biological relevance of the PRTA system. Both critical procedural steps can be adapted to automated platforms, allowing the assay to retain reproducibility and high efficiency through standardization. In addition, re-washing infected cells after treatment with drugs or test compounds eliminates residual free promastigotes from the culture medium. Collectively, these strategies enhance the reliability of PRTA and facilitate its integration into large-scale screening applications (8). Such cell-based models not only provide a more accurate representation of host–parasite interactions but are also well suited for translational research due to their compatibility with high-throughput screening systems. Considering the homogeneity, standardizability, and ethical sustainability of the cell source, differentiated THP-1 macrophages represent a valuable model system for intracellular drug screening targeting the amastigote form of *Leishmania*. Nevertheless, this study has certain methodological limitations. First, only three culture media were evaluated, and a broader parametric analysis encompassing different medium components (e.g., serum types, glucose concentrations, or ionic compositions) was not addressed. Second, the assessment relied solely on RLU measurements without complementary phenotypic or molecular characterization of metacyclic differentiation. Future studies should integrate multi-level approaches, including LPG analysis, infectivity validation through *in vitro* macrophage infections, and gene expression profiling, to provide a more comprehensive understanding

## CONCLUSION

This study compared the effects of different culture media (RPMI-1640, SIM, and M199) on promastigote production, preconditioning, and back-transformation of *L. tropica* amastigotes. The data indicate that culture medium composition is a critical determinant, particularly in the generation of metacyclic promastigotes. Among the tested media, SIM emerged as the most effective medium for producing infective promastigotes under experimental conditions, with significantly higher efficiency than the other media ($p<0.05$). Supporting the PRTA protocol with SIM-based conditions enhanced the physiological relevance of experimental infection models and improved the reliability of *in vitro* drug susceptibility analyses. Moreover, the ability of this model to dynamically track the transformation of intracellular amastigotes highlights its potential as a useful tool in translational research. Overall, the findings underscore the need for standardized and biologically relevant test systems for the evaluation of clinical field isolates. This work provides novel insights that may contribute to the refinement of experimental approaches in Leishmania biology and the optimization of therapeutic strategies.

### *Ethics

**Ethics Committee Approval:** This study was conducted with the approval of the Health Sciences Ethics Committee of the Manisa Celal Bayar University Faculty of Medicine (date: 07.05.2025, decision no: 20.478.486/3098).

**Informed Consent:** Patient consent was not required, as the study was performed using *Leishmania tropica* strains and THP-1 cells obtained from a parasite bank, without direct use of human clinical specimens.

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### *Footnotes*

### **Authorship Contributions*

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

**Conflict of Interest:** One of the authors of this article (A.E.) is a member of the Editorial Board of this journal. He was completely blinded to the peer review process of the article.

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## REFERENCES

1. Kato H. Epidemiology of leishmaniasis: risk factors for its pathology and infection. Parasitol Int. 2025; 105: 102999.
2. Özbel Y, Töz S, Muñoz C, Ortuño M, Jumakanova Z, Pérez-Cutillas P, et al. The current epidemiology of leishmaniasis in Turkey, Azerbaijan and Georgia and implications for disease emergence in European countries. Zoonoses Public Health. 2022; 69: 395-407.
3. Ponte-Sucre A, Gamarro F, Dujardin JC, Barrett MP, López-Vélez R, García-Hernández R, et al. Drug resistance and treatment failure in leishmaniasis: a 21st century challenge. PLoS Negl Trop Dis. 2017; 11: e0006052.
4. Clos J, Grünebast J, Holm M. Promastigote-to-amastigote conversion in *Leishmania* spp.-A Molecular View. Pathogens. 2022; 11: 1052.
5. Dias-Lopes G, Zabala-Peñafiel A, de Albuquerque-Melo BC, Souza-Silva F, Menaguali do Canto L, Cysne-Finkelstein L, et al. Axenic amastigotes of *Leishmania* species as a suitable model for *in vitro* studies. Acta Trop. 2021; 220: 105956.
6. Chanmol W, Jariyapan N, Preativatanyou K, Mano C, Tippawangkosol P, Somboon P, et al. Timulation of metacyclogenesis in *Leishmania (Mundinia) orientalis* for mass production of metacyclic promastigotes. Front Cell Infect Microbiol. 2022; 12: 992741.
7. da Luz RI, Vermeersch M, Dujardin JC, Cos P, Maes L. *In vitro* sensitivity testing of *Leishmania* clinical field isolates: preconditioning of promastigotes enhances infectivity for macrophage host cells. Antimicrob Agents Chemother. 2009; 53: 5197-203.
8. Jain SK, Sahu R, Walker LA, Tekwani BL. A parasite rescue and transformation assay for antileishmanial screening against intracellular *Leishmania donovani* amastigotes in THP1 human acute monocytic leukemia cell line. J Vis Exp. 2012; 30: 4054.
9. Bates PA, Tetley L. *Leishmania mexicana*: induction of metacyclogenesis by cultivation of promastigotes at acidic pH. Exp Parasitol. 1993; 76: 412-23.
10. Li J, Zheng ZW, Natarajan G, Chen QW, Chen DL, Chen JP. The first successful report of the *in vitro* life cycle of Chinese *Leishmania*: the *in vitro* conversion of *Leishmania* amastigotes has been raised to 94% by testing 216 culture medium compound. Acta Parasitol. 2017; 62: 154-63.
11. Özbilgin A, Çavuş İ, Yıldırım A, Kaya T, Ertabaklar H. Evaluation of *in vitro* and *in vivo* drug efficacy over *Leishmania tropica*: a pilot study. Turkiye Parazitol Derg. 2018; 42: 11-9.
12. Yıldırım A, Aksoy T, Kayalar H, Balcıoğlu İC. Semen cannabis and oleum hyperici: antileishmanial activity against *Leishmania tropica* promastigotes and intracellular amastigotes. Parasitol Int. 2024; 103: 102950.
13. Aksoy T, Kilimcioğlu AA. Thymol's antileishmanial activity and its impact on host cytokine profiles: *In vitro* and *ex vivo* studies on *Leishmania tropica*. Parasitol Int. 2026; 110: 103139.
14. Santarém N, Cunha J, Silvestre R, Silva C, Moreira D, Ouellette M, et al. The impact of distinct culture media in *Leishmania infantum* biology and infectivity. Parasitology. 2014; 141: 192-205.
15. Siripattanapipong S, Boontanom P, Leelayoova S, Mungthin M, Tan-Ariya P. *In vitro* growth characteristics and morphological differentiation of *Leishmania martiniquensis* promastigotes in different culture media. Acta Trop. 2019; 197: 105039.
16. Reimão JQ, Coser EM, Lee MR, Coelho AC. Laboratory diagnosis of cutaneous and visceral leishmaniasis: current and future methods. Microorganisms. 2020; 8: 1632.
17. Castelli G, Oliveri E, Valenza V, Giardina S, Facciponte F, La Russa F, et al. Cultivation of Protozoa parasites *in vitro*: growth potential in conventional culture media versus RPMI-PY medium. Vet Sci. 2023; 10: 252.
18. Zhou T, Reji R, Kairon RS, Chiam KH. A review of algorithmic approaches for cell culture media optimization. Front Bioeng Biotechnol. 2023; 11: 1195294.
19. Santos VC, Araujo RN, Machado LA, Pereira MH, Gontijo NF. The physiology of the midgut of *Lutzomyia longipalpis* (Lutz and Neiva 1912): pH in different physiological conditions and mechanisms involved in its control. J Exp Biol. 2008; 211: 2792-8.
20. Croft SL, Sundar S, Fairlamb AH. Drug resistance in leishmaniasis. Clin Microbiol Rev. 2006; 19: 111-26.
21. Sifontes-Rodríguez S, Escalona-Montaño AR, Sánchez-Almaraz DA, Pérez-Olvera O, Aguirre-García MM. Detergent-free parasite transformation and replication assay for drug screening against intracellular *Leishmania* amastigotes. J Microbiol Methods. 2023; 215: 106847.
22. Kimcharoensuk S, Leelayoova S, Mungthin M, Nawattanapaibool N, Tan-Ariya P, Siripattanapipong S. *In vitro* drug susceptibility using a parasite-rescue and transformation assay of *Leishmania (Mundinia) martiniquensis* and *Leishmania (Mundinia) orientalis* amastigotes against antileishmanial drugs. Acta Trop. 2024; 249: 107081.

*Original Investigation*
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# Seroprevalence of *Toxoplasma gondii* among Children with Beta-thalassemia Major and its Effect on Selected Immune Parameters

## *Beta-Talassemi Major Hastası Çocuklarda Toxoplasma gondii Seroprevalansı ve Bazı Bağışıklık Parametreleri Üzerindeki Etkisi*

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## ABSTRACT

**Objective:** The seroprevalence of anti-*Toxoplasma gondii* antibodies among children with beta-thalassemia major is not explored yet. The effect of *T. gondii* infection on the immune response of patients and to analyse its potential association with selected immune parameters, namely interleukin (IL)-10, IL-12, IL-17, interferon-gamma (IFN-γ), and the concentrations of $CD4^+$ and $CD8^+$ cluster of differentiation markers in serum is examined. The study aimed to investigate the seroprevalence of anti-*T. gondii* antibodies and the immunological impact in children with β-thalassemia major.

**Methods:** The study included 90 children (71 BTM and 19 controls), aged 6-15 years. Serum, obtained from 5 mL blood samples, was used to detect anti-*T. gondii* immunoglobulin (Ig)G and IgM antibodies by rapid tests, ELISA, and VIDAS, while cytokine levels were measured by ELISA. After serological testing, participants were classified into 4 groups: thalassemia + parasite, thalassemia only, parasite only, and healthy control. Data were analysed using chi-square and Kruskal-Wallis tests ($p<0.05$), with results expressed as mean ± standard error for statistical comparison between study groups.

**Results:** The total seroprevalence of *T. gondii* was 30.0% (27/90); it was 35.21% among thalassemia patients and 10.53% (2/19) in the control group. Among thalassemia patients, IgM and IgG seropositivity rates were 8.45% (6/71) and 26.76%, respectively; in controls, they were 0.0% and 10.53% (2/19). BTM children showed significantly lower levels of IL-10, IL-12, IL-17, $CD4^+$, and $CD8^+$ ($p<0.05$), whereas IFN-γ ($p>0.12$).

**Conclusion:** Children with BTM showed a significantly higher seroprevalence of *T. gondii* than healthy controls. Infection was associated with significant alterations in immune parameters, indicating increased immune dysregulation in these patients.

**Keywords:** *Toxoplasma gondii*, beta-thalassemia major, interleukins, IFN-γ

## ÖZ

**Amaç:** Majör beta-talasemi hastası çocuklarda anti-*Toxoplasma gondii* antikorlarının seroprevalansı henüz araştırılmamıştır. Bu çalışmada, *T. gondii* enfeksiyonunun hastaların bağışıklık tepkisi üzerindeki etkisi ve seçilmiş bağışıklık parametreleri, yani interlökin (IL)-10, IL-12, IL-17, interferon-gamma (IFN-γ) ile serumdaki $CD4^+$ ve $CD8^+$ farklılaşma kümesi belirteçlerinin konsantrasyonları arasındaki potansiyel ilişki analiz edilmiştir. Çalışma, β-talasemi majör hastası çocuklarda anti-*T. gondii* antikorlarının seroprevalansını ve bunun immünolojik etkisini araştırmayı amaçlamıştır.

**Yöntemler:** Çalışmaya 6-15 yaş aralığındaki 90 çocuk (71'i β-talasemi majör hastası, 19'u sağlıklı kontrol grubu) dahil edildi. 5 mL kan örneklerinden elde edilen serum, hızlı test, ELISA ve VIDAS yöntemleriyle anti-*T. gondii* immünoglobulin (Ig)G ve IgM antikorlarını saptamak için kullanılırken, sitokin düzeyleri ELISA ile ölçüldü. Serolojik testlerin ardından, katılımcı çocuklar dört gruba ayrıldı: talasemi + parazit, sadece talasemi, sadece parazit ve sağlıklı kontrol. Veriler, ki-kare ve Kruskal-Wallis testleri ($p<0.05$) kullanılarak analiz edildi ve sonuçlar, çalışma grupları arasında istatistiksel karşılaştırma amacıyla ortalama ± standart error olarak ifade edildi.

**Sonuç:** *T. gondii*'nin toplam seroprevalansı %30,0 (27/90) olarak saptandı; talasemi hastalarında bu oran %35,21 iken, sağlıklı kontrol grubunda %10,53 idi. Talasemi hastalarında. IgM ve IgG seropozitifliği sırasıyla %8,45 (6/71) ve %26,76 iken, kontrol grubunda bu oranlar %0,0 ve %10,53 (2/19) idi. BTM'li çocuklarda IL-10, IL-12, IL-17, $CD4^+$ ve $CD8^+$ düzeyleri istatistiksel olarak anlamlı derecede daha düşüktü ($p<0.05$), buna karşın IFN-γ ($p>0.12$).

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**Sonuç:** Majör β-talasemi hastası çocuklarda, sağlıklı kontrol grubuna kıyasla *T. gondii* seroprevalansı istatistiksel olarak anlamlı derecede daha yüksek bulunmuştur. Enfeksiyon, bağışıklık parametrelerinde istatistiksel olarak anlamlı değişikliklerle ilişkilendirilmiş olup, bu da söz konusu hastalarda bağışıklık düzensizliğinin arttığını göstermektedir.
**Anahtar Kelimeler:** *Toxoplasma gondii*, majör beta-talasemi, interlökinler, IFN-γ

## INTRODUCTION

*Toxoplasma gondii* is an obligate intracellular protozoan parasite that infects more than one-third of the world's population and causes one of the most prevalent parasitic diseases globally (1). The parasite possesses three morphological stages (tachyzoite), (bradyzoite), and (oocysts) and three common major genotypes (I, II, and III) associated with human infection (2,3) and all these strains have been registered in Iraq in Maysan province (3). Toxoplasmosis is of considerable global public health significance. While immunocompetent individuals may present with mild or no symptoms, the parasite can cause serious complications in immunocompromised patients, particularly those with thalassemia who receive regular blood transfusions (4,5). Recent regional studies have also highlighted the continuing importance of parasitic infections in Maysan Province and southern Iraq. A previous sero-epidemiological study in Maysan reported the presence of *T. gondii* antibodies among men and pregnant women, indicating that toxoplasmosis remains an important local public health issue (6).

Beta-thalassemia major is the most prevalent hereditary haemoglobin disorder, resulting from mutations in the genes encoding beta-globin chains, leading to severe haemolytic anemia and immune dysfunction (7,8). The tachyzoite stage remains viable in stored blood at 4 °C for up to 50 days (9), and the seroprevalence of *T. gondii* among blood donors is estimated at 33% (10). Cytokines constitute a central axis of the immune response against *T. gondii*. Infection induces notable changes in interleukin (IL)-10, IL-12, IL-17, IFN-γ, $CD4^+$, and $CD8^+$ levels (11,8). The chronic immune suppression resulting from repeated blood transfusions has been linked to increased susceptibility of thalassemia patients to *T. gondii* infection (12). Despite the recognized susceptibility of patients with transfusion-dependent beta-thalassemia major to infection with *T. gondii*, data on its seroprevalence and associated immune responses in children, particularly in southern Iraq, remain scarce. Therefore, the present study aimed to determine the seroprevalence of *T. gondii* among children with BTM major and to evaluate its association with selected immune parameters.

## METHODS

### *Sample Collection and Study Design*

A total of 90 venous blood samples (5 mL each) were collected from beta-thalassemia major patients (n=71). Samples were obtained from selected children with beta-thalassemia major who had documented medical histories; cases with conditions other than *T. gondii* infection were excluded. Healthy controls (n=19) have no medical history. Aged 6-15 years, between November 2025 and mid-February 2026, from the Center for Hereditary and Haemorrhagic Blood Diseases (Thalassemia Center), from Maysan Teaching Children's and Maternity Hospital, Maysan, Iraq. Because antibodies to toxoplasmosis were detected in two children in the control group of children without thalassemia, the study population was divided into four groups: (i) thalassemia with *T. gondii* (thalassemia + parasite); (ii) thalassemia only; (iii) non-thalassemia with toxoplasmosis (parasite only); and (iv) non-thalassemia without toxoplasmosis (healthy control). Blood samples for thalassemia patients were drawn before transfusion, placed in gel tubes, centrifuged at 3,000 rpm for 10 minutes to obtain serum, and stored at -20°C until use.

Ethical approval for this study was obtained from the Research and Knowledge Management Unit, Training and Human Development Center, Misan Health Directorate, Ministry of Health, Republic of Iraq (approval no: 709; date: 27.11.2025). Written informed consent was obtained from the parents or legal guardians of all participating children prior to their inclusion in the study.

### *Serological Diagnosis of T. gondii Infection*

Three diagnostic techniques are employed according to the manufacturer's instructions:

(a) Rapid antigen detection test: an immunochromatographic flow assay for initial qualitative detection of immunoglobulin (Ig) G and IgM,

(b) VIDAS is an automated system employing enzyme-linked fluorescent assay,

(c) Enzyme-linked immunosorbent assay (ELISA): indirect sandwich ELISA for qualitative detection of IgG and IgM (Nipigon Co., Canada) and for quantitative measurement of the concentrations of human interleukins and cluster of differentiation markers (IL-10, IL-12, IL-17, IFN-γ, $CD4^+$ and $CD8^+$). Uniform procedures were used in the ELISA protocol for all serological and immunological markers in this study. Results were obtained using a semi-automatic ELISA system (Bio kit, USA), with optical density (OD) measured at 450 nm.

### *Assessment of Immune Parameters: IL-10, IL-12, IL-17, IFN-γ, $CD4^+$, $CD8^+$*

Sandwich-ELISA was used to quantify the levels of certain cytokines. It was performed according to the manufacturer's instructions provided with each kit (SunLong Biotech Co., China). Each Microelisa strip plate provided in this kit was pre-coated with one of the human interleukins or cluster differentiation markers (IL-10, IL-12, IL-17, IFN-γ, $CD4^+$, and $CD8^+$). The cells were loaded at the bottom of the wells with 50 µl of the serum samples diluted 1:5 (10 µl serum: 40 µl diluent), mixed well, and shaken. Then each microtiter plate was sealed with Closure plate membrane and incubated at 37 °C for 30 minutes. T he membrane was carefully removed from each microtiter plate and washed five times with a washing solution. Then, 50 µl of horseradish peroxidase-conjugated antibodies specific for human interleukins or cluster differentiation markers (IL-10, IL-12, IL-17, IFN-γ, $CD4^+$, and $CD8^+$) were added to each well except the blank control well, and the microtiter plates were incubated at 37 °C for 30 minutes. The microtiter plates were washed as described above. Then, 50 µl of chromogen A and 50 µl of chromogen B were added and carefully mixed and incubated at 37 °C for 15 minutes in the dark. Finally, the reaction was terminated by adding 50 microliters of stop solution.

The OD of the samples was measured spectrophotometrically at a wavelength of 450 nm using the ELISA reader. The OD value was proportional to the concentration of each parameter. You can estimate the concentration of each parameter in the samples by comparing sample ODs to the standard curve.

### *Statistical Analysis*

Statistical analyses were performed using SPSS version 27. Chi-square test ($\chi^2$) and Kruskal-Wallis test (H statistic) were applied using a significance level of 0.05 to assess statistical significance. Results are expressed as mean ± standard error.

## RESULTS

### *T. gondii Infection Among Beta-thalassaemia Major Patients*

The current results (Table 1) show a seroprevalence of *T. gondii* of 35.21% (25/71) among children with beta-thalassemia major, which is higher than the seroprevalence recorded in non-infected children with thalassemia (control group), 10.53% (2/19). A statistically significant relationship was found between beta-thalassemia major and toxoplasmosis ($\chi^2$=4.349, p<0.05).

### *Distribution of IgM and IgG Antibodies among Children Population*

The results of the current study (Table 2) showed that the overall seroprevalence of anti-*T. gondii* IgM and IgG antibodies among the children participating in the study was 30% (27/90). The participating children with thalassemia recorded the highest seroprevalence of the two antibodies (anti-*T. gondii* IgM + anti-*T.* gondii IgG*)*, at 35.21% (25/71), compared with 10.53% (2/19) among children without thalassemia. Table 2 also shows that the seroprevalence of the anti-*T. gondii* IgM antibody among children with thalassemia was 8.45%, which is lower than the seroprevalence of the anti-*T. gondii* IgG antibody (26.76%). On the other hand, the seroprevalence of the anti-*T. gondii* IgG antibody among children without thalassemia was 10.53%; in contrast, no seropositivity for anti-*T. gondii* IgM was detected in sera from children without thalassemia. This study did not record the presence of both antibodies in any of the children's sera.

### *Effect of T. gondii on the Levels of Some Cytokines, IL-10, IL-12, IL-17, and IFN-γ, Among Children with Thalassemia*

The mean serum IL-10 levels of the study groups are shown in Table 3: IL-10 levels were 18.342±1.423 pg/mL in the thalassemia + parasite group and 20.461±2.063 pg/mL in the thalassemia only group, values that were close to the healthy control group (19.876±1.053 pg/mL), but all were lower than the parasite only group, which showed the highest mean level (31.700±1.700 pg/mL).

The distribution of the IL-10 levels among participating children in the four groups is shown in Figure 1. There are statistically significant differences in mean IL-10 levels among the four groups (H=8.896, p<0.05). The study also found (Table 3) that the mean of IL-12 levels in the serum of the children group: "thalassemia + parasite", and the children group: "thalassemia only" were 10.074±1.473 and 13.202±2.172 pg/mL, respectively, which are close to each other but lower than the level of 22.123±2.359 pg/mL in "healthy control". In contrast, the mean of IL-12 level in the children group: "parasite only" of 45±5.700 pg/mL, is higher than among the other three groups of children (see Table 3). The distribution of the IL-12 levels among participating children across the four groups was shown in Figure 2, which confirms these results. There are statistically significant differences in mean IL-10 levels among all groups. Statistically significant differences in mean IL-12 levels were observed among the groups (H=24. 217, p<0.001).

**Table 1.** Relationship between *Toxoplasma gondii* and beta-thalassemia major

| | | ***Toxoplasma gondii*** | | | | **Total** |
|---|---|---|---|---|---|---|
| | | **Infected** | | **Non-infected** | | |
| | | **No.** | **%** | **No.** | **%** | |
| **Thalassemia** | **Infected** | 25 | 35.21 | 46 | 64.8 | 71 |
| | **Non-infected** | 2* | 10.53 | 17 | 89.47 | 19 |
| | **Total** | 27 | 30 | 63 | 70 | 90 |
| | | **$\chi^2$=4.349, p<0.05** | | | | |

*: From a group of healthy control children with no history of genetic or other diseases, only two cases of toxoplasmosis were detected

**Table 2.** Distribution of anti-*Toxoplasma gondii* IgM and IgG antibodies among participated children in this study

| | | ***Toxoplasma gondii* seropositive** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **anti-*Toxoplasma gondii* IgM** | | | | **anti-*Toxoplasma gondii* IgG** | | | | **Total** | | | |
| | | **Yes** | **No** | **Total** | **Infected %** | **Yes** | **No** | **Total** | **Infected%** | **Yes** | **No** | **Total** | **Infected %** |
| **Thalassemia** | Yes | 6 | 65 | 71 | 8.45 | 19 | 52 | 71 | 26.76 | 25 | 56 | 71 | 35.21 |
| | No | 0 | 19 | 19 | 0.00 | 2 | 17 | 19 | 10.53 | 2 | 17 | 19 | 10.53 |
| | Total | 6 | 84 | 90 | 6.67 | 21 | 69 | 90 | 23.33 | 27 | 63 | 90 | 30 |

IgM: Immunoglobulin M, IgG: Immunoglobulin G

The results also showed (Table 3) that the mean of IL-17 levels in the serum of the children group: "thalassemia + parasite", and the group: "thalassemia only" were 112.944±6.886 and 127.021±8.624 pg/mL, respectively, which are lower than the mean level of 194.647±13.162 pg/mL in the "healthy control". In contrast, the mean level of IL-17 of 305±12 pg/ml in the children group: "parasite only" is higher than those in all three other groups of children. The distribution of IL-17 levels among the participating children across the four groups is shown in Figure 3 and supports these results. There are statistically significant differences in mean IL-17 levels among all groups of children in this study (H=27.38, p<0.001). The results of the study also showed (Table 3) that the mean of IFN-γ level in the serum of the children group: "thalassemia + parasite", "healthy control" and "parasite only", are 90.271±17.988, 103.200±2.200 and 101.17±9.712 pg/mL, respectively, are lower than that of 129.578±33.233 pg/mL of the children group: "thalassemia only". The distribution of the IFN-γ levels among participating children in the four groups is shown in Figure 4. There are no statistically significant differences between the mean IFN-γ levels for the different groups of children (H=10.936, p>0.12).

### *CD4⁺ and CD8⁺ T-cell Levels Across Study Groups*

The current study (Table 4) shows the means of the levels of the cluster differentiation markers of $CD4^+$ and $CD8^+$ in serum of both children thalassemia groups: thalassaemia + parasite and thalassaemia only, are 0.825±0.055 and 0.790±0.067ng, for $CD4^+$ respectively, and 4.136±0.369 and 3.800±0.308ng for $CD8^+$ respectively are lower than their means among control children groups who were not infected with thalassemia, whether infected with toxoplasma (parasite only) or not (healthy control), which were 1.064±0.048, 1.064±0.048 ng for $CD4^+$ and 4.450±0.650 and 4.517±0.247 ng for $CD8^+$ respectively. The distribution of the $CD4^+$ and $CD8^+$ levels among participating children in the four groups was shown in Figures 5 and 6, respectively, There are statistically significant differences between the means of the $CD4^+$ and the means of the $CD8^+$ levels of the four children groups, respectively [H=20.634 and H=13.281, (p<0.05 and 0.05)] respectively.

**Table 3.** The mean of cytokine levels (mean ± SE) pg/mL among different groups of participating children

| Group | No. | IL-10 mean ± SE | IL-12 mean ± SE | IL-17 mean ± SE | IFN-γ mean ± SE |
|---|---|---|---|---|---|
| Thalassaemia + parasite | 25 | 18.342±1.423 | 10.074±1.473 | 112.944±6.886 | 90.271±17.988 |
| Thalassaemia only | 46 | 20.461±2.063 | 13.202±2.172 | 127.021±8.624 | 129.578±33.233 |
| Parasite only | 2 | 31.700±1.700 | 45.000±5.700 | 305.000±12.000 | 103.200±2.200 |
| Healthy control | 17 | 19.876±1.053 | 22.123±2.359 | 194.647±13.162 | 101.170±9.712 |
| Total | 90 | 20.012±1.155 | 14.725±1.416 | 139.840±6.714 | 112.716±17.989 |
| H statistics value | | 8.896 | 24.217 | 27.380 | 10.936 |
| p | | <0.05 | <0.001 | <0.001 | >0.12 |

H: Kruskal-Wallis test value, SE: Standard error, IL: Interleukin, IFN-γ: Interferon-gamma

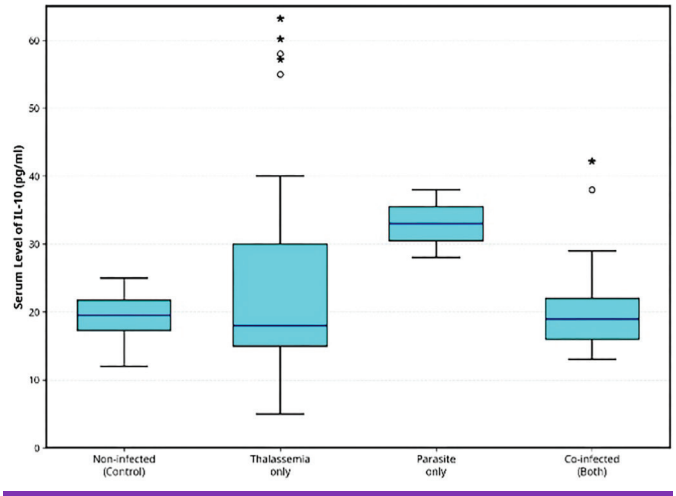


**Figure 1.** Show the distribution of interleukin-10 levels among the four study groups

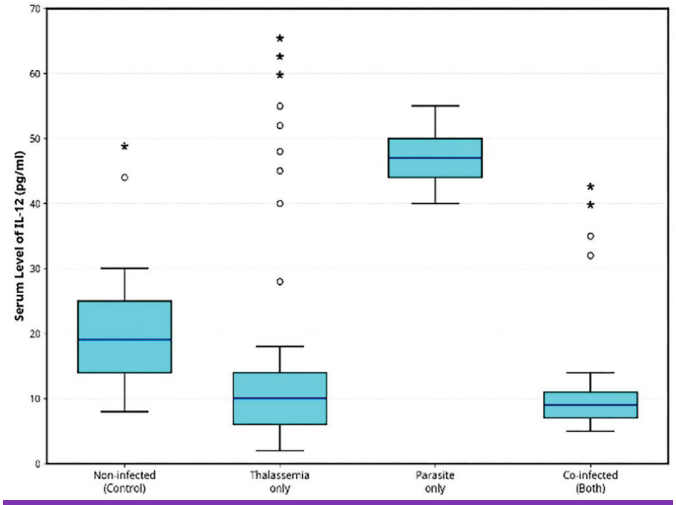


**Figure 2.** Show the distribution of interleukin-12 levels among the four study groups

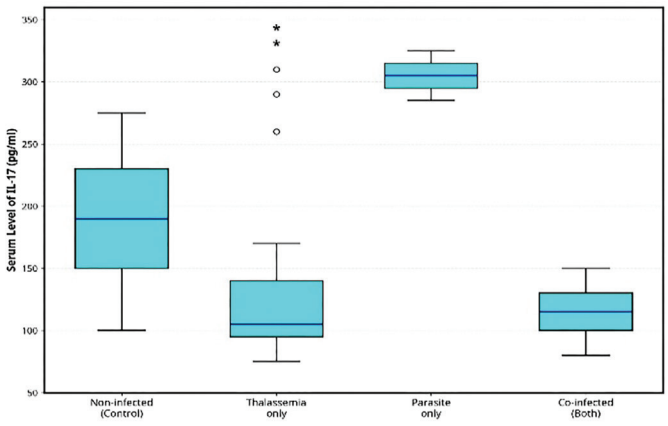


**Figure 3.** Show the distribution of interleukin-17 levels among the four study groups

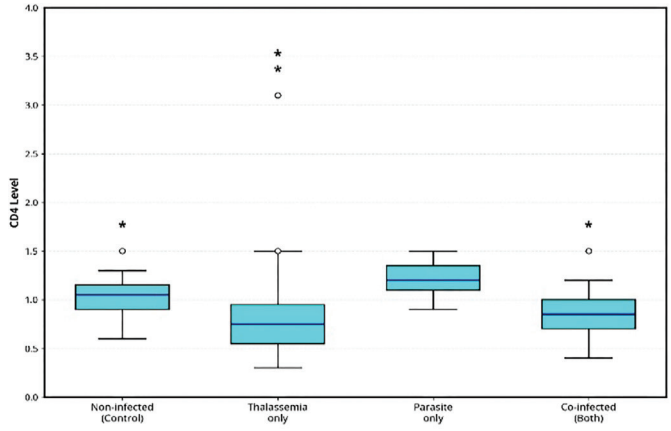


**Figure 5.** Show the distribution of CD4⁺ levels among the four study groups

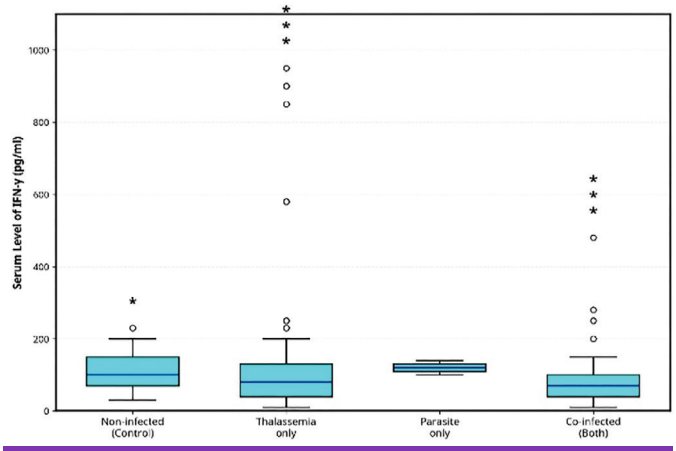


**Figure 4.** Show the distribution of IFN-γ levels among the four study groups
*IFN-γ: Interferon-gamma*

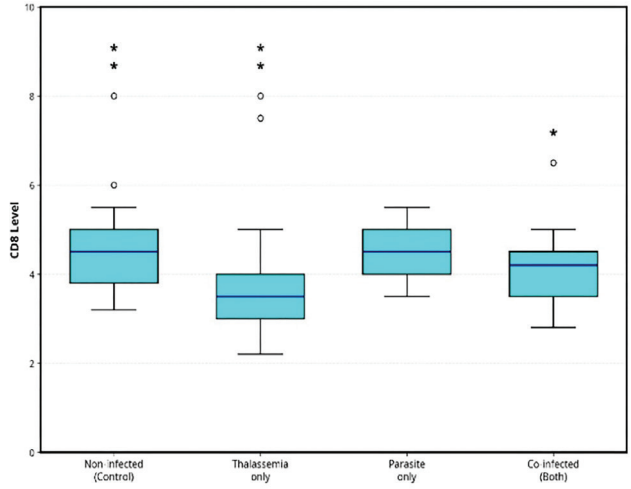


**Figure 6.** Show the distribution of CD8⁺ levels among the four study groups

**Table 4.** CD4⁺ and CD8⁺ T-cell levels (ng/mL, mean ± SE) across study groups

| Groups | No. | CD4⁺ mean ± SE ng/mL) | CD8⁺ mean ± SE (ng/mL) |
|---|---|---|---|
| Thalassaemia + parasite | 25 | 0.825±0.055 | 4.136±0.369 |
| Thalassaemia only | 46 | 0.790±0.067 | 3.800±0.308 |
| Parasite only | 2 | 1.170±0.270 | 4.450±0.650 |
| Healthy control | 17 | 1.064±0.048 | 4.517±0.247 |
| Total | 90 | 0.860±0.040 | 4.043±0.194 |
| H statistic | | 20.634 | 13.281 |
| p | | <0.05 | <0.05 |
| Normal range | | 0.08-4 ng/mL | 3-20 ng/mL |

H: Kruskal-Wallis test value, SE: Standard error

## DISCUSSION

### *T. gondii Infection in Beta-thalassaemia Major Patients*

The significant elevation of seroprevalence in thalassemia patients (35.2%) relative to controls (10.5%) is consistent with numerous studies confirming heightened toxoplasmosis susceptibility in this cohort owing to immune deficiency and repeated transfusions (13-15). An immunohistochemical study from Maysan Province documented the highest IgG seroprevalence (66.7%) in placental tissue of women who had experienced at least one abortion (3). An Egyptian study reported IgG seropositivity at 53.6% in beta-thalassemia major children and 18% in healthy controls (13). The elevation rate in thalassemia patients likely reflects the partial immune dysregulation induced by chronic transfusion dependency, which raises the probability of acquiring or reactivating *T. gondii* infection (12,8).

### *Distribution of IgM and IgG Antibodies*

The predominance of chronic (IgG-positive) over acute (IgM-positive) infection is consistent with an Iranian study reporting 30.67% IgG seropositivity using ELISA (14). with a Mosul-based study recording IgG positivity at 28% (15). and The current findings showed higher seroprevalence of anti-*T. gondii* IgG and IgM antibodies than those reported in an Egyptian study, where 10% (10/100) and 2% (2/100) of thalassemia patients were positive for IgG and IgM antibodies, respectively (16). The absence of cases with both antibodies simultaneously is noteworthy, suggesting that patients are either in the acute or the chronic phase of infection, with no concurrent transition stage observed. The higher proportion of chronic infection among thalassemia patients reflects the persistent immunosuppression characteristic of this disease, which permits the parasite to establish and maintain latent infection in tissue cysts (17). This pattern aligns with a Turkish study reporting 19.4% IgG and 5.5% IgM seropositivity in beta-thalassemia major patients (18). The current findings showed higher anti-*T. gondii* IgG seroprevalence than that reported by an Iranian study, in which IgG positivity was 13.6% (15/110) among thalassemia patients and 26.3% (29/110) among healthy controls (19).

### *Effect of T. gondii Infection on IL-10*

T-cell-derived IL-10 contributes to limiting excessive inflammation and supporting host survival. Therefore, chronic *T. gondii* infection, together with the immunosuppressive state associated with beta-thalassemia major, may impair T-cell function and consequently reduce IL-10 production (20-22). The reduction in IL-10 may also be related to immune dysregulation caused by repeated blood transfusions, iron overload, and splenectomy in patients with beta-thalassaemia major. Recurrent transfusions can alter cytokine profiles and impair dendritic cell function, thereby weakening cytokine regulation. In addition, splenectomised thalassaemic patients have been reported to produce significantly lower IL-10 concentrations than healthy controls, with values of 7.5±4.1 pg/mL compared with 9.2±3.4 pg/mL, supporting the role of splenectomy in reducing anti-inflammatory cytokine output (23,24). These findings are further supported by an Iraqi serological-genetic study conducted among children attending a thalassemia centre in Baghdad, which reported that children carrying seven to eight high-risk genetic variant alleles had a markedly increased disease risk, estimated at 12.35-fold (95% confidence interval: 7.18-21.25; $p<0.001$). This finding suggests that genetic susceptibility may contribute to impaired IL-10 regulation in children with beta-thalassemia major and may partly explain the lower IL-10 levels observed in the present study (25).

These discrepancies may reflect differences in disease stage, inflammatory burden, transfusion history, splenectomy status, and the degree of immune exhaustion among the studied cohorts (26,27). The discrepancy between the present study and reports of elevated IL-10 in active toxoplasmosis may also be attributed to differences in the phase of infection. Acute *T. gondii* infection is usually associated with active immune regulation and increased cytokine production, whereas chronic infection may lead to progressive immune exhaustion and reduced regulatory cytokine output. This interpretation is supported by the finding that women with active toxoplasmosis showed higher IL-10 concentrations than controls, with values of 10.9±1.7 pg/mL and 5.11±0.23 pg/mL, respectively. Thus, the lower IL-10 level in the present thalassemic groups may reflect the combined effects of chronic infection, immune suppression, and thalassemia-related immune dysfunction rather than toxoplasmosis (28).

### *Effect of T. gondii Infection on IL-12*

The reduced IL-12 observed in the present thalassemic groups may not reflect the usual immune response to *T. gondii* alone, but rather the combined effect of parasitic infection and beta-thalassemia-related immunosuppression. The contrast between the present findings and the elevated IL-12 reported in recent toxoplasmosis studies suggests that iron overload, chronic transfusion-related immune disturbance, and impaired macrophage function may override the expected IL-12-mediated Th1 activation in beta-thalassemia major patients (8,29,30).

This interpretation is supported by recent studies in non-thalassemic or immunologically distinct populations, in which *T. gondii* infection was associated with increased, rather than decreased IL-12. In an Iraqi study conducted in Thi-Qar province, IL-12 levels were higher in cardiac patients infected with toxoplasmosis than in controls, reaching 82.85 pg/mL in patients compared with 60.97 pg/mL in controls, indicating immune activation during infection. Similarly, a serological-immunological study from Al-Najaf reported increased IL-12 among breast cancer patients infected with *T. gondii*, with levels of 21±2.4 pg/mL compared with 8±1.04 pg/mL in controls (30).

### *Effect of T. gondii Infection on IL-17A*

IL-17A, primarily produced by Th17 cells, contributes to host defence against infectious agents by promoting neutrophil recruitment and proinflammatory responses. The significant decrease in IL-17A in both thalassemia groups may reflect impaired Th17-mediated immunity during chronic *T. gondii* infection and beta-thalassemia-associated immune suppression (31). This finding agrees with the chronic-phase pattern reported by (32), where IL-17A was much lower in chronic toxoplasmosis (12.96±6.039 pg/mL) than in acute infection (202.0±24.80 pg/mL). This supports the interpretation that the reduced IL-17A in the current study may be related to the predominance of chronic infection and immune exhaustion (32) and not agree with (33). Who reported higher IL-17A levels in women with recurrent abortion associated with toxoplasmosis (6.584±0.854 pg/mL) compared with healthy controls (4.284±0.728 pg/mL). This variation may be related to differences in study population, clinical condition, and immune status (33).

### *Effect of T. gondii Infection on IFN-γ*

IFN-γ is a key cytokine in cellular immunity against *T. gondii*. The non-significant increase observed in the present study may reflect a modest immune response to the infection in children with beta-thalassemia major (34).

The present findings agree with a recent Iraqi study reporting higher IFN-γ levels in thalassaemic groups, with the highest concentration recorded in beta-thalassemia patients only (23.900±0.218 pg/mL), followed by beta-thalassaemia patients infected with *T. gondii* (23.404±0.235 pg/mL). Lower levels were recorded in *T. gondii*-positive controls (14.394±0.264 pg/mL) and healthy controls (10.958±0.178 pg/mL), supporting the role of IFN-γ activation in thalassaemia-associated immune responses (35). In a complementary context related to *T. gondii* infection, the

present findings agree with an Iraqi study that reported elevated serum IFN-γ levels in women with toxoplasmosis-associated miscarriage, reaching 47.080±4.213 pg/mL compared with 25.298±4.751 pg/mL in healthy controls. This increase supports the role of IFN-γ in activating cell-mediated immunity against *T. gondii* infection (36). The results also agree with a recent Brazilian study showing no significant difference in serum IFN-γ among pregnant women with acute toxoplasmosis (29.00±15.25 pg/mL), chronic toxoplasmosis (33.61±28.79 pg/mL), and uninfected controls (29.79±19.16 pg/mL). This suggests that IFN-γ may remain elevated or relatively sustained across infection phases, unlike cytokines that decline clearly during chronic infection (32).

### *Effect of T. gondii Infection on CD4⁺ and CD8⁺ T-cells*

Chronic *T. gondii* infection may further aggravate immune suppression through exhaustion of CD4⁺ and CD8⁺ T-cells. This immune exhaustion can reduce the ability of T-cells to maintain effective anti-parasitic responses and may contribute to parasite persistence or reactivation, particularly in immunocompromised thalassemia patients exposed to chronic immune stimulation from repeated transfusions (21).

The present findings are consistent with a recent Iraqi study that reported altered T-cell counts in patients with beta-thalassemia major infected with *T. gondii*. CD4⁺ T-cells were lower in infected thalassemia patients, with a mean of 530.63±84.15, compared with 804.62±1.71 in controls. However, the reported CD8⁺ T-cell values appear higher in infected thalassemia patients, 870.67±1.25, compared with 597.78±3.96 in controls (37).

**Clinical significance:** The increased seroprevalence of *T. gondii* and associated immune alterations observed in children with beta-thalassemia major support routine serological screening and immunological monitoring as part of their clinical management to reduce infection-related complications. Approval of a pre-transfusion blood test for toxoplasmosis as a routine screening test for thalassemia patients

**Scientific contribution:** This study provides novel evidence from Maysan Province that children with beta-thalassemia major exhibit a higher seroprevalence of *T. gondii,* accompanied by significant immune alterations, thereby advancing current understanding of the immunological impact of toxoplasmosis in this vulnerable population and supporting routine serological and immunological monitoring.

### *Study Limitations*

We encountered considerable difficulty convincing parents of healthy children to participate in this study, and at times even persuading the patients themselves.

## CONCLUSION

The findings of the present study suggest that beta-thalassemia major may increase children's susceptibility to *Toxoplasma gondii* infection through transfusion-related immune dysregulation and impaired cellular immunity. The observed changes in cytokine profiles and T-cell responses also indicate weakened immune control of the parasite and possible immune exhaustion, highlighting the need to strengthen blood screening and preventive measures to reduce the risk of infection in this immunocompromised group.

### *Ethics

**Ethics Committee Approval:** Ethical approval for this study was obtained from the Research and Knowledge Management Unit, Training and Human Development Center, Misan Health Directorate, Ministry of Health, Republic of Iraq (approval no: 709; date: 27.11.2025).

**Informed Consent:** Written informed consent was obtained from the parents or legal guardians of all participating children prior to their inclusion in the study.

### *Footnotes

### *Authorship Contributions

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

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

**Financial Disclosure:** The authors declared that this study received no financial support.

## REFERENCES

1. Schultz AJ, Carruthers VB. *Toxoplasma gondii* LCAT primarily contributes to tachyzoite egress. mSphere. 2018; 3: e00073-18.
2. Dubey JP. Advances in the life cycle of *Toxoplasma gondii*. Int J Parasitol. 1998; 28: 1019-24.
3. Allamy RH, Alsaady HAM. Using antibody rapid test, RT-qPCR, and RFLP/PCR for detecting *Toxoplasma gondii* in the placenta of abortion women and determining the genotype in Maysan province, Iraq. J Wasit Sci Med. 2023; 16: 28-43.
4. Augusto L, Martynowicz J, Amin PH, Alakhras NS, Kaplan MH, Wek RC, et al. *Toxoplasma gondii* co-opts the unfolded protein response to enhance migration and dissemination of infected host cells. mBio. 2020; 11: e00915-20.
5. Al-Aubaidi IK, Saeed SA, Jaaffar AI. Blood lymphocytes detection in Iraqi diabetic type 2 patients infected with chronic toxoplasmosis by using flow cytometry. Indian J Forensic Med Toxicol. 2020; 14: 2297-303.
6. Alsaady HAM, Abboodı AL, Abbood ES. Sero-epidemiology of *Toxoplasma gondii* among men and pregnant women in Maysan Province, south of Iraq. Iran J Ichthyol. 2021; 8(Suppl 1): 27-37.
7. Galanello R, Origa R. Beta-thalassemia. Orphanet J Rare Dis. 2010; 5: 11.
8. Gluba-Brzózka A, Franczyk B, Rysz-Górzyńska M, Rokicki R, Koziarska-Rościszewska M, Rysz J. Pathomechanisms of immunological disturbances in β-thalassemia. Int J Mol Sci.. 2021; 22: 9677.
9. Shkarin VV. O sokhraniaemosti toksoplazm v konservirovannoĭ krovi donora e ee komponentakh [Survival of toxoplasma in preserved donor blood and its components]. Med Parazitol (Mosk). 1968; 37: 433-5. Russian.
10. Foroutan-Rad M, Majidiani H, Dalvand S, Daryani A, Kooti W, Saki J, et al. Toxoplasmosis in blood donors: a systematic review and meta-analysis. Transfus Med Rev. 2016; 30: 116-22.
11. Özçelik S, Alim M, Özpınar N. Detection of *Toxoplasma gondii* infection among diabetic patients in Turkey. Clin Epidemiol Glob Health. 2020; 8: 899-902.
12. Ehsanipour F, Faranoush P, Foroughi-Gilvaee MR, Sadighnia N, Fallahpour M, Motamedi M, et al. Evaluation of the immune system in patients with transfusion-dependent beta-thalassemia in Rasoul-e-Akram Hospital in 2021: a descriptive cross-sectional study. Health Sci Rep. 2022; 5: e871.
13. El-Tantawy N, Darwish A, Eissa E. Seroprevalence of *Toxoplasma gondii* infection among β-thalassemia major pediatric population: implications

for transfusion-transmissible toxoplasmosis. Pediatr Infect Dis J. 2019; 38: 236-40.

14. Yousefi E, Foroutan M, Salehi R, Khademvatan S. Detection of acute and chronic toxoplasmosis amongst multi-transfused thalassemia patients in southwest of Iran. J Acute Dis. 2017; 6: 120-5.
15. Zko SS, Al-Naftachi MT, Al-Jawadi WA, Alghrer MM. Seroprevalence of *Toxoplasma gondii* among thalassemia patients and its relation with some biochemical parameters. J Nat Sci Biol Med. 2024; 15: 118-23.
16. Abd El-Latif NF, Salem AI, Sadek NA, Salah SAM, Shalaby DG, Elhadad H. Serological and molecular detection of *Toxoplasma gondii* in ß. thalassemia patients. J Parasit Dis. 2023; 47: 778-86.
17. Shihab RN, Al-Aubaidi IK. Toxoplasmosis in thalassemic Iraqi patients: serological and hematological study. Ro J Infect Dis. 2024; 27: 100-6.
18. Karakaş S, Ozlem S, Tellioğlu AM, Ertabaklar H, Ertuğ S. Aydın ilinde beta talasemi majorlü olgularda anti-*Toxoplasma gondii* IgG ve IgM antikorlarının araştırılması [Investigation of anti-*Toxoplasma gondii* IgG and IgM antibodies in beta-thalassemia major patients in Aydın province]. Turkiye Parazitol Derg. 2012; 36: 133-6. Turkish.
19. Dabirzadeh M, Yusefnia H, Azizi H, Raeghi S, Mirahmadi H. Comparison of nested PCR and ELISA techniques in diagnosis of toxoplasmosis infection in thalassemia patients by targeting the RE gene. Ann Parasitol. 2022; 68: 447-52.
20. Hwang S, Cobb DA, Bhadra R, Youngblood B, Khan IA. Blimp-1-mediated CD4 T cell exhaustion causes CD8 T cell dysfunction during chronic toxoplasmosis. J Exp Med. 2016; 213: 1799-818.
21. Ivanova DL, Krempels R, Denton SL, Fettel KD, Saltz GM, Rach D, et al. NK cells negatively regulate CD8 T cells to promote immune exhaustion and chronic *Toxoplasma gondii* infection. Front Cell Infect Microbiol. 2020; 10: 313.
22. Doherty CM, Patterson PR, Emeanuwa JA, Belmares Ortega J, Fox BA, Bzik DJ, et al. T lymphocyte-dependent IL-10 down-regulates a cytokine storm driven by *Toxoplasma gondii* GRA24. mBio. 2024; 15: e01455-24.
23. Moshtaghi-Kashanian GR, Gholamhoseinian A, Hoseinimoghadam A, Rajabalian S. Splenectomy changes the pattern of cytokine production in beta-thalassemic patients. Cytokine. 2006; 35: 253-7.
24. Sae-Khow K, Charoensappakit A, Leelahavanichkul A. Neutrophil diversity (immature, aged, and low-density neutrophils) and functional plasticity: possible impacts of iron overload in β-thalassemia. Int J Mol Sci. 2024; 25: 10651.
25. Hoidy WH, Chyad DH, Essa AM, Essa SM, Al-Saadi MH. Interleukin gene polymorphisms (IL-6, IL-10, IL-17 a, IL-18) predict beta-thalassemia major susceptibility and inflammatory profiles in Iraqi children: a genetic risk score analysis. Cytokine. 2026; 203: 157156.
26. Ruaa K. Surhan, Mayyada Darweesh, Atheer Basil Al-Obiadi. IL-10-1082A\G gene polymorphism and production in β-thalassemia major and association with HCV infection. Research J. Pharm. and Tech 2018; 11: 2603-8
27. Jabbar MR, Darweesh MF. Evaluate IL-10 level and gene polymorphism in β-Thalassemia patients with related to microbial infection and disease outcome. International Journal of Health Sciences. 2022; 6(S6): 5697-707.
28. Al-Taei ZKM, Jasim MM. A study of the relationship between toxoplasmosis and its effect on the level of the complete blood count and the level of interleukin 10 among aborted women in the city of Tikrit. Turk J Physiother Rehabil. 2021; 32: 14976-81.
29. Abdullhusein HS, Al-Aboody B, Mohammed AH, Al-Adhami RAM. The regulation of immune system by anti-inflammatory interleukins in heart disease patients infected with toxoplasmosis. Int J Res Sci Technol. 2020; 10: 16-23.
30. Al-Kufi AKG. Relationship between toxoplasmosis and breast cancer, and detection of some immunological and hormonal indicators in women in Al-Najaf Al-Ashraf Province [master's thesis]. Kufa (Iraq): University of Kufa, Faculty of Education for Girls. 2020.
31. Ham DW, Kim SG, Seo SH, Shin JH, Lee SH, Shin EH. Chronic *Toxoplasma gondii* infection alleviates experimental autoimmune encephalomyelitis by the immune regulation inducing reduction in IL-17A/Th17 via upregulation of SOCS3. Neurotherapeutics. 2021; 18: 430-47.
32. Evangelista FF, Nishi L, Colli CM, Sant'Ana PL, Higa LT, Muniz LHG, et al. Increased levels of IL-17A in serum and amniotic fluid of pregnant women with acute toxoplasmosis. Acta Trop. 2021; 222: 106019.
33. Baqer NN, Saheb EJ, Ahmed NS. Genetic polymorphism of IL-17A (rs2275913) in Iraqi women with recurrent abortion and its relationship with susceptibility to toxoplasmosis. Meta Gene. 2021; 29: 100939.
34. Merli P, Quintarelli C, Strocchio L, Locatelli F. The role of interferon-gamma and its signaling pathway in pediatric hematological disorders. Pediatr Blood Cancer. 2021; 68: e28900.
35. Shihab RN. Study some biological parameters in Iraqi thalassemic patients infected with Toxoplasma gondii [dissertation]. Baghdad: University of Baghdad, College of Education for Pure Science (Ibn Al-Haitham), Department of Biology; 2024.
36. Ismael AK, Salih TA. Studying the role of IFN-γ, vitamin C, SOD, LH, FSH, APA and ACA in *Toxoplasma gondii*-infected miscarriage women. Baghdad Sci J. 2019; 16: 697-706.
37. Shihab RN, Ali Saeed S, Sulaiman EH. CD4 and CD8 T-lymphocyte cell count in β-thalassemia major patients infected with toxoplasmosis. Iran J Med Microbiol. 2025; 19: 203-9.

*Original Investigation*
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# Investigation of the Detection Frequency of *Demodex* spp. in Marmara University Faculty of Medicine Students According to Mask Use and Personal Hygiene Habits During the COVID-19 Pandemic

## *COVID-19 Pandemisi Sırasında Maske Kullanımı ve Kişisel Hijyen Alışkanlıklarına Göre Marmara Üniversitesi Tıp Fakültesi Öğrencilerinde Demodex spp. Saptanma Sıklığının İncelenmesi*

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## ABSTRACT

**Objective:** Despite the existence of studies evaluating the effect of personal hygiene habits on *Demodex* mites, which are permanent ectoparasites of humans, there is a paucity of research examining the effect of mask use on the prevalence of *Demodex* mites during the period of the SARS-CoV-2 pandemic. The present study set out to investigate the impact of mask use during the pandemic and personal hygiene habits on the prevalence of *Demodex* spp.

**Methods:** Standardized skin surface biopsy were obtained from Marmara University Faculty of Medicine students' foreheads and cheeks during the pandemic. Two areas were sampled: foreheads and cheeks. A quantity of immersion oil (ranging from one to two drops) was deposited on the sampled slide area. The slide was covered with a coverslip, then a microscope was invetigated (x4, x10 and x40). Infestation was indicated by the presence of five or more *Demodex* per $cm^2$. Volunteers were asked to complete a questionnaire about their personal hygiene habits. SPSS v.16.0 (IBM Corp., Armonk, NY, USA) software was used to analyse the results.

**Results:** A total of 104 volunteers, comprising 43 women and 61 men, participated in the study. *Demodex* spp. was detected in 49.0% (n=51) of the participants, 51.2% (n=22) of women and 47.5% (n=29) of men. *Demodex* spp. were identified in 46.2% (n=48) of the samples obtained from the cheek and 13.5% (n=14) of the samples obtained from the forehead. It was determined that all participants with mites were using masks. The detection frequency exhibited an upward trend in proportion to the duration of mask change. *Demodex* spp. was identified in 52.8% of subjects with a documented history of acne, and a significant correlation was observed between the frequency of facial cleansing and the presence of *Demodex* spp. (p=0.036).

**Conclusion:** The research indicates that the increase in *Demodex* spp. in the cheeks of individuals using medical masks during the pandemic is attributable.

**Keywords:** Ectoparasite, SARS-CoV-2, mask

## ÖZ

**Amaç:** İnsanda daimi bir ektoparazit olan *Demodex* spp. akarları ile ilgili kişisel hijyen alışkanlıklarının etkisini değerlendiren çalışmalar literatürde yer almakla birlikte, COVID-19 pandemisi döneminde maske kullanımının *Demodex* spp. yaygınlığı üzerindeki etkisini inceleyen araştırmalar sınırlıdır. Bu çalışmada COVID-19 maske kullanımı ve kişisel hijyen alışkanlıklarının *Demodex* spp. saptanma sıklığını araştırmak amaçlanmıştır.

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**Yöntemler:** Marmara Üniversitesi Tıp Fakültesi öğrencilerinden alın ve maske altında kalan yanak bölgesinden standart yüzeyel deri biyopsi örnekleri alınmıştır. Örnekler, lam yüzeyine siyanoakrilat damlatılarak etkenin araştırılacağı yüz bölgesine yapıştırılıp bir dakika beklenerek alınmıştır. Gönüllülerden alın ve yanak olmak üzere iki bölgeden örnek alınmıştır. Lamın örnek alınan kısmına 1-2 damla immersiyon yağı damlatılıp lamel ile kapatılarak ışık mikroskobunda x4, x10 ve x40'lık objektiflerde parazit varlığı araştırılmıştır. $Cm^2$'de 5 ve daha fazla *Demodex* spp. görülmesi enfestasyon olarak değerlendirildi. Örnek alımı sırasında gönüllülerden tıbbi maske kullanımı, yüz yıkama sıklığı, makyaj kullanımı gibi kişisel hijyen alışkanlıklarının sorulduğu bir anket doldurmaları istenmiş, bu anket soruları ile örneklerin sonuçları SPSS 16.0 (IBM Corp., Armonk, NY, USA) ile analiz edilmiştir.
**Bulgular:** Çalışmaya 43 kadın, 61 erkek toplam 104 gönüllü katılmıştır. Katılımcıların %49,0 (n=51)'inde *Demodex* spp. saptanmış olup, kadınların %51,2'si (n=22), erkeklerin %47,5'tir (n=29). Yanaktan alınan örneklerin %46,2 (n=48), alından alınan örneklerin %13,5'ünde (n=14) *Demodex* spp. bulunmuştur. Akar saptanan tüm katılımcıların maske kullandığı belirlenmiştir. Maske değiştirme süresi uzadıkça enfestasyon oranı artış göstermiştir. Akne öyküsü olan katılımcıların %52,8'inde *Demodex* spp. saptanmış olup, yüz yıkama sıklığı ile *Demodex* spp. varlığı arasında anlamlı bir bağlantı bulunmuştur (p=0,036).
**Sonuç:** Araştırmamız sonucunda yanak bölgesinde *Demodex* spp. saptanma sıklığının daha yüksek olması COVID-19 pandemisi nedeniyle bireylerin uzun süre tıbbi maske kullanımına bağlı olabileceği sonucuna varılmıştır.
**Anahtar Kelimeler:** Ektoparazit, SARS-CoV-2, maske

## INTRODUCTION

*Demodex*, a permanent ectoparasite in humans, was first detected by Henle in 1841 and described by German dermatologist Gustav Simon in 1842, who demonstrated its ability to exist in pilosebaceous follicles (1). Two distinct types of *Demodex folliculorum* and *Demodex brevis* have been identified in human populations. *Demodex* species are generally found in the face, scalp, hair follicles on the upper chest and sebaceous glands of healthy individuals without any clinical symptoms (2-4). *Demodex* spp. mites have been commonly found to occur in all age groups with the exception of newborns. In the neonatal period, infestation with the mite occurs through direct contact. However, the mite is less prevalent in newborns and children, likely attributable to reduced sebum production in these age groups. Furthermore, an increase in mite density has been observed with age (5,6). *Demodex* prevalence has been observed to increase in advanced age and in patients with immunocompromised. It has been established that *Demodex* spp. density >5/$cm^2$ is associated with pathogenicity (3,5,6).

The symptoms appear as the number of mites in the follicles or glands reaches a certain threshold. Mites also penetrate the tissue in immunosuppression and bacterial superinfection cases (7,8). Lesions on the face cause itching, redness and enlarged blood vessels. During the inflammatory phase, these become papules, blisters and pustules. The presence of mites is crucial for diagnosis (7-9).

Skin lesions, especially those present on the face, are characterised by pruritus, erythema, telangiectasia, papules, blisters, and pimples in the area where the mites are found during the inflammatory period. These lesions are important in clinical and diagnostic studies (10,11). Although infestation is most commonly observed in the facial region (forehead, cheeks, nose, chin, nasolabial region) in humans, cases have also been reported in various other parts of the body, including the neck, scalp, ear, trunk, and genital region (3,12-14).

The number of studies planned to reveal the reasons for this difference in *Demodex* spp. prevalence and evaluate risk factors such as sociodemographic data, living conditions, hygiene habits, and skin characteristics of the selected population is limited.

During the COVID-19 pandemic, medical masks were widely used for a long time, and it has been reported that this may lead to changes in skin flora (15-18).

The objective of this study was to ascertain the frequency of *Demodex* infestation in standard superficial skin biopsy (SSSB) samples obtained from Marmara University Faculty of Medicine students. Moreover, the objective was to ascertain the impact of factors such as the use of medical masks and the diverse personal hygiene practices of the students during the course of the pandemic of the novel strain of SARS-CoV-2 on *Demodex* infestation.

## METHODS

This study is a clinical research aiming to determine the prevalence of *Demodex* spp. infestation in Marmara University Faculty of Medicine students and the personal hygiene habits of these individuals. The study was conducted between September 2021 and June 2022 on volunteers randomly selected among Marmara University Faculty of Medicine students.

Participants were asked to complete an questionnaire that included personal hygiene details such as mask use and face-washing habits. Subsequently, SSSB were obtained from cheek regions covered by the mask and the forehead. The samples were collected by applying a drop of cyanoacrylate to the surface of a slide and then pressing the slide once onto the corresponding facial area. Two distinct samples were collected from each participant: one from the forehead and one from the cheek.

A quantity of immersion oil measuring 1-2 drops was deposited on the surface of the sampled slide, which was then covered with a coverslip. The presence of *Demodex* spp. was investigated under x4, x10 and x40 magnification lenses on a light microscope. The presence of more than five mites per square centimetre was considered to indicate an infestation.

This study was conducted in accordance with the principles of good clinical practice as set out in the Declaration of Helsinki. The study was approved by the Marmara University Faculty of Medicine Clinical Research Ethics Committee (ethics committee no: 09.2022.315, date: 11.02.2022). Written informed consent was obtained from all participants.

### *Statistical Analysis*

The results obtained from the questionnaire data and the samples obtained from the participants were analysed with the SPSS 16.0 (IBM Corp., Armonk, NY, USA) package programme. The investigation focused on the correlation between the independent variables present within the personal information form and the subsequent findings derived from the samples. The dependent variables, i.e. the sampling site (cheek and forehead) were then compared using the McNemar's test. $P<0.05$ was accepted as statistically significant.

## RESULTS

The present study comprised a total of 104 participants, including 43 females and 61 males. *Demodex* infestation was detected in 51 (49.0%) of the 104 participants. The prevalence of infestation in samples collected from male students was 47.5% (n=29), while in female students it was 51.2% (n=22). The distribution of the participants' survey data and infestation rates is presented in Table 1. The presence of *Demodex* spp. was determined in 29.8% (n=62) of all samples (n=208), 13.5% (n=14) of forehead samples (n=104) and 46.2% (n=48) of cheek samples (n=104) (p<0.0001). The presence of infestation was determined in 29.8% (n=62) of all samples (n=208). The light microscopy image of the mites is given in Figure 1.

In the course of the research project, 104 volunteers participated. Of these, 19 (18.3%) volunteers confirmed their use of make-up products. Of these, 18 were female and one was male. Of the 85 respondents who provided a negative response to that question, 25 were female and 60 were male. The infestation rate was 47.4% (n=9) in the samples taken from 19 participants who used make-up products, and 49.4% (n=42) in the samples taken from 85 participants who did not use make-up products. No statistically significant results were found (Table 1). An evaluation was conducted to ascertain the correlation between mask usage and the frequency of mask change, as well as the frequency of *Demodex* infestation. The findings revealed that the majority of participants (n=96) used masks for a duration exceeding four hours, with only eight individuals opting for change intervals of one to four hours. The percentage of infestation in the forehead area was 14.6% (n=14) and in the cheek area was 47.9% (n=46) in the participants whose mask use time was more than 4 hours (n=96). No statistically significant difference was found (p=0.212).

## DISCUSSION

The *Demodex* spp. are characterised by a prevalence of colonisation, particularly in the facial region, a phenomenon attributable to a number of factors. It has been established that *D. folliculorum* and, albeit less frequently, *D. brevis* are endemic to humans (1,6). The use of masks has been demonstrated to increase sebum production and to provide a warm and humid environment, thus increasing the frequency and density of *Demodex* mites (15-18).

In order to explain the differences in the prevalence of *Demodex* spp., various studies have been conducted to evaluate risk factors such as sociodemographic characteristics, living conditions,

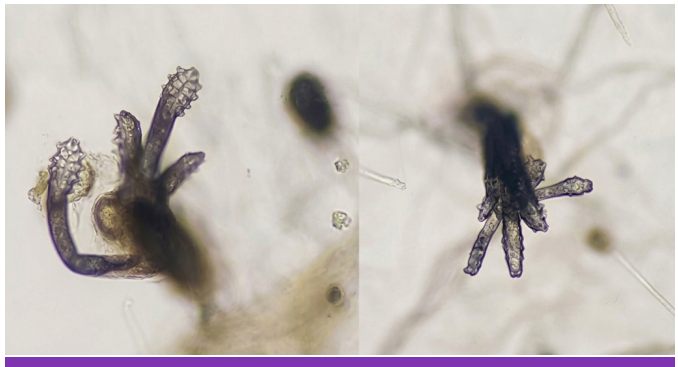

**Figure 1.** *Demodex* spp. under light microscope x40 magnification

**Table 1.** Frequency of *Demodex* spp. existance according to different variables

| | Positive | | p-value |
|---|---|---|---|
| | n | % | |
| **Sex** | | | 0.132 |
| **Female** | 22 | 51.2 | |
| **Male** | 29 | 47.5 | |
| **Variable** | n | Positivity (%) | |
| **Total number of samples** | 208 | 29.8 (n=62) | - |
| **Samples from forehead** | 104 | 13.5 (n=14) | <0.001* |
| **Samples from cheek** | 104 | 46.2 (n=48) | - |
| **Age group** | | | 0.991 |
| <21 age | 49 | 48.9 (24) | |
| ≥21 age | 55 | 49 (27) | |
| **Face-washing frequency** | | | 0,531 |
| Once a day | 17 | 47.1 (n=8) | |
| More than once a day | 87 | 49.4 (n=43) | |
| **Face-washing product usage** | | | 0.578 |
| Yes | 66 | 46.9 (n=31) | |
| No | 38 | 52.6 (n=20) | |
| **Preferred water temperature for face-washing** | | | 0.457 |
| Warm | 64 | 45.3 (n=29) | |
| Room temperature | 36 | 55.6 (n=20) | |
| **Skin care product usage** | | | 0.067 |
| Yes | 38 | 47.4 (n=18) | |
| No | 66 | 50.0 (n=33) | |
| **Makeup usage** | | | 0.678 |
| Yes | 19 | 47.4 (n=9) | |
| No | 85 | 49.4 (n=42) | |
| **Skin type** | | | 0.507 |
| Dry | 12 | 33.3 (n=4) | |
| Combination | 71 | 50.7 (n=36) | |
| Oily | 21 | 52.4 (n=11) | |
| **Epilation-laser history** | | | 0.580 |
| Yes | 12 | 58.3 (n=7) | |
| No | 92 | 47.8 (n=44) | |
| **Shaving frequency** | | | 0.812 |
| Yes | 64 | 48.4 (n=31) | |
| Never | 40 | 50.0 (n=20) | |
| **Bath frequency** | | | 0.109 |
| 1-3 times a week | 65 | 55.4 (n=36) | |
| Daily | 39 | 38.5 (n=15) | |
| **Shared towel usage** | | | 0.773 |
| Yes | 13 | 53.8 (n=7) | |
| No | 91 | 48.4 (n=44) | |
| **Self-reporterd skin conditions** | | | 0.377 |
| Yes | 63 | 52.4 (n=33) | |
| No | 41 | | |
| **Acne treatment** | | | 0.672 |
| Yes | 31 | 45.2 (n=14) | |
| No | 75 | 50.7 (n=38) | |
| **Antibiotic usage** | | | 0.628 |
| Yes | 8 | 62.5 (n=5) | |
| No | 96 | 47.9 (n=46) | |

*: Significant difference

hygiene habits, and skin characteristics of the selected population. Karaman et al. (19) investigated the relationship between gender, age, skin type, frequency of skin care, and the prevalence of *Demodex* spp. in a study involving a total of 300 university students aged 18 to 30. The study reported a prevalence of *Demodex* spp. of 37%, and no significant association was found between *Demodex* spp. prevalence and gender, age, skin type, or frequency of skin care (19).

The number of studies examining the relationship between the frequency of *Demodex* spp. and mask use is quite limited. In one study, the effect of mask type, demographic characteristics, background and face-washing habits of patients on *Demodex* prevalence was examined. It was reported that the use of masks in individuals without underlying skin disease did not create a suitable microenvironment for *Demodex* spp. (20). However, studies have been conducted that suggest a link between mask use and the development of various facial diseases, as well as an increased prevalence of *Demodex* mites (15-18). As stated in the case report, the long-term utilisation of masks has the potential to modify the microenvironment of the skin, thereby increasing the prevalence of *Demodex*-related atopic dermatitis, seborrheic dermatitis, acne vulgaris and rosacea (15).

In studies examining the distribution of mites in the body, it has been reported that infestation in humans is most commonly seen in areas such as the face, forehead, cheeks, nose, chin and nasolabial region, but they can also exist in various other parts of the body such as the neck, scalp, ears, chest, back, breast, buttocks and genitals (3,12-14). The fact that sebum secretion is more intense in the forehead and nose regions causes the follicles in these regions to enlarge more compared to other regions; this has been reported to cause *Demodex* spp. to settle in these regions frequently (21). Studies examining the distribution of the presence of *Demodex* spp. in different regions of the face have yielded different results. In a study by Tilki et al. (22), it was reported that the prevalence of *Demodex* spp. in samples taken from the cheek region was statistically significantly higher than in the nasolabial and chin regions. In our study, similar to the study of Tilki et al. (22) the infestation rate in the cheek region was found significantly higher.

The global prevalence of mask-wearing has increased significantly since the onset of the pandemic, with individuals facing extended periods of mask-use. It has been hypothesised that this situation may result in alterations to the skin's microbial flora (15-18).

Different results were obtained in studies investigating the prevalence of *Demodex* spp. Yazar et al. (23) reported that *Demodex* spp. were detected in 2.9% of 171 university students with the cellophane-tape method and Kaya et al. (24) reported that *Demodex* spp. were detected in 2.7% of 347 male students with the same method. On the other hand, Kaplan et al. (25) reported that parasites were detected in 10.07% of 258 university students, Zeytun et al. (26) in 50.1% of university students (n=538), Özdemir et al. (27) in 47.4% of 270 university students with the SBBB method. In the study conducted by Çetinkaya et al. (28) before the COVID pandemic, the prevalence of the parasite was found to be 35.9% (33/92). They reported that parasites were detected in a single region in 48.5% of individuals with parasites, in two different regions in 42.4%, in three different regions in 6.1%, and in four different regions in 3% (28). It was reported that the presence of parasites was statistically significantly lower in those who used facial cleansing products ($p<0.05$) (28). In our study, the rate of parasites detected in the cheek region was found to be higher than the samples taken from the forehead region. In our study, we found infestation in a single site (forehead=3, cheek=37) in 40 individuals and in two sites in 11 volunteers. In our study, we found *Demodex* infestation in 51 (49.0 %) of 104 participants in total. *Demodex* infestation has been reported in the literature at rates ranging from 10% to 75% (22). Erdal and Albayrak (4) reported *Demodex* infestation in 21 (14.5%) of 144 patients. However, no significant correlation was found between the frequency of mask change and *Demodex* frequency in our study.

The data obtained from the forehead and cheek regions in the present study indicate a higher existance of *Demodex* spp. (47.9%) in the forehead and cheek regions of 96 volunteers who frequently changed their masks (4+ hours) compared to 8 volunteers who changed their masks (1-4 hours) frequently. In summary, the frequent change of the mask may have resulted in a reduced quantity of *Demodex* in the positive samples obtained from the cheek area. Consequently, the regular replacement of masks during the course of the pandemic may have contributed to a reduction in the prevalence of *Demodex* spp. in humans.

There are studies investigating the relationship between demographic characteristics and parasite prevalence in individuals with *Demodex* spp. infestation. In a study conducted in medical students in China, *Demodex* spp. was found in 21.16% of females and 16.05% of males (29). Tilki et al. (22) found the presence of *Demodex* spp. in 65.5% of men and 60.3% of women in their study investigating the relationship between skin type and characteristics and demographic characteristics of individuals. In our study, *Demodex* spp. infestation was found to be 47.5% in males and 51.2% in females. In previous studies examining the relationship between age and *Demodex* spp. presence, it was reported that the presence of parasite increased with increasing age (2,30). However, no significant relationship was found between age and *Demodex* spp. infestation in our study. It is thought that this may be due to the limited age distribution of the volunteers included in the study.

In the literature, there are different findings and interpretations about the relationship between the frequency of face-washing and the presence of *Demodex* spp. According to the study conducted by Kahraman et al. (31) it was thought that frequent face-washing may lead to dryness in the skin and may decrease the rate of *Demodex* spp. in clean skin. Song et al. (32) found that the prevalence of *Demodex* mites was significantly higher in students who used warm water instead of room temperature water to wash their faces. Yasak Guner et al. (33) reported that there was no significant relationship between the frequency of face-washing and the presence of *Demodex* spp. in their study ($p=0.12$). In our study, no statistically significant difference was found between the frequency of face-washing and water temperature and the frequency of *Demodex* spp. infestation.

The use of make-up products is thought to contribute to the destruction of *Demodex* mites by mechanically occluding the follicle, preventing the migration and respiration of the parasite, and also by toxic effect since they contain antiseptics such as alcohol (33). In our study, no significant difference was observed between the prevalence of *Demodex* spp. in participants who did not wear skin make-up compared to participants who wore make-up.

In a study conducted by Sevgen and Mor (34) it was reported that a higher rate of *Demodex* spp. was detected in individuals who did not have epilation or laser application. However, according to our findings, a higher rate of parasite presence was detected in the samples taken from volunteers who had epilation-laser application compared to those who did not, but this difference was not found to be statistically significant. In this respect, our study is inconsistent with the results of Sevgen and Mor (34). The main limitation of our study are as follows: failure to differentiate *Demodex* mites at species level.

## CONCLUSION

In our study, it was evaluated that the higher frequency of *Demodex* spp. infestation in the cheek area may be related to the use of masks, which became widespread during the pandemic period. We think that more long-term studies with a wider participation should be carried out in order to reveal the effect of mask use on the prevalence of *Demodex* spp. infestation more clearly.

### *Ethics

**Ethics Committee Approval:** The study was approved by the Marmara University Faculty of Medicine Clinical Research Ethics Committee (ethics committee no: 09.2022.315, date: 11.02.2022).

**Informed Consent:** Written informed consent was obtained from all participants.

### Footnotes

### *Authorship Contributions

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

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## REFERENCES

1. Yazısız H, Çekin Y, Koçlar FG. The presence of *Demodex* mites in patients with dermatologic symptoms of the face. Turkiye Parazitol Derg. 2019; 43: 143-8.
2. Rychlik K, Sternicka J, Zabłotna M, Nowicki RJ, Bieniaszewski L, Purzycka-Bohdan D. The prevalence of *Demodex* spp. infestation in dermatological patients in Northern Poland. Life (Basel). 2024; 14: 1196.
3. Aktaş Karabay E, Aksu Çerman A. *Demodex folliculorum* infestations in common facial dermatoses: acne vulgaris, rosacea, seborrheic dermatitis. An Bras Dermatol. 2020; 95: 187-93.
4. Erdal B, Albayrak H. Investigation of the prevalence of *Demodex* spp. in dermatological diseases. Turkiye Parazitol Derg. 2022; 46: 54-9.
5. Chudzicka-Strugała I, Gołębiewska I, Brudecki G, Elamin W, Zwoździak B. Demodicosis in different age groups and alternative treatment options-a review. J Clin Med. 2023; 12: 1649.
6. Chioveanu FG, Niculet E, Torlac C, Busila C, Tatu AL. Beyond the surface: understanding demodex and its link to blepharitis and facial dermatoses. Clin Ophthalmol. 2024; 18: 1801-10.
7. Forton FMN. Rosacea, an infectious disease: why rosacea with papulopustules should be considered a demodicosis. A narrative review. J Eur Acad Dermatol Venereol. 2022; 36: 987-1002.
8. Forton FMN. The pathogenic role of *Demodex* mites in rosacea: a potential therapeutic target already in erythematotelangiectatic rosacea? Dermatol Ther (Heidelb). 2020; 10: 1229-53.
9. Geng RSQ, Sood S, Hua N, Chen J, Sibbald RG, Sibbald C. Efficacy of treatments in reducing inflammatory lesion count in rosacea: a systematic review. J Cutan Med Surg. 2024; 28: 352-9.
10. Serarslan G, Makbule Kaya Ö, Dirican E. Scale and pustule on dermoscopy of rosacea: a diagnostic clue for *Demodex* species. Dermatol Pract Concept. 2021; 11: e2021139.
11. Micheletti RG, James WD, Elston DM, McMahon PJ. Andrews' Diseases of the Skin Clinical Atlas. 1st ed. Philadelphia (PA): Elsevier; 2021.
12. Márquez FJ, López-Montoya AJ, Sánchez-Carrión S, Dimov I, de Rojas M. Epidemiological survey of two morphotypes of *Demodex folliculorum* (Prostigmata: Demodicidade) in young people from southern Spain. Parasite Epidemiol Control. 2024; 27: e00381.
13. Wei F, Li L, Kong Y, Yan X, Varghese KJ, Zhang S, et al. Evidence for the clinical association between *Demodex* and rosacea: a review. Dermatology. 2024; 240: 95-102.
14. Zeytun E, Yazici M. Human *Demodex* mites as a possible etiological factor in rosacea: a cross-sectional study from Turkey. Syst Appl Acarol. 2024; 29: 527-38.
15. Paichitrojjana A. Demodicosis Associated with wearing a face mask: a case report. Case Rep Dermatol. 2022; 14: 19-23.
16. Yu J, Chen JK, Mowad CM, Reeder M, Hylwa S, Chisolm S, et al. Occupational dermatitis to facial personal protective equipment in health care workers: a systematic review. J Am Acad Dermatol. 2021; 84: 486-94.
17. Thatiparthi A, Liu J, Martin A, Wu JJ. Adverse effects of COVID-19 and face masks: a systematic review. J Clin Aesthet Dermatol. 2021; 14(9 Suppl 1): S39-45.
18. Chiriac AE, Wollina U, Azoicai D. Flare-up of rosacea due to face mask in healthcare workers during COVID-19. Maedica (Bucur). 2020; 15: 416-7.
19. Karaman Ü, Kolören Z, Enginyurt Ö, Özer A. Ordu ilinde yurtlarda kalan üniversite öğrencilerinde *Demodex* türlerinin epidomiyolojisi [The epidemiology of *Demodex* mites at the college students living in dormitories in the city of Ordu]. Turkiye Parazitol Derg. 2014; 38: 166-71. Turkish.
20. Aktas ND. Could wearing face masks during the pandemic have created an environment for *Demodex* mites? Sanamed. 2024; 19: 155-61.
21. Ertuğ S, Tilekliođlu E, Yıldız İ, Malatyalı E, Ertabaklar H. The retrospective analysis of *Demodex* spp. results in Aydın Adnan Menderes University. Turkiye Parazitol Derg. 2020; 44: 72-7.
22. Tilki E, Zeytun E, Doğan S. Prevalence and density of *Demodex folliculorum* and *Demodex brevis* in Erzincan Province. Turkiye Parazitol Derg. 2017; 41: 80-6.
23. Yazar S, Özcan H, Çetinkaya Ü. Üniversite öğrencilerinde selofan-bant yöntemi ile *Demodex* sp. araştırılması [Investigation of *Demodex* sp. using cellophane tape method among university students]. Turkiye Parazitol Derg. 2008; 32: 238-40. Turkish.
24. Kaya M, Hamamcı B, Çetinkaya Ü, Yaman O, Yazar S. Bir lisede öğrenim gören yabancı uyruklu erkek öğrencilerde selofan-bant yöntemi ile *Demodex* sp. araştırılması [Investigation of *Demodex* sp. using cellophane tape method in foreign male students in a high school]. Turk Hij Den Biyol Derg. 2010; 67: 73-7. Turkish.
25. Kaplan M, Keleştemur N, Başpınar S. *Demodex* spp. prevalence among university students. Kafkas Univ Vet Fak Derg. 2012; 18(Suppl-A): A43-6.
26. Zeytun E, Tilki E, Doğan S, Mumcuoğlu KY. The effect of skin moisture, pH, and temperature on the density of *Demodex* folliculorum and *Demodex brevis* (Acari: Demodicidae) in students and staff of the Erzincan University, Turkey. Int J Dermatol. 2017; 56: 762-6.
27. Özdemir H, Özer E, Özdemir S, Alkanat M. Sağlık bilimleri fakültesi öğrencilerinde demodeks türlerinin görülme sıklığı [The prevalence of *demodex* species in faculty of health sciences students]. Turkderm. 2015; 49: 139-41. Turkish.

28. Çetinkaya Ü, Oğuzkaya Artan M, Baykan Z. Sağlık hizmetleri meslek yüksekokulu öğrencilerinde *Demodex* spp. prevalansı ve risk faktörleri [Prevalence and risk factors for *Demodex* spp. in students of vocational school of healthcare services]. Turk Mikrobiyol Cemiy Derg. 2020; 50: 63-9. Turkish.

29. Wang LM, Wang WQ, Shen LJ, Jia XM, Yang ZQ, Li CY, et al. [Investigation on Demodex infections among university students in Kunming City]. Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi. 2020; 32: 428-31. Chinese.

30. Paichitrojjana A. *Demodex*: The worst enemies are the ones that used to be friends. Dermatol Reports. 2022; 14: 9339.

31. Kahraman M, Akın Polat Z, Esen M, Çoban Gül F. The association between the presence of human facial mites *Demodex* (Acari, Demodicidae) and dermatological symptoms in rosacea patients in Central Anatolia, Türkiye. Turkiye Parazitol Derg. 2025; 48: 240-4.

32. Song ZY, Zhang HR, Jiang M, Wang JJ, Guang YJ, Sun H, et al. Prevalence and influencing factors of *Demodex* infection among college students in Zhengzhou, PR China. *Southeast A*sian J Trop Med Public Health. 2025; 56: 144-59.

33. Yasak Guner R, Tosun M, Akyol M, Hayta SB. *Demodex* infestation as a cause of sensitive skin in a dermatology outpatient clinic. J Cosmet Dermatol. 2022; 21: 1610-5.

34. Sevgen SN, Mor N. Üniversite öğrencilerinde Demodex spp. yaygınlığının araştırılması: sağlık bilimleri fakültesi örneği [The investigation of prevalence of *Demodex* spp. in university students: the case of the faculty of health sciences]. Turkiye Parazitol Derg. 2019; 43: 198-203. Turkish.

*Original Investigation*
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# Evaluation of Dermatologists' Opinions in the Face of Increasing Scabies Cases: A Survey Study

## *Artan Uyuz Olguları Karşısında Dermatologların Görüşlerinin Değerlendirilmesi: Bir Anket Çalışması*

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## ABSTRACT

**Objective:** Scabies is a common and highly contagious parasitic skin disease that continues to pose a significant public health burden worldwide and in Türkiye. This study aimed to evaluate the approaches, experiences, and challenges of dermatologists in Türkiye regarding the diagnosis and treatment of scabies.
**Methods:** A cross-sectional survey was conducted among 107 dermatologists. Data were collected anonymously via an online questionnaire.
**Results:** Sulfur-based magistral formulations were the preferred treatments among adults, pregnant women, and children over two years of age, whereas permethrin remained the first-line choice for children under two years of age. Although permethrin has traditionally been considered the gold standard, 47.7% of dermatologists reported inadequate treatment responses, while 41.1% found it effective. Conversely, 74.8% of respondents reported successful outcomes with sulfur therapy. Nearly 60% of dermatologists reported using oral ivermectin as a second-line therapy, while 20% prescribed it as a first-line therapy in appropriate cases. Approximately 70% of participants reported frustration in managing scabies cases. Most emphasised the need to reduce hospital burden, extend consultation times, and involve family physicians in screening and follow-up.
**Conclusion:** Scabies remains a growing healthcare problem in Türkiye. Strengthening primary care involvement, improving patient education, and reconsidering treatment strategies are essential for effective disease control.
**Keywords:** Sarcoptes scabiei, dermatologists, treatment outcome, drug resistance, ivermectin

## ÖZ

**Amaç:** Skabies, dünya genelinde ve Türkiye'de önemli bir halk sağlığı yükü oluşturmaya devam eden yaygın ve oldukça bulaşıcı bir paraziter deri hastalığıdır. Bu çalışma, Türkiye'deki dermatologların skabies tanı ve tedavisine ilişkin yaklaşımlarını, deneyimlerini ve karşılaştıkları zorlukları değerlendirmeyi amaçlamaktadır.
**Yöntemler:** Yüz yedi dermatolog arasında kesitsel bir anket çalışması gerçekleştirilmiştir. Veriler anonim olarak çevrim içi bir anket aracılığıyla toplanmıştır.
**Bulgular:** Erişkinler, hamileler ve iki yaş üzerindeki çocuklarda en sık tercih edilen tedavi sülfür bazlı majistral preparatlar olmuştur. İki yaş altındaki çocuklarda ise ilk basamak tedavi olarak permethrin tercih edilmiştir. Permethrin geleneksel olarak altın standart kabul edilmesine rağmen, dermatologların %47,7'si yetersiz tedavi yanıtı bildirmiş, yalnızca %41,1'i etkili bulmuştur. Buna karşılık, katılımcıların %74,8'i sülfür tedavisinde başarılı sonuçlar elde ettiklerini ifade etmiştir. Dermatologların yaklaşık %60'ı oral ivermektini ikinci basamak tedavi olarak, %20'si ise uygun olgularda ilk basamak olarak reçete ettiklerini belirtmiştir. Katılımcıların yaklaşık %70'i skabies olgularının yönetiminde hayal kırıklığı yaşadığını bildirmiştir. Çoğu hekim, hastane yükünün azaltılması, muayene sürelerinin uzatılması ve aile hekimlerinin tarama ve takip sürecine dahil edilmesi gerektiğini vurgulamıştır.
**Sonuç:** Skabies, Türkiye'de büyüyen bir sağlık sorunu olmaya devam etmektedir. Birinci basamağın sürece daha etkin katılımının sağlanması, hasta eğitiminin geliştirilmesi ve tedavi stratejilerinin yeniden gözden geçirilmesi, hastalığın etkin kontrolü için gereklidir.
**Anahtar Kelimeler:** Sarcoptes scabiei, dermatolog, tedavi sonuçları, ilaç direnci, ivermektin

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## INTRODUCTION

Scabies is a pruritic and highly contagious cutaneous infestation caused by *Sarcoptes scabiei* (1). It is recognised as a significant global public health concern, affecting approximately 200 million individuals at any given time and an estimated 400 million people annually. Although more prevalent in developing countries, an increasing incidence has been reported in high-income regions in recent years (2). In 2017, the World Health Organisation officially classified scabies as a neglected tropical disease (3).

Current clinical guidelines recommend several treatments for scabies, including topical permethrin, benzyl benzoate, sulfur, malathion, crotamiton, synergised pyrethrins, and both topical and oral ivermectin (4-6). Although topical permethrin remains recommended as the first-line therapy in the European guidelines, multiple reports from different European countries have documented growing resistance to permethrin (7-9).

Although the introduction of oral ivermectin therapy in Türkiye in recent years has contributed to progress in the management of scabies, dermatologists continue to employ a variety of treatment approaches. While 5% topical permethrin remains widely regarded by most physicians as an effective and safe first-line therapy, some dermatologists consider it ineffective and therefore do not prescribe it. In this study, we investigated dermatologists' general approaches, experiences, and challenges in diagnosing and treating scabies across different regions of Türkiye.

## METHODS

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments, or with comparable ethical standards. Ethical approval for this study was obtained from the Clinical Research Ethics Committee of Atatürk University Faculty of Medicine (file number: B.30.2.ATA.0.01.00/336, decision no: 117, date: 31.03.2022).

A cross-sectional study was conducted using an online survey. Between June 15 and July 15, 2024, participants were invited to complete a questionnaire developed by the researchers on Google Forms. A study announcement poster was prepared and disseminated through the researchers' social media accounts. Before completing the survey, all participants were informed about the content of the questionnaire. No personal data were collected, and responses were recorded anonymously. Participation in the study was entirely voluntary, and participants retained the option to withdraw their consent at any point until the completion of the survey.

Participants were included if they met the following inclusion criteria: (a) completion of all survey questions; and (b) employment as a dermatologist in either a public or private hospital in Türkiye. A total of 107 participants who fulfilled these criteria were enrolled in the study.

### *Statistical Analysis*

Statistical analyses were performed using SPSS version 28.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics included mean, standard deviation, median, minimum, maximum, frequency, and percentage values. The distribution of variables was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. For quantitative independent variables not normally distributed, the Mann-Whitney U test was applied. For qualitative independent variables, the chi-square test was used; when the assumptions of the chi-square test were not met, Fisher's exact test was applied.

## RESULTS

The participants' ages ranged from 25 to 80 years (median: 35). The majority were women, with 85 women (79.4%) and 22 men (20.6%) (Table 1). The mean age of dermatologists working in private institutions was significantly higher than that of those working in public institutions ($p<0.05$). The gender distribution did not differ significantly between employees of public and private institutions ($p>0.05$).

**Table 1.** Demographic data and treatment choices for scabies

| | | Min-max | Median | Mean ± SD, n (%) | |
|---|---|---|---|---|---|
| **Age** | | 25.0-80.0 | 35.0 | 38.9±10.1 | |
| **Sex** | Male | | | 22 | 20.6% |
| | Female | | | 85 | 79.4% |
| **In which region do you work?** | **Mediterranean Region** | | | 3 | 2.8% |
| | Eastern Anatolia Region | | | 16 | 15.0% |
| | **Aegean Region** | | | 18 | 16.8% |
| | **Southeastern Anatolia Region** | | | 5 | 4.7% |
| | **Central Anatolia Region** | | | 23 | 21.5% |
| | Black Sea Region | | | 10 | 9.3% |
| | Marmara Region | | | 32 | 29.9% |
| **What type of hospital are you currently working in?** | Training and Research Hospital | | | 36 | 33.6% |
| | State Hospital | | | 27 | 25.2% |
| | Private Hospital | | | 21 | 19.6% |
| | University Hospital | | | 17 | 15.9% |
| | Private Practice | | | 4 | 3.7% |
| | City Hospital | | | 1 | 0.9% |
| | Private Medical Center | | | 1 | 0.9% |

**Table 1.** Continued

| | Min-max | Median | Mean ± SD, n (%) | |
|---|---|---|---|---|
| **Average number of scabies patients seen per day** | 0.0-50.0 | 5.0 | 8.0±8.5 | |
| **First-line treatment preferences in scabies patients** | | | n | % |
| **Sulfur-based magistral preparations** | | | 65 | 60.7% |
| **Permethrin-containing creams or lotions** | | | 50 | 46.7% |
| Oral ivermectin | | | 37 | 34.6% |
| **Sulfur-tar combination (Wilkinson's ointment)** | | | 30 | 28.0% |
| Balsam of Peru mixtures | | | 18 | 16.8% |
| **Treatment preferences in pregnant scabies patients** | | | | |
| Sulfur-based magistral preparations | | | 59 | 55.1% |
| Permethrin-containing creams or lotions | | | 56 | 52.3% |
| Balsam of Peru mixtures | | | 20 | 18.7% |
| Sulfur-tar combination (Wilkinson's ointment) | | | 5 | 4.7% |
| **Preferred treatment for scabies in infants (0-2 years)** | | | | |
| Permethrin-containing creams or lotions | | | 46 | 43.0% |
| Sulfur-based magistral preparations | | | 39 | 36.4% |
| Balsam of Peru mixtures | | | 35 | 32.7% |
| Sulfur-tar combination (Wilkinson's ointment) | | | 27 | 25.2% |
| Xabion lotion | | | 3 | 2.8% |
| **Preferred treatment for scabies in children (>2 years)** | | | | |
| Sulfur-based magistral preparations | | | 67 | 62.6% |
| Permethrin-containing creams or lotions | | | 56 | 52.3% |
| Balsam of Peru mixtures | | | 20 | 18.7% |
| **Sulfur-tar combination (Wilkinson's ointment)** | | | 17 | 15.9% |
| Oral ivermectin | | | 14 | 13.1% |

Note: Participants were allowed to select multiple options
SD: Standard deviation

In the treatment of scabies, magistral sulfur preparations were the preferred agents for adults, pregnant women, and children over 2 years of age (60.7%, 55.1%, and 62.6%, respectively). For children aged 0-2 years, permethrin was the most preferred treatment (43%) (Table 1). Overall, magistral sulfur preparations were also the preferred first-line treatment for scabies patients (Figure 1). Eighty-nine point seven percent of participants attributed the lack of treatment response to patients' improper application of therapy and to asymptomatic family members' non-compliance with treatment recommendations. Additionally, 52.3% of participants attributed incomplete treatment to the irritant effects of topical agents, which led to treatment discontinuation. Furthermore, 47.7% of dermatologists reported that permethrin was ineffective in achieving an adequate therapeutic response. Approximately 47% of respondents associated treatment failure with misdiagnosis in primary care and insufficient patient education due to limited consultation time (Table 2).

Sixty per cent of participants reported switching to oral ivermectin for patients who were unresponsive to other therapies. Seventy per cent indicated that they examined scabies for diagnosis and management within 2-7 minutes (Table 3).

Around 70% reported difficulties convincing asymptomatic family members to undergo treatment, expressed uncertainty about whether non-attending family members had completed therapy since they could not prescribe for them, and emphasised that explaining the correct use of topical therapies was highly time-consuming. Approximately 70% of participants reported feeling frustrated with the management of scabies. In addition, 90% stated that scabies treatment burden in hospitals should be reduced and that family physicians should be better informed and involved in patient screening and family follow-up (Table 4).

The average number of daily scabies patients seen was significantly lower among dermatologists working in private institutions than among those in public institutions ($p<0.05$) (Table 5, Figure 2). The use of systemic ivermectin as a first-line treatment in appropriate patients, particularly those unresponsive to other therapies, was significantly more frequent in the private institution group than in the public institution group ($p<0.05$). Conversely, the proportion of dermatologists reporting difficulty advising patients on environmental decontamination after treatment was significantly lower in private than in public institutions ($p<0.05$). No significant difference was observed between employees of public and private institutions in the proportion of participants reporting frustration with the diagnosis, treatment, and follow-up of scabies ($p>0.05$).

**Table 2.** Opinions on treatment response/non-response

| | | |
|---|---|---|
| Incorrect application of treatment by patients | 96 | 89.7% |
| Non-compliance of asymptomatic family members with recommended treatment | 96 | 89.7% |
| I consider sulfur-based magistral preparations to be effective | 80 | 74.8% |
| Continued contact with carrier but untreated individuals | 78 | 72.9% |
| I consider oral ivermectin to be effective | 68 | 63.6% |
| Treatment discontinuation due to irritant properties of therapeutic agents | 56 | 52.3% |
| I consider sulfur-tar combination therapy to be effective | 54 | 50.5% |
| I consider permethrin therapy to be ineffective | 51 | 47.7% |
| Misdiagnosis in primary care | 51 | 47.7% |
| Inadequate patient counseling and treatment information | 51 | 47.7% |
| Insufficient patient understanding due to limited consultation time | 50 | 46.7% |
| Drug resistance | 47 | 43.9% |
| I consider permethrin therapy to be effective | 44 | 41.1% |
| I consider balsam of Peru mixtures to be effective | 44 | 41.1% |
| Failure to adequately clean environment and clothing | 40 | 37.4% |
| I consider oral ivermectin to be ineffective | 5 | 4.7% |
| I consider balsam of Peru mixtures to be ineffective | 4 | 3.7% |
| I observed that oral ivermectin alone is insufficient | 2 | 1.9% |
| I consider sulfur-tar combination therapy to be ineffective | 2 | 1.9% |
| It is not appropriate to state that a specific therapy is definitively effective or ineffective; response varies between patients | 1 | 0.9% |
| Responds to combination of permethrin and sulfur therapy | 1 | 0.9% |
| Oral ivermectin is effective only when combined with topical therapy | 1 | 0.9% |
| I consider that maintenance therapy is essential on days 7 and 10 following oral ivermectin | 1 | 0.9% |
| In resistant cases, i use oral ivermectin in combination with sulfur-based magistral preparations | 1 | 0.9% |
| I consider sulfur-based magistral preparations to be ineffective | 1 | 0.9% |
| I consider re-infection to be the most important problem | 1 | 0.9% |
| Patients concealing their disease | 1 | 0.9% |
| Due to improper instructions, many patients apply the medication daily only to itching areas | 1 | 0.9% |
| Patients are not informed that treatment must be repeated after one week | 1 | 0.9% |
| Patients not believing they have scabies and refusing treatment | 1 | 0.9% |
| There may be parasite-related factors | 1 | 0.9% |
| Frequent use of scabies medications in primary care due to misdiagnosis | 1 | 0.9% |

**Table 3.** Opinions on the use of systemic ivermectin in scabies treatment

| | | n | % |
|---|---|---|---|
| **Opinions on the use of systemic ivermectin in scabies treatment** | I prefer it in patients unresponsive to other therapies | 63 | 58.9 |
| | It is my first choice in eligible patients | 23 | 21.5 |
| | I achieve satisfactory outcomes with current topical therapies | 19 | 17.8 |
| | There is an absolute need for it | 2 | 1.9 |
| **Time spent for patient counseling (diagnosis and treatment)** | 2-4 minutes | 33 | 30.8 |
| | 5-7 minutes | 42 | 39.3 |
| | 8-10 minutes | 17 | 15.9 |
| | ≥10 minutes | 15 | 14.0 |

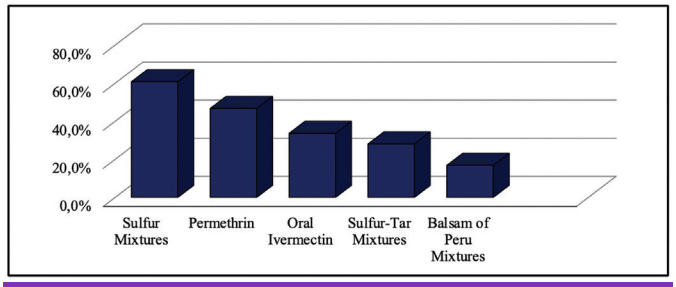


**Figure 1.** The most commonly preferred first-line treatment agents in patients with scabies

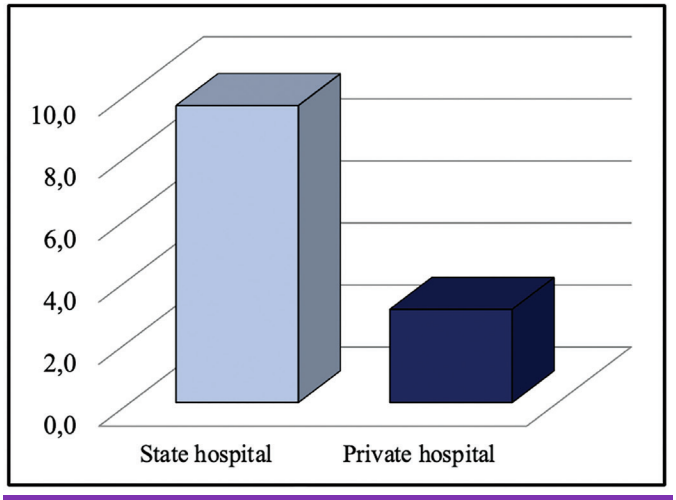


**Figure 2.** Daily average scabies cases

**Table 4.** Challenges faced regarding scabies

| | | n | % |
|---|---|---|---|
| I struggle with making the diagnosis | Disagree | 92 | 86.0 |
| | Uncertain | 15 | 14.0 |
| I struggle to convince patients to accept the diagnosis and treatment | Disagree | 44 | 41.1 |
| | Uncertain | 25 | 23.4 |
| | Agree | 30 | 28.0 |
| | Strongly agree | 8 | 7.5 |
| I struggle to convince non-itching family members to receive treatment | Disagree | 14 | 13.1 |
| | Uncertain | 15 | 14.0 |
| | Agree | 48 | 44.9 |
| | Strongly agree | 30 | 28.0 |
| I am uncertain about the adequacy of treatment in family members who do not present for examination since i cannot prescribe for them | Disagree | 18 | 16.8 |
| | Uncertain | 13 | 12.1 |
| | Agree | 39 | 36.4 |
| | Strongly agree | 37 | 34.6 |
| I struggle to instruct patients about post-treatment environmental hygiene | Disagree | 43 | 40.2 |
| | Uncertain | 30 | 28.0 |
| | Agree | 23 | 21.5 |
| | Strongly agree | 11 | 10.3 |
| Explaining how to apply topical therapy is highly time-consuming | Disagree | 15 | 14.0 |
| | Uncertain | 13 | 12.1 |
| | Agree | 39 | 36.4 |
| | Strongly agree | 40 | 37.4 |
| Difficult application, unpleasant odor, and irritant effects of topical medications extend the duration of treatment | Disagree | 7 | 6.5 |
| | Uncertain | 21 | 19.6 |
| | Agree | 44 | 41.1 |
| | Strongly agree | 35 | 32.7 |
| I have difficulty treating individuals living in communal settings such as military barracks, boarding schools, dormitories, or nursing homes | Disagree | 2 | 1.9 |
| | Uncertain | 17 | 15.9 |
| | Agree | 31 | 29.0 |
| | Strongly agree | 57 | 53.3 |
| I feel fatigued regarding the diagnosis, treatment, and follow-up of scabies | Disagree | 19 | 17.8 |
| | Uncertain | 16 | 15.0 |
| | Agree | 34 | 31.8 |
| | Strongly agree | 38 | 35.5 |
| I believe that scabies has become a major public health problem today, and that reducing the burden on hospitals, informing family physicians, and implementing household screening are essential for outbreak control | Disagree | 4 | 3.7 |
| | Uncertain | 10 | 9.3 |
| | Agree | 28 | 26.2 |
| | Strongly agree | 65 | 60.7 |
| I believe that systemic therapies, which are easier to explain and administer than topical treatments, are necessary and should be prescribed as the first choice for all eligible patients | Disagree | 24 | 22.4 |
| | Uncertain | 38 | 35.5 |
| | Agree | 28 | 26.2 |
| | Strongly agree | 17 | 15.9 |

## DISCUSSION

Scabies is a highly prevalent parasitic infestation in low- and middle-income tropical countries, affecting individuals of all ages, races, genders, and socioeconomic groups. Conditions such as war, natural disasters, famine, overcrowding, malnutrition, migration, homelessness, and poor hygiene contribute to scabies outbreaks. Among refugee populations, scabies has been reported as the third most common infectious disease (10). In Türkiye, the rising prevalence of scabies is well documented. A retrospective cross-sectional study conducted between 2017 and 2019 demonstrated a gradual increase in prevalence from 3.2% to 6.2% (11). In a multicenter study conducted at 12 tertiary dermatology clinics across different geographic regions of the country, 17,803 patients (14,574 adults and 3,229 children) were diagnosed with scabies between 2014 and 2019. Notably, the number of cases increased nearly seven-fold between 2017 and 2018, and approximately thirty-fold between 2017 and 2019 (12). This surge in scabies incidence in Türkiye is thought to be associated not only with irregular migration movements over the past decade but also with additional factors such as the coronavirus disease 2019 pandemic and major earthquakes that have affected millions of people (10-13).

The increase in scabies prevalence has also brought specific challenges in its diagnosis and management. In clinical practice, scabies is most often diagnosed based on characteristic features such as pruritus, distribution of lesions, the presence of burrows, and family history. At the same time, parasitological examination is rarely required (10). Eighty-six percent of participants reported no difficulty establishing a diagnosis of scabies. However, 58.9% reported challenges convincing patients to accept the diagnosis and treatment. Although scabies can generally be diagnosed easily, patient acceptance of the diagnosis may be challenging because the diagnosis can evoke feelings of dirtiness, shame, and social stigma. Moreover, 78% of respondents reported difficulty persuading asymptomatic family members to undergo treatment, while 76% expressed doubts about the adequacy of therapy for family members who did not attend a clinical evaluation. These factors may contribute to treatment failure and ongoing transmission, thereby preventing effective outbreak control.

Permethrin (5% cream or lotion) has been considered the gold standard treatment for scabies; however, recent studies and case reports have demonstrated high levels of resistance of *Sarcoptes scabiei* to this agent (7-9,14-16). Some studies, however, suggest that this resistance may be overestimated and that treatment failure could instead be attributed to application errors (17). In our study, physicians most frequently preferred sulfur as the first-line therapy for scabies (60.7%), while permethrin was selected less frequently (46.7%). Among pregnant women and children older than 2 years of age, sulfur was also the most common first-line treatment, while permethrin remained the preferred treatment in children under 2 years of age. 74.8% of participants reported successful outcomes with sulfur therapy, whereas only 41.1% reported that permethrin was effective. Notably, 47.7% of physicians stated that they did not observe a satisfactory response to permethrin. Despite permethrin's designation as the gold standard, prescriptions in our study indicated a shift toward sulfur treatment, which may reflect an observational signal of increasing resistance to permethrin in our country. However, this is a questionnaire-based study relying on physicians' opinions, and

**Table 5.** Comparison between ministry (public) and private hospitals

| | | Ministry Hospital (n=81) | | | Private Hospital (n=26) | | | p | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean ± SD, n (%) | | Median | Mean ± SD, n (%) | | Median | | |
| **Age** | | 37.1±10.2 | | 33.0 | 44.3±7.7 | | 43.5 | **0.000** | m |
| **Sex** | Male | 19 | 23.5% | | 3 | 11.5% | | 0.191 | X2 |
| | Female | 62 | 76.5% | | 23 | 88.5% | | | |
| **Average number of scabies patients seen per day** | | 9.6±9.2 | | 5.0 | 3.0±2.1 | | 2.0 | **0.000** | m |

m: Mann-Whitney U test, X2: Chi-square test

no direct measurement of resistance was performed. Therefore, this result should not be interpreted as confirmed resistance but rather as an observational finding that may indicate resistance. Similarly, a report from Türkiye described cases of recurrence following permethrin therapy that were successfully treated with oral ivermectin (15). A nother study from Türkiye demonstrated that 10% sulfur ointment was significantly more effective than 5% permethrin cream for the treatment of scabies (14). Nevertheless, in our study, 96% of participants attributed treatment failure to patients' incorrect application of therapy and to the refusal of asymptomatic family members to undergo treatment.

Improvements in healthcare services and increased accessibility to care have led to a significant rise in demand for medical care in Türkiye in recent years (18). This has led to an increased outpatient workload and limited the consultation time available per patient. In our study, 85% of physicians working in public hospitals reported allocating 2-7 minutes per patient, whereas 77% of those working in private institutions reported spending 10 minutes or more. Furthermore, the average daily number of scabies patients seen was nearly 10 in public hospitals compared with approximately 3 in private institutions. Eighty per cent of physicians reported that explaining the proper use of topical therapy was highly time-consuming. Nearly half of the participants believed that insufficient patient education and understanding, primarily due to limited consultation time, contributed to treatment failure. These findings suggest that the time allocated to patients is markedly insufficient to provide adequate counselling regarding topical and other treatment modalities.

The main limitations to the use of medical therapies were identified as treatment discontinuation due to irritation and unpleasant odour (10,17). A total of 79% of participants reported that the complexity of use, odour, and irritation, all associated with topical agents, prolonged the treatment process. Scabies poses a particularly high risk for outbreaks in institutional settings such as military barracks, boarding schools, dormitories, and nursing homes (19). Approximately 90% of participants reported difficulties treating individuals residing in communal environments.

In a study assessing the level of knowledge regarding the diagnosis and treatment of scabies in primary healthcare, it was reported that although physicians had sufficient knowledge about scabies, they experienced difficulties in its management (20). In our study, 47.7% of participants indicated that misdiagnosis and mistreatment occurred at the primary care level. Furthermore, 93% of respondents stated that scabies has become a significant public health problem, emphasising the need to reduce the burden on hospitals and to inform family physicians about the disease, thereby enabling them to perform household screening. In addition, 72% of physicians reported feelings of frustration due to the difficulties associated with the diagnosis, treatment, and follow-up of scabies.

Oral ivermectin is generally considered in cases where topical therapy fails. In Türkiye, oral ivermectin has been approved since 2022 for the treatment of scabies in patients unresponsive to topical agents (10). In our study, 60% of participants reported using oral ivermectin as a second-line treatment for nonresponse to topical therapy. In comparison, 20% indicated that they prescribed it as a first-line agent. Given the challenges associated with topical treatments, many participants expressed a preference for oral therapy as a first-line option due to its ease of administration and of explanation to patients.

### *Study Limitations*

This study has certain limitations. As the data were obtained through a self-reported online survey, responses may be subject to recall and response biases, and the recruitment of 107 dermatologists via social media may not fully capture the diversity of the broader dermatology community. The findings primarily reflect physicians' experiences rather than objectively measured clinical outcomes. Moreover, the study was conducted over a short time frame and included only hospital-based dermatologists, which may limit the generalisability of the results to other institutions and populations.

## CONCLUSION

Our findings indicate that dermatologists experience significant challenges in the management of scabies. The majority of participants reported no response to permethrin and expressed a stronger preference for sulfur-based formulations. Limited consultation times restricted physicians' ability to provide patients with detailed instructions and complicated treatment for family members, often leading to incomplete therapy and continued transmission. These issues, combined with high patient burden, contributed to feelings of fatigue and frustration among physicians. Therefore, extending consultation times, improving the training of family physicians in the diagnosis and management of scabies, and implementing systematic household screening should be emphasised as key strategies to achieve more effective disease control.

### *Ethics*

**Ethics Committee Approval:** The study protocol was approved by the Clinical Research Ethics Committee of Atatürk University Faculty of Medicine (file number: B.30.2.ATA.0.01.00/336, decision no: 117, date: 31.03.2022).

**Informed Consent:** Informed consent was obtained from all participants involved in the study. Participation in this study was completely voluntary and anonymous.

### *Footnotes*

### **Authorship Contributions*

Concept: N.M., E.U., Design: N.M., E.U., Z.U., E.K., Data Collection or Processing: N.M., E.U., Analysis or Interpretation: N.M., E.U., E.K., Literature Search: N.M., E.U., E.K., Writing: N.M., E.U., Z.U., E.K.

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

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## REFERENCES

1. Micali G, Lacarrubba F, Verzì AE, Chosidow O, Schwartz RA. Scabies: advances in noninvasive diagnosis. PLoS Negl Trop Dis. 2016; 10 :e0004691.
2. Karimkhani C, Colombara DV, Drucker AM, Norton SA, Hay R, Engelman D, et al. The global burden of scabies: a cross-sectional analysis from the Global Burden of Disease Study 2015. Lancet Infect Dis. 2017; 17: 1247-54.
3. Marks M, Engelman D, Romani L, Mason D, Sokana O, Kama M, et al. Exploration of a simplified clinical examination for scabies to support public health decision-making. PLoS Negl Trop Dis. 2018; 12: e0006996.
4. Salavastru CM, Chosidow O, Boffa MJ, Janier M, Tiplica GS. European guideline for the management of scabies. J Eur Acad Dermatol Venereol. 2017; 31: 1248-53.
5. Sunderkötter C, Feldmeier H, Fölster-Holst R, Geisel B, Klinke-Rehbein S, Nast A, et al. S1 guidelines on the diagnosis and treatment of scabies - short version. J Dtsch Dermatol Ges. 2016; 14: 1155-67.
6. Workowski KA, Bolan GA; Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2015. MMWR Recomm Rep. 2015; 64: 1-137. Erratum in: MMWR Recomm Rep. 2015; 64: 924.
7. Meyersburg D, Kaiser A, Bauer JW. Loss of efficacy of topical 5% permethrin for treating scabies: an Austrian single-center study. J Dermatolog Treat. 2020; 33: 1-4.
8. Luque-Luna M, Corbella-Bagot L, Giavedoni P. Permethrin-resistant scabies. Med Clin (Barc). 2024; 162: 403-4.
9. Mayer KE, Biedermann T, Posch C. European scabies challenge: what about permethrin-resistant mites? J Eur Acad Dermatol Venereol. 2022; 36: e712-3.
10. Uzun S, Durdu M, Yürekli A, Mülayim MK, Akyol M, Velipaşaoğlu S, et al. Clinical practice guidelines for the diagnosis and treatment of scabies. Int J Dermatol. 2024; 63: 1642-56.
11. Turan Ç, Metin N, Utlu Z. Epidemiological evaluation of scabies cases encountered in the last three years as a tertiary health center. Turkiye Parazitol Derg. 2020; 44: 77-82.
12. Özden MG, Ertürk K, Kartal SP, Yayli S, Göktay F, Doğramacı CA, et al. An extraordinary outbreak of scabies in Turkey. J Eur Acad Dermatol Venereol. 2020; 34: e818-20.
13. Turan Ç, Metin N. Impact of pandemic in the frequency of scabies: possible scabies outbreak scenario aftermath COVID-19. Turkiye Parazitol Derg. 2021; 45: 190-4.
14. Ertugrul G, Aktas H. Comparison of sulfur ointment and permethrin treatments in scabies. Dermatol Ther. 2022; 35: e15897.
15. Aydemir S, Kul Z, Barlik F, Saygin M, Ekici A, Yilmaz H. Increase in scabies cases and permethrin nonresponse in Türkiye. Indian J Public Health. 2025; 69: 63-6.
16. Mang R, Kremer A, Lehmann P, Assmann T. Skabies – klinische therapieresistenz auf permethrin : fallbeschreibungen und eine kritische auseinandersetzung mit den aktuellen therapieempfehlungen [Scabies-clinical resistance to permethrin therapy: case reports and a critical discussion of current treatment recommendations]. Hautarzt. 2021; 72: 595-9. German.
17. Yürekli A. Is there a really resistance to scabies treatment with permethrin? In vitro killing activity of permethrin on *Sarcoptes scabiei* from patients with resistant scabies. Dermatol Ther. 2022; 35: e15260.
18. Beştemir A, Aydın H. 300 million patient examinations per year; evaluation of emergency and polyclinic services of 2nd and 3rd stage public health facilities in Türkiye. Sakarya Medical Journal. 2022; 12: 496-502.
19. Mounsey KE, Murray HC, King M, Oprescu F. Retrospective analysis of institutional scabies outbreaks from 1984 to 2013: lessons learned and moving forward. Epidemiol Infect. 2016; 144: 2462-71.
20. Etgu F, Onder S. Evaluating knowledge level about scabies in primary care physicians during the scabies outbreak of Turkey. Turk J Dermatol. 2023; 17: 57-63.