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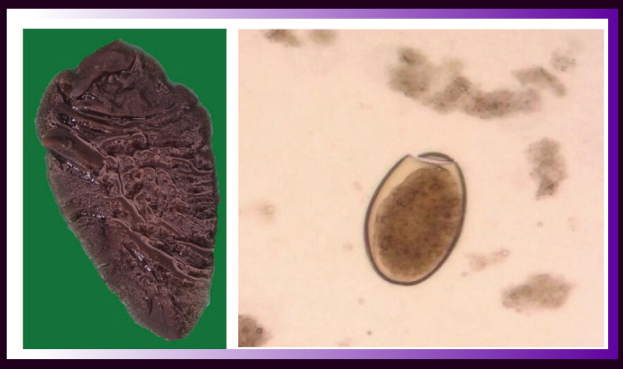

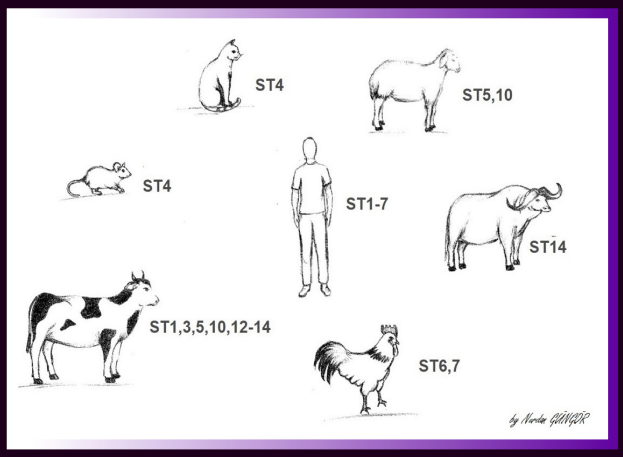

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EDİTÖRDEN

Bu yılın üçüncü sayısında 9 özgün araştırma, 2 derleme ve bir olgu sunumu yer almaktadır. Özgün makalelerde, *Acanthamoeba*'nın moleküler tanısından leishmaniasis ve scabies ile ilgili makalelerin bibliyometrik ve scientometrik analizine, balık parazitlerinden, Ağrı ilinde sığırlardaki fascioliasis yaygınlığına, bazı yağların cryptosporidiosis üzerine etkinliğine ve Orta ile Doğu Karadeniz'de ev tozu akarlarının yaygınlığına oldukça geniş bir yelpazede araştırmalar bulunmaktadır. Derleme makalelerde ise ülkemizde *Blastocystis* alt tiplerine ve kedilerde görülen parazitlerin yer aldığı çalışmalar sunulmuştur. Olgu sunumunda ekstrem bir kist hidatik olgusuna yer verilmiştir.

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Prof. Dr. Yusuf Özbel

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Telefon/Phone: +90 (212) 621 99 25
Faks/Fax: +90 (212) 621 99 27
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Contact

Editorial Office

Editor in Chief: Yusuf Özbel, MD, Prof

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

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

Fax: +90 232 388 13 47

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

İÇİNDEKİLER/CONTENTS

ÖZGÜN ARAŞTIRMALAR / ORIGINAL INVESTIGATIONS

Türkiye PARAZİTOLOJİ Dergisi

İÇİNDEKİLER/CONTENTS

Turkiye Parazitol Derg 2023;47(3):129-35
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Acanthamoeba'nın Hızlı Tespiti için *18S rRNA* Genine Özgü Hızlı Döngü Aracılı İzotermal Amplifikasyon (LAMP) Testinin Geliştirilmesi ve Hassasiyetinin Belirlenmesi

Development and Sensitivity Determination of 18S rRNA Gene-specific Fast Loop-mediated Isothermal Amplification (LAMP) Assay for Rapid Detection of Acanthamoeba

Mehmet Aykur[1], Muhammet Karakavuk[2,3], Mesut Akıl[4], Hüseyin Can[3,5], Mert Döşkaya[3,6], Adnan Yüksel Gürüz[3,6], Hande Dağcı[6], Aysu Değirmenci Döşkaya[3,6]

[1]Tokat Gaziosmanpaşa Üniversitesi Tıp Fakültesi, Parazitoloji Anabilim Dalı, Tokat, Türkiye
[2]Ege Üniversitesi Ödemiş Meslek Yüksekokulu, Laborant ve Veteriner Sağlık Bölümü, İzmir, Türkiye
[3]Ege Üniversitesi Aşı Geliştirme, Uygulama ve Araştırma Merkezi, İzmir, Türkiye
[4]İstanbul Medeniyet Üniversitesi Tıp Fakültesi, Parazitoloji Anabilim Dalı, İstanbul, Türkiye
[5]Ege Üniversitesi Fen Fakültesi, Moleküler Biyoloji Anabilim Dalı, Biyoloji Bölümü, İzmir, Türkiye
[6]Ege Üniversitesi Tıp Fakültesi, Parazitoloji Anabilim Dalı, İzmir, Türkiye

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ÖZ

Amaç: Serbest yaşayan amiplerden biri olan *Acanthamoeba*, başta su, toprak ve hava olmak üzere birçok çevresel örnekte tespit edilmiştir. *Acanthamoeba*'nın neden olduğu en önemli klinik tablolar içinde *Acanthamoeba* keratiti ve granülomatöz amibik ensefalit bulunmaktadır. Bu çalışmada klinik ve çevresel örneklerde *Acanthamoeba*'nın varlığının daha hızlı bir şekilde saptanabilmesi için *Acanthamoeba 18S rRNA* gen bölgesine özgü primerler ile geliştirilen hızlı döngü aracılı izotermal amplifikasyon (LAMP) testinin hassasiyetinin belirlenmesi amaçlanmıştır.

Yöntemler: Kültürde çoğaltılan *Acanthamoeba* spp. suşu 200 µL'de 1x10^6 trofozoit olacak şekilde sulandırılıp DNA izolasyonu yapılarak DNA miktarı Nano-Drop Spektrofotometre ile belirlenmiştir. Saflaştırılan DNA'lar 1000 pg'den 0,001 pg'ye kadar seyreltilip, kolorimetrik ve floresan tabanlı iki farklı LAMP reaksiyonunda kullanılmıştır. LAMP reaksiyon karışımı toplam 25 µL hacimde 63 °C'de 60 dk olacak şekilde inkübe edilmiştir.

Bulgular: Geliştirilen testlerin hassasiyetinin saptanmasında elde edilen *Acanthamoeba* spp. genomik DNA'sını hem kolorimetrik hem de floresan tabanlı LAMP testlerinde 1, 10, 100 ve 1000 pg/reaksiyon pozitiflik gözlenmiştir. Hem kolorimetrik hem de floresan LAMP testinin en düşük analitik hassasiyeti 1 pg/reaksiyon olarak tespit edildi. Ayrıca, testin özgüllüğünü değerlendirmek için diğer parazit DNA'ları ile uygulanan LAMP reaksiyonu sonucunda kolorimetrik ve %1'lik agaroz jel elektroforezinde pozitif kontrol dışında LAMP ürünü tespit edilmemiş olup, geliştirilen testin özgüllüğü %100 olarak tespit edilmiştir.

Sonuç: *Acanthamoeba*'nın *18S rRNA* gen bölgesi hedef alınarak geliştirilen LAMP testinin 1 pg genomik DNA saptama limitine sahip olduğu ortaya konulmuştur. LAMP testinin kültür yöntemine göre daha hassas ve hızlı olmasının yanı sıra basit, ucuz ve yüksek hassasiyete sahip olması umut vericidir. Bu nedenle geliştirilen testin çevresel ve klinik örneklerde *Acanthamoeba* spp. tanısı için uygulanabileceği düşünülmektedir.

Anahtar Kelimeler: *Acanthamoeba* spp., *18S rRNA* geni, hassasiyet, kolorimetrik LAMP, floresan LAMP

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Yazar Adresi/Address for Correspondence: Mehmet Aykur, Tokat Gaziosmanpaşa Üniversitesi Tıp Fakültesi, Parazitoloji Anabilim Dalı, Tokat, Türkiye
Tel/Phone: +90 534 575 11 46 **E-Posta/E-mail:** mehmetaykur@gmail.com **ORCID ID:** orcid.org/0000-0002-6100-1037

Türkiye PARAZİTOLOJİ Dergisi

ABSTRACT

Objective: *Acanthamoeba*, one of the free-living amoeba, has been detected in many environmental samples, mainly in water, soil and air. *Acanthamoeba* keratitis and granulomatous amoebic encephalitis are among the most important clinical manifestations caused by *Acanthamoeba*. In this study, it was aimed to determine the sensitivity of the rapid loop mediated isothermal amplification (LAMP) test designed with primers specific to *Acanthamoeba 18S rRNA* gene to detect more rapidly the presence of *Acanthamoeba* in clinical and environmental samples.

Methods: *Acanthamoeba* strain grown in culture was diluted in 200 µL as $1x10^6$ trophozoites and DNA was isolated, and the amount of DNA was determined by Nano-Drop Spectrophotometer. The purified DNAs were diluted from 1000 pg to 0.001 pg and used in colorimetric and fluorescence-based LAMP reactions. The LAMP reaction mixture was incubated for 60 minutes at 63 °C in a total volume of 25 µL.

Results: To determine the sensitivity of the test, positivity of *Acanthamoeba* genomic DNA was observed at 1, 10, 100 and 1000 pg/reaction in both colorimetric and fluorescence-based LAMP tests. The lowest analytical sensitivity of both calorimetric and fluorescent LAMP assay was determined as 1 pg/reaction. In addition, as a result of LAMP reaction applied with other parasite DNAs to evaluate the specificity of the test, no LAMP product was detected in calorimetric and 1% agarose gel electrophoresis, except for positive control, and the specificity of the test was determined as 100%.

Conclusion: It has been demonstrated that the LAMP assay designed by targeting *18S rRNA* gene of *Acanthamoeba* has a detection limit of 1 pg of genomic DNA. It is promising that LAMP test is more sensitive and faster than culture method, as well as simple, inexpensive and highly sensitive. For this reason, it is thought that developed test can be applied in the diagnosis of *Acanthamoeba* in environmental and clinical samples.

Keywords: *Acanthamoeba* spp., *18S rRNA* gene, sensitivity, colorimetric LAMP, fluorescence LAMP

GİRİŞ

Serbest yaşayan amiplerden biri olan *Acanthamoeba* cinsindeki türler, toprak, tatlı su kaynakları ve denizler gibi doğal ekosistemlerin yanı sıra havalandırma sistemleri, havuzlar, ameliyathaneler gibi insan yapımı alanlarda da yaşayabilmektedir (1-3). Ayrıca, kontakt lenslerden, lens saklama kapları ve solüsyonlarından da *Acanthamoeba* izole edilmiştir. Bu fırsatçı amip, merkezi sinir sistem hastalığı olan granülomatöz amibik ensefalitte (GAE), gözde görmeyi tehdit eden bir göz hastalığı olan *Acanthamoeba* keratitine (AK) ve özellikle bağışıklık sistemi baskılanmış kişilerde *Acanthamoeba* pnömonisi ve deri lezyonları gibi çeşitli klinik semptomlara yol açabilmektedir (4). Ayrıca, bu hastalıklar yalnızca insanlarda değil aynı zamanda da hayvanlarda görülmektedir (5). Köpek, sığır, kuş ve at gibi bazı hayvanlarda da bu fırsatçı patojenin enfeksiyonlara neden olabildiği bildirilmiştir (6-8).

Acanthamoeba'nın yaşam döngüsünde trofozoit ve kist olmak üzere iki formu vardır. Trofozoit formu aktif olarak beslenen, büyüyen, çoğalan ve hareket eden bir formudur. Kist formu ise olumsuz dış çevre koşullarına karşı daha dayanıklı olan formudur (1). Trofozoitler, sıcaklık ve pH uygun ve gıda yeterli ise yaşayabilirler. Ancak bu faktörlerin yokluğunda endokist ve ektokistten oluşan çift duvarlı bir kiste dönüşür. Zorlu koşullara dayanıklı olan kistler antibiyotiklere, klora ve dezenfektanlara karşı dirençlidirler. Koşullar tekrar uygun olduğunda, eksitasyon ile trofozoit formuna dönüşebilirler (1,9).

Acanthamoeba, özellikle bağışıklığı baskılanmış hastalarda nadir görülen ancak ölümcül GAE'ye neden olur. GAE'nin ölüm oranı %97-98 civarındadır. Bugüne kadar, tüm dünyada yaklaşık 200'den fazla *Acanthamoeba*'nın neden olduğu GAE olgusu rapor edilmiştir (10). Amerika Birleşik Devletleri'nde her yıl *Acanthamoeba*'nın neden olduğu yaklaşık 10 GAE olgusu bildirilmektedir (11). Türkiye'de bugüne kadar *Acanthamoeba*'nın sebep olduğu toplam dört GAE olgusu rapor edilmiştir (12). Özellikle gelişmiş ülkelerde AK olgularının küresel insidans oranı artmaya devam etmekte olup ve en önemli risk faktörü olarak kontakt lens kullanıcıları olguların %85-88'ini oluşturduğu bildirilmiştir (13). Son zamanlarda, miyopi ve kozmetik amaçlı kontakt lens kullananların sayısı artmış ve kornea lazer refraktif cerrahi sonrası AK, nadir de olsa bildirilmiştir (14). Kontakt lens kullanımının daha az olduğu gelişmekte olan ülkelerde çoğu olgunun travma ile ilişkili olmasının yanı sıra, ana bulaş kaynağı kontamine su, toprak ve toz olduğu belirtilmiştir (13,15,16).

AK'nin erken teşhisi etkili tedavi için önemlidir. Ancak, AK sıklıkla başlangıçta herpes simpleks virüsü, bakteriyel veya fungal keratite benzerlik gösterdiğinden yanlış teşhis edilebilir. Bu yüzden tedavide gecikmeler olur ve sıklıkla önemli görme kaybına veya kornea nakli ameliyatına sebep olabilir (16). *Acanthamoeba*'nın klinik örneklerde tanısında mikroskobik inceleme, kültür yöntemi gibi yöntemlerle doğrudan patojenin varlığının tanımlanması hem zaman alıcı hem de teknik uzmanlık gerektiren bir yöntemdir. Ayrıca, bu yöntemler genellikle hassas, uygun veya kesin bir teşhis için yeterince uygulanabilir değildir (13,17). *Acanthamoeba*'nın saptanmasını daha da iyileştirmek için polimeraz zincir reaksiyonu (PZR), gerçek zamanlı PZR gibi moleküler yöntemler kullanılması daha yüksek hassasiyette ve daha kısa süre gerektirmesine rağmen pahalı laboratuvar ekipmanlarına duyulan ihtiyaç nedeniyle hala yaygın olarak kullanılmamaktadır (17-19). Bu nedenle *Acanthamoeba* tanısı için basit, hızlı ve hassas bir tanısal teste ihtiyaç olduğu düşünülmektedir.

Döngü aracılı izotermal amplifikasyon (LAMP) yöntemi teşhis amacıyla kullanılan ilk izotermal moleküler tabanlı bir yöntemdir. Bu yöntem, geleneksel DNA tabanlı teşhis yöntemlerine umut verici bir alternatiftir. Çünkü hızlı DNA amplifikasyonuna dayalı, basit ve oldukça yüksek duyarlılık ve özgüllüğe sahip bir testtir. LAMP yönteminde izotermal koşullar altında *Bacillus stearothermophilus* DNA polimerazı ve spesifik olarak tasarlanmış 4-6 primer kullanarak bir saatten daha kısa sürede hedef DNA'nın 10^9 kopya sayısına kadar çoğaltılabilmektedir (20). LAMP tekniğinde kullanılan primer sayısının PZR'de kullanılandan daha fazla olması amplifikasyonun özgüllüğünü ve hızını iyileştirir, böylece çoklu bantlardan oluşan tipik bir merdiven modeli üreten döngü yapılı bir amplikon oluşturur (21). Pozitif bir reaksiyon, bulanıklık olarak gözle kolorimetrik veya floresan boyaların eklenmesiyle kolayca belirlenir (22).

Bu çalışmada *Acanthamoeba* tanısını daha hızlı bir şekilde gerçekleştirmek için *Acanthamoeba 18S rRNA* gen bölgesine özgü primerler tasarlanarak hem kolorimetrik hem de floresan tabanlı hızlı LAMP testinin hassasiyetinin belirlenmesi amaçlanmıştır.

YÖNTEMLER

Acanthamoeba Kültürü ve Hazırlanması

Bu çalışmada kullanılan *Acanthamoeba* suşu, Ege Üniversitesi Tıp Fakültesi Hastanesi'nde AK'li bir hastanın gözünden izole edilmiş ve bu izolat genotipi önceki çalışmamızda T4 (GenBank no: ON600789.1) olarak tanımlanmıştır (23). Kriyopreservasyon (-80 °C) işlemi ile saklanmış olan *Acanthamoeba* suşunu tekrar canlandırmak için daha önce hazırlanmış olan %1,5'lik non-nutrient agarın (NNA) inaktive edilmiş *Escherichia coli* (ATCC 25992) ile yüzeyi kaplanan agar plakların üzerine eklenerek 37 °C'de inkübe edilmiştir. NNA plaklarında çoğalma meydana geldikten sonra *Acanthamoeba* suşu aksenik kültür olan proteaz pepton %0,75, maya ekstresi %0,75 ve %1,5 glikoz içeren (PYG) ortamda 30 °C'de kültüre edilmiştir. Amipler toplanıp 500 g'de 10 dk santrifüj edilip ve PBS ile üç kez yıkanmıştır.

DNA İzolasyonu

Kültüre edilmiş *Acanthamoeba*'nın total genomik DNA'sı QIAamp DNA Mini Kit'i (Qiagen, Almanya) kullanılarak üretici firmanın protokolüne göre izole edilmiştir. Kısaca; 200 µL'sinde $1x10^6$ trofozoit içeren kültür sıvısı üzerine 20 µL proteinase K ve 200 µL buffer AL eklenip ardından 56 °C'de 10 dk inkübe edilmiştir. İnkübasyondan sonra 200 µL etanol eklenip vorteks yapılıp ve QIAamp filtreli kolon tüplerine aktarılmıştır. Daha sonra QIAamp filtreli kolon tüpleri iki kez sırasıyla 500 µL Buffer AW1 ve Buffer AW2 ile yıkanmıştır. DNA'yı elde etmek için 100 µL Buffer AE eklenerek santrifüj yapılmıştır. Ekstrakte edilen DNA miktarı Nano-Drop spektrofotometre ile ölçüldükten sonra çalışılıncaya kadar -20 °C'de saklanmıştır.

LAMP Primer Tasarımı

Acanthamoeba'ya özgü *18S rRNA* geni hedefleyen altı farklı bölgeyi tanıyan iki iç ve iki dış primer setleri PrimerExplorer V5 yazılımı (http://primerexplorer.jp/lampv5e/index.html) kullanılarak tasarlanmıştır (Tablo 1). Tasarlanan primerlerin *Acanthamoeba*'ya spesifik olduğu, Ulusal Biyoteknoloji Bilgi Merkezi (NCBI) BLAST programı kullanılarak (https://blast.ncbi.nlm.nih.gov/Blast.cgi) kontrol edilmiştir.

LAMP Reaksiyonun Optimizasyonu

LAMP testinin optimizasyonu için ilk olarak Kolorimetrik LAMP reaksiyonları, WarmStart Colorimetric LAMP 2X Master Mix with UDG (New England Biolabs, Ipswich, ABD) kullanılarak gerçekleştirilmiştir. 25 µL LAMP reaksiyon içeriği; 12,5 µL WarmStart Colorimetric LAMP 2X Master mix, 2,5 µL LAMP primer karışımı (final konsantrasyon FIP ve BIP: 1,6 µM, F3 ve B3: 0,2 µM, LF ve LB 0,4 µM), 1 µL total genomik DNA ve 9 µL nükleaz içermeyen su ile hazırlanmıştır. İkinci olarak, WarmStart Fluorescent LAMP (DNA&RNA) kiti (New England Biolabs, Ipswich, ABD) kullanılarak toplam hacmi 25 µL olan reaksiyon; 12,5 µL, WarmStart LAMP 2X Master mix, 2,5 µL LAMP primer karışımı (final konsantrasyon FIP ve BIP: 1,6 µM, F3 ve B3: 0,2 µM, LF ve LB 0,4 µM), 0,5 µL floresan boya, 1 µL total genomik DNA ve 8,5 µL nükleaz içermeyen su ile hazırlanmıştır. LAMP reaksiyonları termal blokta 59, 61, 63 ve 65 °C sıcaklıklarda optimal sıcaklığın ve sürenin belirlenmesi için 30, 45 ve 60 dakika olacak şekilde test edilmiştir. Elde edilen LAMP ürünleri %1 agaroz jel elektroforezi üzerinde yürütülüp ve SafeView Classic (Abm, Kanada) eklenerek DNR Bio-görüntüleme sistemleri altında görüntülenmiştir.

Acanthamoeba'nın Analitik Hassasiyeti

Kolorimetrik ve floresan LAMP testlerinin tanımlayabileceği minimum DNA miktarını belirlemek ve LAMP reaksiyonun duyarlılığının değerlendirmesi için, *Acanthamoeba* DNA'sının 10 kat seri sulandırmaları (1000, 100, 10, 1, 0,1, 0,01, 0,001 pg/µL) kullanılmıştır. Kolorimetrik ve floresan LAMP reaksiyonları yukarıda tespit edilen (63 °C'de 60 dakika) optimal koşullara göre yapılmıştır. Kolorimetrik LAMP testinin sonuçlarının değerlendirilmesinde indikatörlerin pH değişikliğine bağlı olarak pembe renkten sarı renge dönüşmektedir. Böylece pembe renk negatif durumu gösterirken sarı renk pozitif durumu ifade edecek şekilde yorumlanmıştır. Floresan LAMP testinin değerlendirilmesinde ise ultraviyole (UV) ışığı altında floresan yansımalar incelenmiştir.

Ayrıca kolorimetrik LAMP testinin özgüllüğünü saptamak için diğer parazit etkenlerinden (*Giardia intestinalis, Leishmania infantum, Blastocystis, Toxoplasma gondii* ve *Naegleria fowler*i) elde edilen genomik DNA'lar LAMP ile amplifiye edilip %1 agaroz jel elektroforezi üzerinde yürütülmüştür.

İstatistiksel Analiz

Bu çalışmada istatistiksel analiz yapılmamıştır.

BULGULAR

Acanthamoeba Kültürü ve DNA İzolasyonu

Acanthamoeba suşu NNA plaklarında çoğalması görüldükten sonra aksenik kültür olan PYG besiyerine alınarak çoğalması için 30 °C'de inkübe edilmiştir. İnkübasyondan 7 gün sonra *Acanthamoeba*'nın %95'i trofozoit formunda ve %5'i ise kist fomunda tespit edilmiştir (Şekil 1). Amipler toplanıp hemositometre kullanılarak mikroskop altında sayılmıştır. Amipler DNA izolasyonu için 200 µL'de $1x10^6$ olacak şekilde hesaplanmıştır. DNA izolasyonu yapıldıktan sonra Nano-Drop spektrofotometre ile ölçülerek total genomik *Acanthamoeba* DNA konsantrasyonu 1000, 100, 10, 1, 0,1, 0,01, 0,001 pg olacak şekilde sulandırılmıştır.

Tablo 1. *Acanthamoeba* spp. *18S rRNA* genine özgü LAMP testi için tasarlanan primerler

Primerler	Hedef gen	Sekans (5´ → 3´)
F3	*18S rRNA*	GGGGATCGAAGACGATCAGA
B3		CCCCGGAACCCAAAGACT
FIB		GCGCCGATGGTGGTGTTTTGTAAACCATAAACGATGCCGACC
BIP		GGCGTCTGTCCCTTTCAACGGCTGCTAGGGGAGTCATTCAC
LF		TTCAACGTCTCCTAATCGCT
LB		GGTTTAGCCCGGTGGCA

LAMP Primer Tasarımı

LAMP yöntemi ile *Acanthamoeba*'yı saptamak için PrimerExplorer V5 programı kullanılarak tasarlanan *Acanthamoeba castellanii Neff* suşun (GenBank No: U07416) 18S rRNA bölgesine özgü 6 LAMP primerleri Tablo 1'de gösterilmiştir. Tasarlanan primerlerin *Acanthamoeba* ile %100 benzerlik gösterdiği ve diğer parazitler ile benzerliği bulunmadığı online NCBI BLAST programı ile değerlendirilmiştir.

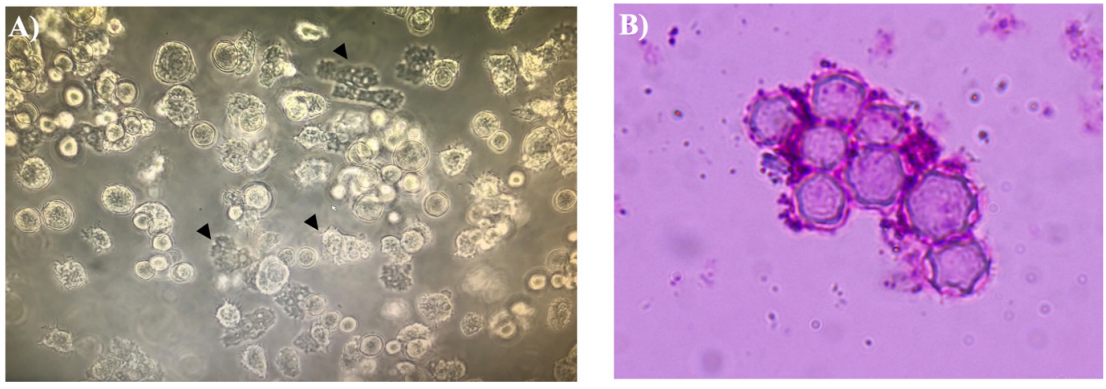
LAMP Reaksiyonun Optimizasyonu

LAMP'ın optimizasyonu için 30, 45 ve 60 dakika olacak şekilde 59, 61, 63 ve 65 °C sıcaklıklarda inkübe edilerek elde edilen LAMP ürünleri değerlendirilmiştir. Her ne kadar 45 dakikalık inkübasyonda LAMP ürünü gözlenmiş olsa da elde edilen ürünün rengi, pembe renk olan negatif kontrol ile karıştırılabilecek olan turuncuya dönüştüğü gözlenmiştir. Bu yüzden elde edilen sonuçlar hem kolorimetrik hem de floresan LAMP testinin 60 dakika süreyle 63 °C'de inkübasyonda en iyi ürün tespit edilmiştir.

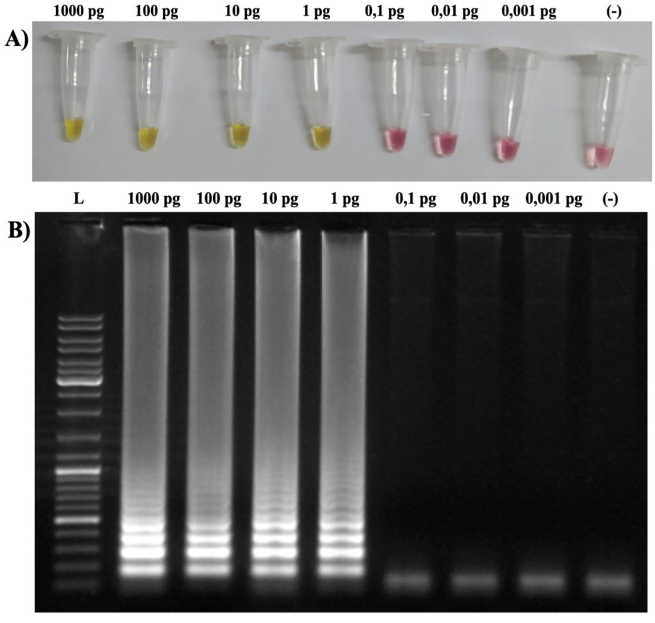
Kolorimetrik LAMP reaksiyonunda pozitif örneklerde pembeden sarıya bir renk değişimi görülürken, negatif örneklerde pembe olarak kalmıştır. Floresan LAMP reaksiyonunda pozitif örnekler UV ışık altında ışıma vererek tespit edilmiştir.

Acanthamoeba'nın Analitik Hassasiyeti

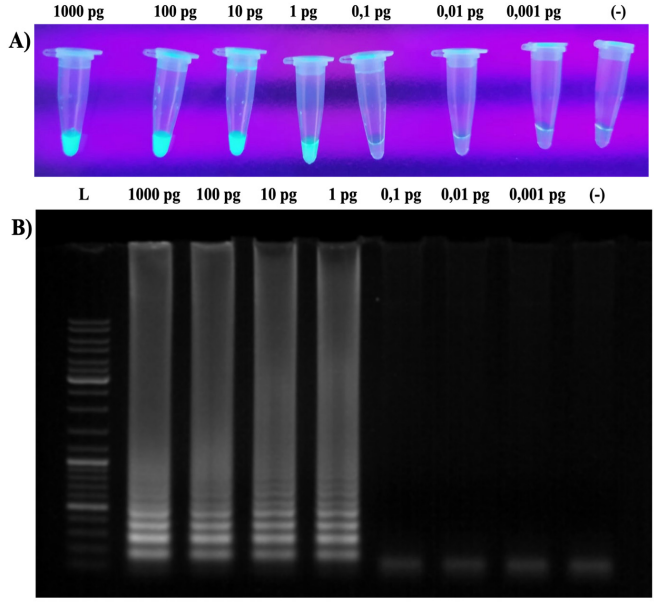
Nano-Drop Spektrofotometre ile ölçülen total genomik *Acanthamoeba* DNA konsantrasyonu 1000, 100, 10, 1, 0,1, 0,01, 0,001 pg olacak şekilde farklı oranlarda sulandırılmıştır. Bir dizi sulandırılan örnekler ile yapılan LAMP reaksiyonu sonucunda kolorimetrik gözlemde 1, 10, 100 ve 1000 pg/reaksiyon tüplerinde rengin pembeden sarıya dönüştüğü gözlenmiştir (Şekil 2A) ve aynı şekilde floresan LAMP reaksiyon sonucunda da UV ışını altında 1, 10, 100 ve 1000 pg/reaksiyon tüplerinde ışıma gözlenmiştir (Şekil 3A). Ayrıca, LAMP ile pozitif olarak tespit edilen testin en düşük konsantrasyon %1'lik agaroz jel elektroforez üzerinde yürütüp oluşan bantlar ile test doğrulanmıştır (Şekil 2B, 3B). Böylece hem kolorimetrik hem de floresan LAMP testinin en düşük analitik hassasiyeti 1 pg/reaksiyon olarak tespit edilmiştir.



Şekil 1. A) PYG besiyerinde çoğalmış *Acanthamoeba* trofozoitleri (siyah ok başı), **B)** Giemsa boyama yöntemi ile boyanmış *Acanthamoeba* kist şekilleri



Şekil 2. LAMP reaksiyonunda *Acanthamoeba* DNA'sının bir seri seyreltilmesinin **A)** Kolorimetrik görüntüleme ile analitik hassasiyetinin gösterilmesi **B)** %1'lik agaroz jelde görüntülenmesi **(-)** Negatif kontrol

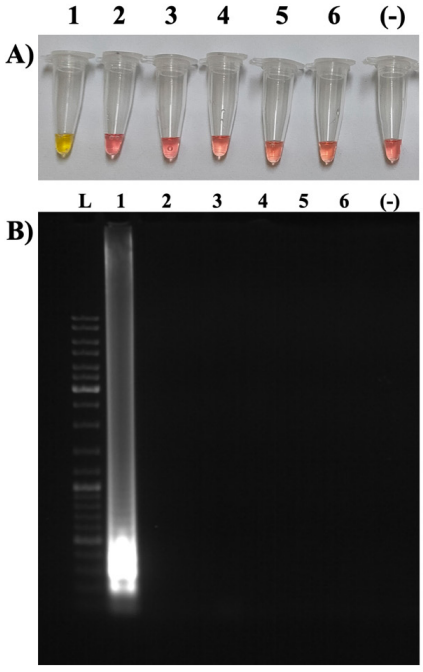


Şekil 3. LAMP reaksiyonunda *Acanthamoeba* DNA'sının bir seri seyreltilmesinin **A)** Ultraviyole altında floresan boya ile analitik hassasiyetinin gösterilmesi **B)** %1'lik agaroz jelde görüntülenmesi **(-)** Negatif kontrol

Çalışmamızda LAMP testinin özgüllüğünü değerlendirmek için *Giardia intestinalis, Leishmania infantum, Blastocystis* sp., *Toxoplasma gondii* ve *Naegleria fowleri*'nin DNA örnekleri kullanılmıştır. Kolorimetrik LAMP testinde pozitif kontrolde sarı renk görülür iken diğer örneklerde herhangi bir renk değişikliği olmayıp pembe renk olarak gözlenmiştir (Şekil 4A). Ayrıca, LAMP ürünü %1'lik agaroz jel elektroforezinde yalnızca pozitif kontrolde (*Acanthamoeba* T4 genotipi, GenBank no: ON600789.1) amplifiye ürünler görülürken diğer parazit DNA'larının ürünlerinde herhangi bir bant görülmemiştir (Şekil 4B).

TARTIŞMA

Acanthamoeba cinsinin birçok türleri ve bazı genotipleri dünyanın birçok yerinde yaygın olarak görülmektedir. Bu amipler suda, toprakta, havada, sebze ve meyvelerde, kontakt lens solüsyonlarında, hayvanlarda, insan dokuları ve boşluklarında tespit edilmiştir (1,4,24,25). *Acanthamoeba* insanlarda merkezi sinir sistemini etkileyen ciddi enfeksiyon olan GAE ve görmeyi tehdit eden AK'ye sebep olmaktadır. Ayrıca, kontakt lens takanlar için büyük bir risk olan AK'nin insidansının son yıllarda arttığı bildirilmiştir (4,16). Bu parazitin sebep olduğu hastalıkların teşhis edilmesi ve yönetilmesi oldukça zordur. Ayrıca, etkili bir tedavi sağlanabilmesi için hızlı, duyarlı, ucuz ve kolayca uygulanabilen ve yorumlanabilen sonuçlar sağlayan bir tanı yöntemi gerektirir. Özellikle AK tanısında erken tanı hayati önem taşımaktadır. Ancak, hastalığın erken klinik görünümü Herpes ve mantar enfeksiyonları ile kolay bir şekilde karışabilmektedir. Bu yüzden yanlış tanıya sebep olarak tedavinin gecikmesine sebep olmaktadır (26,27). Kornea kazıntı örneklerinin doğrudan yaymaları veya kültüre edilmiş örneklerinin mikroskobik tanımlanmaları, AK'nin laboratuvar teşhisinde oldukça yaygın kullanılan bir yöntemdir. Ancak, *Acanthamoeba* trofozoitleri ve kistlerinin genellikle kazınmış hücrelere veya hücre döküntülerine benzer olduğundan dolayı ayırt etmek zaman alıcı ve teknik uzmanlık gerektirmektedir. Konfokal mikroskopu, yakın zamanda, kültür ve mikrobiyolojik analizlere ihtiyaç kalmadan *in vivo* olarak enfeksiyonun hızlı teşhisini sağlamaktadır. Ancak bu yöntem her laboratuvar için uygun olmayan pahalı ve özel uzmanlık gerektiren bir araçtır (28,29). Bu nedenle, *Acanthamoeba*'nın laboratuvar tanısının için hassas, hızlı ve düşük maliyetli bir yöntem gereklidir. Bu çalışmada *Acanthamoeba*'nın *18S rRNA* gen bölgesine özgü primerler tasarlanarak hem kolorimetrik hem de floresan görüntüleme yöntemleri kullanılarak hassas, ucuz ve hızlı LAMP testinin etkinliği gösterilmiştir.



Şekil 4. LAMP testinin özgüllüğünün gösterilmesi **A)** Kolorimetrik olarak elde edilen ürünlerin görüntüsü **B)** %1'lik agaroz jel elektroforez görüntüsü, **1:** *Acanthamoeba* spp., **2:** *Giardia intestinalis*, **3:** *Leishmania infantum*, **4:** *Blastocystis*, **5:** *Toxoplasma gondii*, **6:** *Naegleria fowleri*
L: DNA ladder, **(-)**: Negatif kontrol

Acanthamoeba'nın doku, kornea kazıntıları veya çevresel örneklerde tanımlamak için PZR ve genomik dizileme gibi yöntemler geliştirilmiştir. Ancak, *Acanthamoeba*'nın teşhisi için kullanılan bu yöntemin reaksiyon süresinin uzun olması, maliyetinin yüksek ve karmaşık olması, pahalı laboratuvar cihazları gerektirmesi ve alanında uzman bir kişiye ihtiyaç duyulması açısından dezavantajları bulunmaktadır (19,30). Ancak, PZR'den daha ucuz ve daha verimli olan LAMP yöntemi, 2000 yılında Notomi ve ark. (20) tarafından geliştirilen nispeten yeni bir moleküler tekniktir. LAMP testi için dört primer hedefin altı farklı bölgesi için tasarlanan primerler sayesinde, hedef diziyi yüksek seçicilik ve özgüllükle çoğaltması beklenir. Hemen hemen tüm araştırmalarda LAMP tekniğinin özgüllüğünün %100'e yakın olduğu bildirilmiştir (21). Bakteriyel, viral ve paraziter enfeksiyonların moleküler tespiti dahil birçok klinik uygulama için LAMP tekniği başarılı bir şekilde rapor edilmiştir (31,32). LAMP, pahalı olmayan su banyosu gibi ucuz cihazlar ile gerçekleştirilebilmektedir. Ayrıca, PZR'de kullanılan agaroz jel elektroforezi gibi uygulamalara ihtiyaç duyulmamaktadır. Test sonucu oluşan renk değişikliği çıplak gözle veya floresan boya kullanarak değerlendirilebilmektedir (21,33).

Bu çalışmada *Acanthamoeba*'nın *18S rRNA* genine özgü LAMP primerleri tasarlanmıştır. *Acanthamoeba* genomunda bu gen bölgesi cinse özgü olmasının yanı sıra genotipleme için de yaygın olarak kullanılan değişken bölgedir (19). Ayrıca, daha önceki çalışmalarda klinik ve çevresel örneklerin bu gen bölgesine ait primerlerin kullanılmasından dolayı bu çalışmada da bu bölge seçilmiştir (12,34-37).

Türkiye'de çeşitli çevresel ve klinik örneklerde bazı parazitlerin varlığı LAMP testi ile tespiti gerçekleştirilmiştir (38). Çevresel su örneklerinde *Cryptosporidium* ookistlerin varlığının tespit edilmesinde LAMP yönteminin PZR yönteminden daha duyarlı olduğu ortaya konmuştur (36). Ayrıca, çevresel su örneklerinde *Toxoplasma gondii*'nin *B1* geni ile geliştirilen LAMP yöntemi ile *18S rRNA* genine özgü PZR yöntemin karşılaştırılıp LAMP yönteminin daha duyarlı olduğu rapor edilmiştir (37). Başka bir çalışmada *Toxoplasma gondii*'nin *RE* gen bölgesine özgü LAMP primerleri tasarlanarak testin hassasiyeti 0,05 takizoit olarak tespit edilip PZR'ye göre *RE* gen bölgesi 1,000 kat daha hassas olduğu bildirilmiştir (32).

Acanthamoeba'nın tanısında altın standart olarak kültür yönteminin kabul edilmesine rağmen önceki çalışmalar LAMP testinin, direkt mikroskobik inceleme, kültür yöntemi ve PZR yöntemlerinden duyarlılığının ve özgüllüğünün daha yüksek olduğunu göstermiştir (39,40). Önceki çalışmalarda LAMP

yönteminin duyarlılığı *Acanthamoeba* spp.'nin DNA'sı bir seri sulandırma ile değerlendirilip, 10 pg *Acanthamoeba* DNA'sı floresan boya ile UV ışık altında gösterilmiştir (41). Bir başka çalışmada ise *in vitro* olarak kültüre edilmiş *Acanthamoeba* türlerinden ekstrakte edilen DNA'ların seyreltilerek LAMP testinde minimum genomik DNA konsantrasyonu 1 pg *Acanthamoeba* DNA'sı floresan boya ile tespit edilmiştir (39). Çalışmamızda kültüre ettiğimiz *Acanthamoeba*'dan elde edilen DNA'ların bir seri seyreltildikten sonra minimun genomik DNA konsantrasyonu 1 pg olarak hem kolorimetrik hem de floresan boya ile tespit edilmiştir. Elde edilen sonuçların daha hızlı ve hassas bir şekilde yorumlanabilmesi ve hasta başında uygulanabilir olması açısından kolorimetrik yöntem daha avantajlı olmaktadır. Ayrıca önceki çalışmalarda LAMP yönteminin PZR yönteminden 10 kat daha fazla duyarlı olduğu bildirilmiştir (41). LAMP yönteminin *Acanthamoeba*'yı tespit etmede örneklere ısıl işlem uygulanarak elde edilen DNA ile pozitif tespit edilirken PZR yönteminde sadece kit ile ekstrakte edilmiş DNA kullanıldığında etkili olduğu rapor edilmiştir (39). LAMP ve PZR sonuçları arasındaki bu fark LAMP yönteminde toplam altı primer kullanılarak çalışmasından dolayı daha yüksek hassasiyete sahiptir (42). Bundan dolayı DNA izolasyon basamağına ihtiyaç duymadan özgün primerler kullanılarak tasarlanan LAMP yöntemi ile *Acanthamoeba*'nın tanısı için gereken süre kısaltılabilir.

Acanthamoeba'nın *18S rRNA* genine özgü tasarlanan primerler daha önceki çalışmalarda AK ile benzer klinik gösteren göz enfeksiyonlarına neden olabilecek mantar (*Aspergillus fumigatus, Fusarium solani,* ve *Candida albicans*), bakteri (*Pseudomonas aeruginosa* ve *Escherichia coli*) ve viral (Herpes simpleks virüs tip 1) patojenler ile herhangi bir çapraz reaksiyon gözlenmediği rapor edilmektedir (39,40). Çalışmamızda LAMP testi için tasarlanan *18S rRNA* genine özgü primerlerin çeşitli protozoanlar (*Giardia intestinalis, Leishmania infantum, Blastocystis* sp., *Toxoplasma gondii* ve *Naegleria fowleri*) ile herhangi bir çapraz reaksiyon gözlenmemiştir.

AK tanısında kullanılan kültür ve mikroskobik yöntemlerin her bir testi için yaklaşık 2,5 $ olması açısından en ucuz yöntem olarak önemli bir avantaja sahipken, testlerin uzun zaman alması ve uzman personel gerektirmesi dezavantajlarıdır. Moleküler yöntemlerin gelişmesiyle daha hızlı ve hassas olan PZR yöntemleri her bir test için yaklaşık maliyeti 16,4-30 $ olabilmekle birlikte gerekli olan zaman ise 120-150 dk arasında değişmekte ve pahalı laboratuvar cihazlarına gereksinim duyulmaktadır (43,44). Son yıllarda geliştirilen LAMP yöntemi ise daha duyarlı, hızlı ve ucuz olması açısından önemli bir moleküler testtir. Ayrıca tek bir su banyosu ile uygulanabilen ucuz bir yöntem olması, her bir test için yaklaşık 0,71-2 $ maliyetinin olması, sonuçların 60-90 dk arasında değerlendirmesi gibi avantajlara sahiptir (21). Çalışmamızda uyguladığımız LAMP testi ile 60 dk içinde sonuca varılmıştır. Bu şekilde hastalığın tanısında sonuçlara hızlı bir şekilde ulaşılması sağlanarak, erken tedaviye başlanmasına olanak sağlayacaktır.

SONUÇ

Çalışmamızda, tasarladığımız primerler kullanılarak yapılan LAMP testi *Acanthamoeba*'nın DNA'sının varlığını saptamada kullanılabilecek basit, ucuz ve yüksek hassasiyete sahip umut verici bir tekniktir. LAMP testi, tek bir ekipman ile minimum donanımlı laboratuvarlarda, saha ortamlarında ve hasta başında kullanım için basit, hızlı, uygun maliyetli bir yöntemdir. Bu nedenle LAMP testi, AK tanısının hızlı bir şekilde teşhisine yardımcı olmak için laboratuvar test yöntemi olarak klinik uygulama için dikkate değerdir. Ayrıca, çevresel örneklerde *Acanthamoeba* tarama çalışmalarında daha hızlı bir şekilde uygulanabilir bir testtir.

*** Etik**

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Hasta Onayı: Bu çalışma için geçerli değildir.

Hakem Değerlendirmesi: Editörler kurulu ve editörler kurulu dışında olan kişiler tarafından değerlendirilmiştir.

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Study on the Therapeutic Effect of Coconut Oil Extracts as An Alternative Medicinal Plant in *Cryptosporidium* Infected Mice

Cryptosporidium ile Enfekte Farelerde Alternatif Bir Tıbbi Bitki Olarak Hindistan Cevizi Yağı Ekstraktlarının Terapötik Etkisi Üzerine Çalışma

Heba M El Naggar[1], Basant O Mohammed[1], Tarek Aboushousha[3], Hagar F Abdelmaksoud[2]

[1]Department of Medical Parasitology, Faculty of Medicine, Ain Shams University, Cairo, Egypt
[2]Department of Parasitology, Theodor Bilharz Research Institute, Giza, Egypt
[3]Department of Pathology, Theodor Bilharz Research Institute, Giza, Egypt

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ABSTRACT

Objective: Cryptosporidiosis caused by *Cryptosporidium* sp. is a globally spreading disease. Nowadays, new researches are moving towards an effective treatment without side effects, especially for young and immune-compromised patients. The current study was designed to evaluate the therapeutic effect of the coconut oil extracts as an alternative medicinal plant in *Cryptosporidium* infected immunocompromised mice.

Methods: Sixty white albino mice were classified into six groups; Group I: Infected with *Cryptosporidium* oocysts treated with Nitazoxanide, Group II: Infected with *Cryptosporidium* oocysts and treated with coconut water extract, Group III: Infected with *Cryptosporidium* oocysts and treated with coconut Hexan extract, Group IV: Infected with *Cryptosporidium* oocysts and treated with coconut ethanol extract, Group V: Positive control, Group VI: Negative control. Stool samples were collected and examined; histopathological and immune-histochemical assessment using anti caspase-3 and anti CDX2 monoclonal antibodies were performed.

Results: Coconut oil extracts results revealed a significant decrease of oocyst count, correlated with an amelioration of histopathological and confirmed by immunohistochemical changes in ileal tissue.

Conclusion: The present study has opened fresh avenues for development of natural therapy like coconut oil extracts, which have a potential therapeutic efficacy against Cryptosporidiosis. That was confirmed by different methodologies, parasitological examination, histopathological examination, and immunohistochemical assays. It paves the way for being a promising anti-parasitic agent for infection eradication. However, further studies are still required to gain more knowledge about different coconut extracts in order to reach the best treatment efficacy.

Keywords: *Cryptosporidium* sp., coconut oil, caspase-3, CDX2

ÖZ

Amaç: *Cryptosporidium parvum* (*C. parvum*), Cryptosporidiosis hastalığına neden olan ve tüm dünyada yaygın olarak bulunan protozoan bir parazittir. Günümüzde yeni araştırmalar özellikle genç ve bağışıklık sistemi baskılanmış hastalarda yan etkisi olmayan etkili tedaviye doğru ilerlemektedir. Bu nedenle, mevcut çalışma, kriptosporidium ile enfekte olmuş bağışıklığı baskılanmış farelerde alternatif bir tıbbi bitki olarak hindistan cevizi yağı ekstraktlarının terapötik etkisini değerlendirmek için tasarlanmıştır.

Yöntemler: Altmış beyaz albino fare, altı gruba ayrıldı; Grup I: Nitazoksanid ile tedavi edilen *C. parvum* ookistleri ile enfekte. Grup II: *C. parvum* ookistleri ile enfekte ve hindistan cevizi suyu özü ile tedavi edildi. Grup III: *C. parvum* ookistleri ile enfekte edilmiş ve hindistan cevizi heksan özü ile tedavi edilmiştir. Grup IV: *C. parvum* ookistleri ile enfekte edilmiş ve hindistan cevizi etanol özü ile tedavi edilmiştir. Grup V: Pozitif kontrol. Grup VI: Negatif kontrol. Dışkı örnekleri toplandı ve incelendi, anti-kaspaz-3 ve anti-CDX2 monoklonal antikorları kullanılarak histopatolojik ve immünohistokimyasal değerlendirme yapıldı.

Bulgular: Hindistan cevizi yağı ekstraktlarının sonuçları, histopatolojik iyileşme ile ilişkili ve ileal dokudaki immünohistokimyasal değişikliklerle doğrulanan ookist sayısında önemli bir azalma olduğunu ortaya koydu.

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Address for Correspondence/Yazar Adresi: Basant O Mohammed, Department of Medical Parasitology, Faculty of Medicine, Ain Shams University, Cairo, Egypt
Phone/Tel: +01006339417 **E-mail/E-Posta:** dr-bosy@hotmail.com **ORCID ID:** orcid.org/0000-0002-5122-8772

Türkiye PARAZİTOLOJİ Dergisi

Sonuç: Bu çalışma, farklı metodolojiler parazitolojik inceleme, histopatolojik inceleme ve immünohistokimyasal testler ile onaylanan Cryptosporidiosis'e karşı potansiyel bir terapötik etkinliğe sahip hindistan cevizi yağı özleri gibi doğal terapinin geliştirilmesi için yeni yollar açmıştır ve bu da umut verici bir anti-parazitik ajan olmanın yolunu açmaktadır (enfeksiyon eradikasyonu). Bununla birlikte, en iyi tedavi etkinliğine ulaşmak için farklı hindistan cevizi özleri hakkında daha fazla bilgi vermek için daha fazla çalışmaya ihtiyaç vardır.
Anahtar Kelimeler: *Cryptosporidium* sp., hindistan cevizi yağı, kaspaz-3, CDX2

INTRODUCTION

Cryptosporidium sp. are a group of zoonotic protozoan parasites that infect a wide variety of vertebrate hosts. Mostly it causes asymptomatic infection or diarrhea in patients with strong immune system, but it can cause severe and chronic diarrhea, pancreatic, biliary and respiratory tract infections and even death in immunocompromized (1).

Nitazoxanide is shown to treat diarrhea, which is caused by *Cryptosporidium*, but it has a limited efficacy in immunocompromised or malnourished patients and not licensed for infants younger than 1 year of age (2). Hence, development of new effective drugs for cryptosporidiosis presents a pressing need.

Coconut oil is a natural oil that consists of 92% saturated acids, with a main and most important structure, with many beneficial antimicrobial, antioxidant, and anti-inflammatory properties known as Lauric acid (3,4). Many researches regarding its antimicrobial activity first shown by John Kabara in the 1970s. He found that it had broad-spectrum antimicrobial activities including antibacterial, antiviral and antifungal (5).

In the field of parasitology, Lauric acid proved to be safe, easy to use with excellent tolerability against giardiasis when combined with metronidazole. It has also proven to be a prophylactic measure, especially in cases of traveler's diarrhea. Besides, Lauric acid may have an immune-stimulant effect and so offers a good solution for immune-compromised patients (6).

Long time ago, it was found that *Cryptosporidium* was causing death of epithelial cells in the intestine in the form of villous structure changes with apoptotic epithelial cells found in the intestine. It was stated an important relationship between epithelial apoptosis and cryptosporidiosis pathogenesis (7).

During apoptosis, Caspase-3 is one of the cysteine proteases that are responsible for the morphological changes within the cells. It is considered as a meeting point of the two main apoptotic pathways and cleaves most of the cellular substrates in the apoptotic process (8). Therefore, measurement of Caspase-3 activity is considered as an important reliable determinant of apoptosis (9). Many researches stated that it is an independent prognostic factor for tumors of the digestive system. It is considered an perfect indicator for measuring apoptosis in cancers especially colorectal carcinomas (10).

CDX2 is a homeobox domain-containing transcription factor. It has a valuable role in regulation of the proliferation and differentiation of intestinal cells. CDX2 expression is indicative of intestinal differentiation, as it is expressed inside the nuclei of intestinal epithelial cells starting from the duodenum reaching the rectum. So, It has an important benefit in determining the origin of metastatic tumors (11,12).

By immunohistochemistry, CDX2 is expressed uniformly in most of the colorectal and duodenal adenocarcinoma. unlike carcinomas of the genitourinary, breast, lung, and head and neck (13,14).

The aim of this study was exploring the effect of coconut oil extracts against cryptosporidiosis in infected mice and evaluating it's protective and curative properties using parasitological, histopathological, and IHC anti caspase-3 and anti CDX2 monoclonal antibodies.

METHODS

Oil Preparation

Mature coconuts (Cocos nucifera L.) were purchased from a market at Giza governorate in Egypt. Coconuts were manually dehusked and cracked to collect coconut water, which was carefully filtered using a filter membrane and concentrated using a rotary evaporator (Rotatory evaporator, Buchi, Switzerland) under pressure to obtain a concentrated water extract. Whereas, the coconut pulp was crushed using electric mill to small pieces. 200 g of crushed coconut pulp were soaked in 800 mL of n-hexane and left for one 2 days at room temperature with shaking from time to time followed by filtration using filter paper (Whatmann No.1). Then the filtrate was concentrated under reduced pressure using rotary evaporator at 40 ºC, this process was repeated 3 times to give 10 g dry n-hexane extract. The residue after extraction with n-hexane was soaked in 800 mL 70% EtOH for 2 days at room temperature, then filtered using filter paper. The filtrate was concentrated under reduced pressure using rotary evaporator at 40 ºC (repeated 3 times) to yield 15 g dry 70% EtOH extract. After complete dryness, all extracts were kept in brown dry plastic vials for biological experiments.

Oocyst Preparation

Fecal samples of mice that have *Cryptosporidium* positive infection was examined and oocysts were confirmed by staining with MZN Stain. Samples were irrigated by 10 mL saline and then strained; followed by centrifugation of the suspension was centrifuged at 3000 g/10 minutes. This washing step was recurred two times with removing the supernatant. The sediment re-suspended with Sheather's solution and let aside for 10 minutes. Again the supernatant was raised and washed with saline twice. Finally, the oocysts were counted in the deposit using hemocytometer to calculate the required concentration for infection. The infection dose was 10^3 *Cryptosporidium* oocysts, dissolved in 200 µL of PBS.

Animal Groups

Sixty healthy laboratory-bred male, white Albino mice of CDI strain, about 6-8 weeks old, 25-30 gram, white Albino mice of CDI strain (obtained from Theodor Bilharz Animal house). Mice were

maintained in standard environmental conditions at temperature (24 °C), relative humidity (50%) with a 12:12 light: Dark cycle, and fed a standard commercial diet and water, away from direct sunlight ensuring good sanitary condition.

Immunosuppression: Using nasogastric feeding tube, mice were given administered with 0.25 ug/g/day of dexamethasone sodium phosphate dexazone) daily for two weeks. This step was done before oral inoculation with *Cryptosporidium* oocysts.

Mice were divided into six groups containing ten mice each:

Group I (GI): Mice infected with *Cryptosporidium* oocysts with a dose of 103 oocysts/0.2 mL/mouse orally and treated with Nitazoxanide 65 mg daily orally by using nasogastric feeding tube every day for 7 days post infection. The doses were calculated by extrapolation of human therapeutic doses to animal doses.

Group II (GII): Infected with *Cryptosporidium* oocysts with a dose of 10^3 oocysts/0.2 mL/mouse orally and treated by water extract of coconut a dose of 0.2 Mg/g.

Group III (GIII): Infected with *Cryptosporidium* oocysts with a dose of 10^3 oocysts/0.2 mL/mouse orally treated by hexan extract of coconut oil with a dose of 0.2 Mg/g.

Group IV (GIV): Infected with *Cryptosporidium* oocysts with a dose of 10^3 oocysts/0.2 mL/mouse orally and treated by ethanol extract of coconut oil with a dose of 0.2 Mg/g.

Group V (GV): Infected with *Cryptosporidium* oocysts with a dose of 10^3 oocysts/0.2 mL/mouse orally (positive control).

Group VI (GVI): Non-infected (negative control).

Evaluation of the Experimental Drug Treatment

1- Parasitological examination: At the 7th day after starting treatment administration, samples were collected from each group and subjected to parasitological examination after staining by modified Ziehl-Neelsen stain. Compound microscope was used to confirm mice infection and to calculate the numbers of *Cryptosporidium* oocysts in each sample.

Oocyst count in stool was completed in all groups except GVI, followed by sacrifice of the tested mice by cervical dislocation and for GVI it was done at the same time as previous groups.

2- Histopathological examination: Seven days post infection, sacrificing of all mice was done. The ileocecal region was obtained and fixed in 10% buffered formalin solution then embedded in paraffin wax blocks. Sections of 4 µm thickness were rehydrated and stained with (H&E). All slides were examined by a pathologist which was blinded to the experimental design to assess the pathological changes.

3- Immunohistochemical study: De-paraffinization and rehydration of ileocecal paraffin sections were done. Obtaining of antigen was made by microwaving the sections in citrate buffer, pH 6.0. 3% hydrogen peroxide methanol were added to block endogenous peroxidase and incubated overnight at 4 °C in humid chamber with the primary antibodies: Anti-caspase-3 antibody (31A1067): (sc-56053), or for two hours with anti CDX2 (catalog: M3636; monoclonal; host: Mouse) antibody in a dilution of 1:100-1:400, followed by adding secondary antibody (Biotin-streptavidin link) (DAKO). After that, 3,3'diaminobenzidine tetrahydrochloride (DAB) substrate chromogen solution (Universal Detection Kit, Dako Envision, Denmark) was added to localize antigen. Followed by counterstaining with hematoxylin, dehydration in alcohol and mounting was done.

Regarding the negative control group, all steps were done in the above-mentioned sequences but non-immune immunoglobulin G were added (IgG; DAKO, Glostrup, Copenhagen, Denmark).

Instead of the primary antibodies.

4- Interpretation of immunostaining and scoring analysis: Immunohistochemical analysis were blind-quantified by two pathologists. The sections were examined by using light microscope (Scope A1, Axio, Zeiss, Germany). Photomicrographs were taken using a microscope-camera (AxioCam, MRc5, Zeiss, Germany).

Positive CDX2 was mentioned when nuclei of epithelial cells took brown color, while positivity for Caspase-3 was mentioned when the cytoplasm of epithelial cells took brown color. Calculation of the percentage of positive cells in 10 HPF, follwed by grading the intensity of the color from 1 to 3.

Ethical consideration: According to the NIH guidelines for animal experimentation, animals were reared and sacrificed. According to the valid International Guidelines for animal experimentation, animal protocols were conducted and approved by the Ethical Committee at Theodor Bilharz Research Institute (TBRI).

Statistical Analysis

Using Statistical Package for Social Sciences, Windows version 22., Student's (t)-test and analysis of variance test were used to evaluate the possible dscrepancy among the study groups. P-value <0.05 was considered significant.

RESULTS

1. Parasitological Examination of Stool of Different Study Groups

GI, GII, GIII and GIV showed highly significant statistical difference between mean oocysts count when compared with GV (p-value <0.001). GII showed the best response with GII showed the best response with severe decrease of the mean count of oocysts (Figure 1, Table 1).

2. Histopathological Examination of Sections from Ileocaecal Regions of the Studied Groups

The present study revealed mild amelioration of the histopathological changes following infection including partial villous atrophy with moderate diminution in ratio of villous height to crypt length. A little bit inflammatory infiltration was found in the lamina propria (Figure 2, 3).

GII revealed significant improvement of the histopathological changes with no oocysts found (Figure 4, 5). GIII showing many adherent and separate cryptospores, villous broadening, moderate mucosal cellular infiltration and focal ulceration (Figure 6, 7). GIV showed nearly the same histopathological features compared to GIII but with less ulcerations (Figure 8, 9). So, we found that coconut extract in water (GII) gave the best response compared to the positive control GV (Figure 10, 11) and the negative control GVI (Figure 12, 13).

C- Immunohistochemical results (IHC)

In positive control group, Caspase-3, which is a marker for apoptosis, was detected by finding discrete cytoplasmic positive cells in the intestinal crypt for Caspase. This increased apoptotic activity is due to the oncogenic effect of cryptosporidiosis with nearly negative staining by CDX2 (Figure 14). In negative control group Caspase-3 is nearly negative with remarkable nuclear positivity for CDX2 (Figure 15).

Sections in intestine of group infected then treated with coconut oil extract showing focal negative staining by CDX2 (Figure 16). Thiös could be due to the oncogenic effect of cryptosporidiosis, that could not be -at least- partially antagonized by the coconut extract. Also show few positive cells stained by Caspase-3. Being compared to the positive control group, there is decrease in the apoptotic index after treatment with coconut extract (Figure 17).

Table 1. Analysis of different groups using post-hoc test

		Least significance difference	p-value
PC	**Coconut ethanol**	**126.6**	**<0.001 HS**
	Coconut H_2O	**153**	**<0.001 HS**
	Coconut hexane	**111.4**	**<0.001 HS**
	Nitazoxanide	**105.8**	**<0.001 HS**
Coconut ethanol	**PC**	**-126.6**	**<0.001 HS**
	Coconut H_2O	26.4	0.061 NS
	Coconut hexane	-15.2	0.266 NS
	Nitazoxanide	-20.8	0.133 NS
Coconut H_2O	**PC**	**-153**	**<0.001 HS**
	Coconut ethanol	-26.4	0.061 NS
	Coconut hexane	**-41.6**	**0.005 S**
	Nitazoxanide	**-47.2**	**0.002 S**
Coconut hexane	**PC**	**-111.4**	**<0.001 HS**
	Coconut ethanol	15.2	0.266 NS
	Coconut H_2O	**41.6**	**0.005 S**
	Nitazoxanide	-5.6	0.678 NS
Nitazoxanide	**PC**	**-105.8**	**<0.001 HS**
	Coconut ethanol	20.8	0.133 NS
	Coconut H_2O	**47.2**	**0.002 S**
	Coconut hexane	5.6	0.678 NS

S: p-value <0.05 is considered significant.
NS: p-value >0.05 is considered non-significant.
HS: p-value <0.001 is considered highly significant

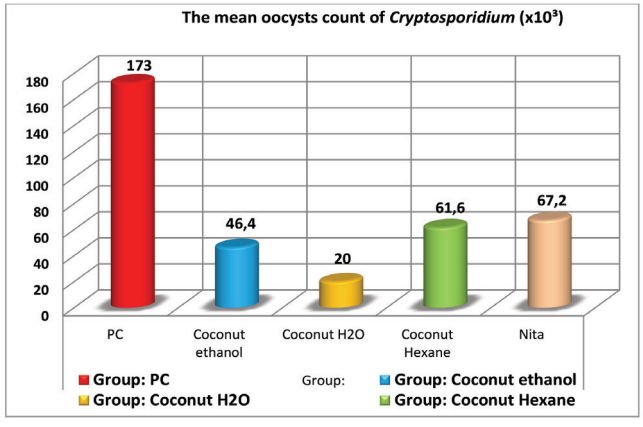


Figure 1. Chart showing comparison between groups as regard the mean oocysts count of *Cryptosporidium*

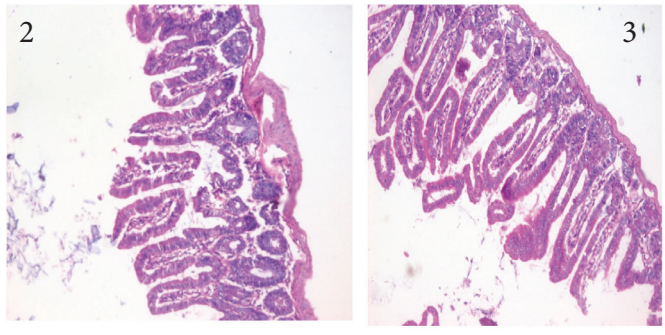
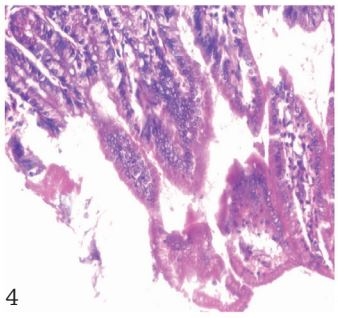


Figure 2, 3. Section of small intestine in GI showing mild blunting of the villi with moderate lowering in villous height to crypt length. slight inflammatory cells appear in lamina propria (H&E stain x200)



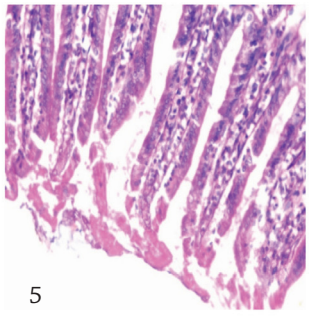
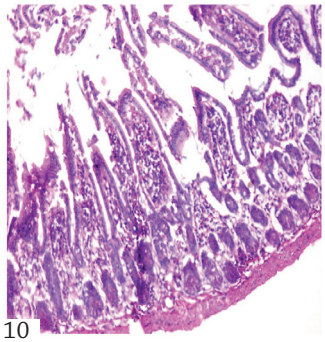


Figure 4, 5. GII group revealed milder inflammation and mild villous broadening compared to GI, minimal surface erosions with normal brush border and goblet cells and slight inflammatory cells in lamina propria without *Cryptosporidium* oocysts (H&E stain x200)



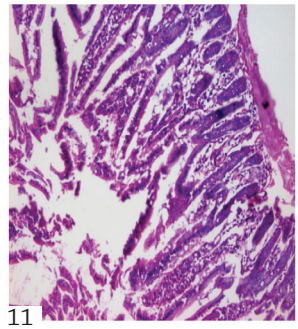
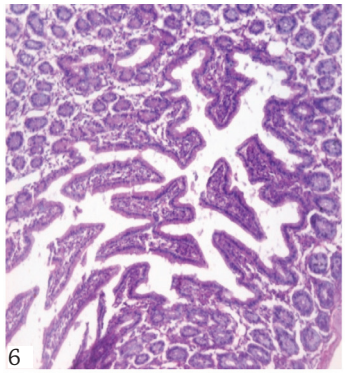


Figure 10, 11. Section of small intestine in GV (positive control) showing many cryptospores, distortion of villi, ulceration and dense cellular infiltration (H&E stain x200)



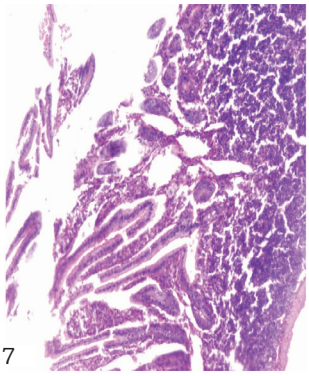
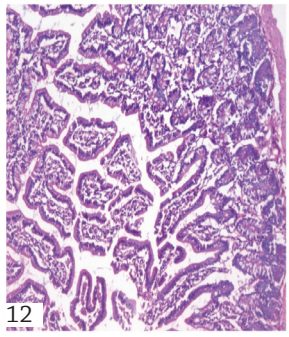


Figure 6, 7. GIII group revealed many adherent and separate cryptospores, villous broadening, moderate mucosal mononuclear inflammatory cells, focal ulceration (H&E stain x200)



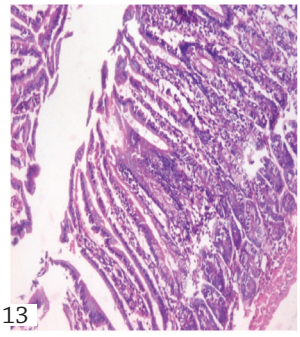
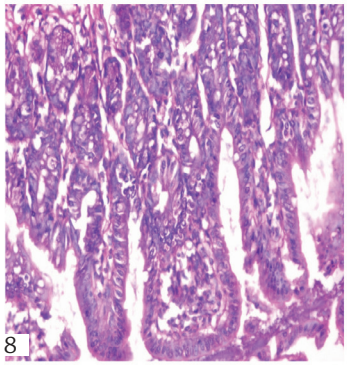


Figure 12, 13. Section of small intestine in GVI (negative control) showing normal architecture of villi with average length and width with intact brush border (H &E stain x200)



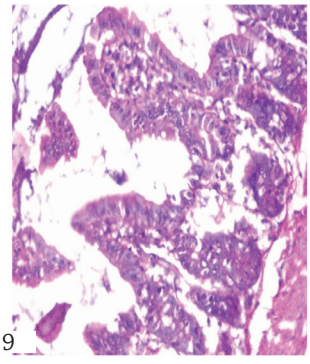


Figure 8, 9. Section of small intestine in GIV showing nearly same features compared to group GIII but with less ulcerations (H&E stain x200)

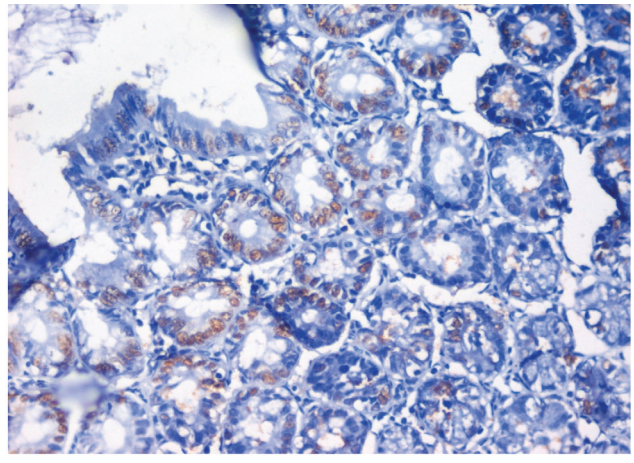


Figure 14. Negative control group revealed obvious positivity for CDX2 in the nuclei (Brownish color) (X400)

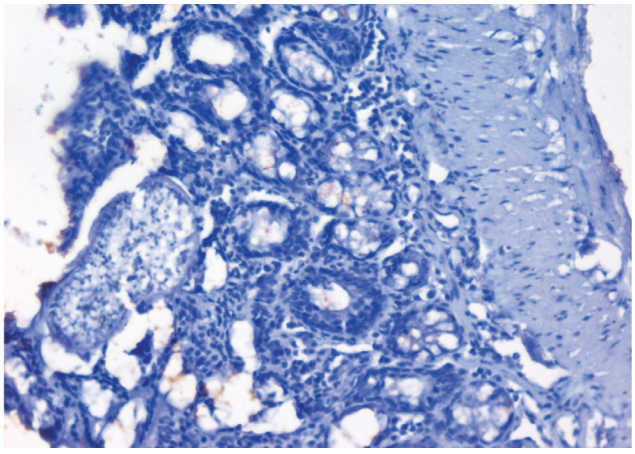


Figure 15. Sections in intestine of group (*C. parvum* infected for 60 days then treated with coconut) showing focal negative staining by CDX2 (X400)

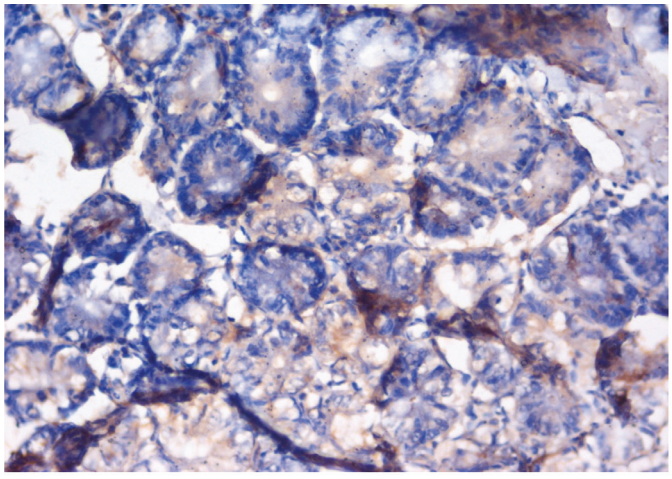


Figure 16. Sections in intestine of (positive control) revealed obvious brownish discoloration in the cytoplasm in the crypts of the intestine indicating Caspase 3 (x400)

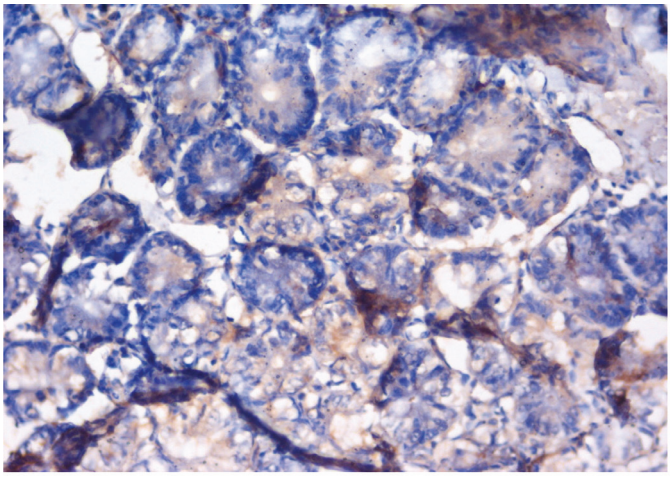


Figure 17. Sections in intestine of Group (Cryptosporidium infected for 2 months and cured by coconut) showing few positive cells stained by Caspase 3 (X400)

DISCUSSION

"Back to nature" is one of the newly notions used in the field of general health. Since the early days of human civilization, using medications from plant origin has been started. In the last three decades, the search for using herbal medicines increased especially with the rise in the resistance of human pathogens to usual treatment (15).

Coconut oil is a natural oil chemically composed of triglyceride compounds that contain large amounts of saturated medium chain fatty acids. Among which is Lauric acid (C12:0) which is the major fatty acid in coconut oil accounting for around 50% of the total fatty acids (3). It has beneficial effects, such as antimicrobial, anti-inflammatory, immunostimulant, and antioxidant actions (16).

In this study, highly statistically significant discrepancy in the *Cryptosporidium* oocysts count was found among nitazoxanide, positive control, coconut ethanol, coconut hexane and coconut H_2O groups with the lowest count was shown in coconut H_2O (20 oocysts/0.001 g feces). These results are congruent with those reported by several authors working on other protozoa. Hassan et al. (17) reported highly significant reduction of concentration of *blastocystis* cells after administration of monolaurin ($p<0.01$). These results were convenient with Rayan et al. (18) who confirmed that Lauric acid has an anti-*Giardia* effect comparing to metronidazole.

These results are compatible with Aly et al. (6) who found found improvement of the therapeutic effect against giardiasis when combined Lauric acid with metronidazole. This agrees with Helmy, who declared decrease in *Giardia* cysts and trophozoites outcomes using both Lauric acid with metronidazole (98.83 to 96.95%) (19). Moreover, Fahmy et al. (20) found same results in *E. histolytica* by high reduction in trophozoite and cyst (90.12%, 92.56%, respectively).

In this study, the experimentally-treated mice groups with coconut water extract kept their normal appearance with varying degrees of histopathological corrections in comparison with the positive control with promising results in treatment of cryptosporidiosis (Figure 4, 5). This coincides with Helmy, who found, by histopathological examination and electron microscopic examination, a complete healing of intestinal mucosa after the combined treatment of metronidazole and Lauric acid for treatment of giardiasis (19).

In birds, Hafeez et al. (21) recorded that inclusion of 2% coconut oil as a supplement improved growth performance and villus length was significantly improved ($p<0.01$) in broiler chicks exposed to experimentally induced coccidiosis caused by *Eimeria*.

Owing to the importance of coconut oil, not only as anti-parasitic, it was included in studies of the pandemic COVID-19 as antiviral and anti-inflammatory. Angeles-Agdeppa et al. (22) evaluated it's effect of on cases of COVID-19 in Philippines and found more rapid relief from symptoms with significant decline in the CRP levels.

It is noteworthy that coconut oil can modulate the cellular immunity and can be a potential alternative to antibiotics, as it has a broad-spectrum activity as an antibacterial and antiviral (23,24).

Aforethought, many studies investigated the appearance of Caspase-3 in ileal epithelial cells of infected mice and it's important role of apoptosis of epithelial and stromal cells during the course

of Cryptosporidiosis. Activation of Caspase-3 is considered to compel the cell toward irreversible apoptotic death (25).

In the present study, IHC results of Caspase-3 showed, before treatment with coconut oil, sections of intestine of positive control group showed scattered cytoplasmic positive cells in the intestinal crypt for Caspase-3. This is due to the increased apoptotic activity due to oncogenic effect of cryptosporidiosis. This is reconcilable with previous studies, which found that Caspase-dependent apoptosis was increased by *C. parvum in vitro* and *in vivo* infecion (26,27).

Following treatment with coconut oil in the present study, few positive cells stained by Caspase-3 were found. This indicate that there is decrease in the apoptotic index after treatment with coconut oil with promising results in treatment of cryptosporidiosis.

In contrast, Samaka et al. (28) stated that there was no significant association between dual treatment of infected mice with phenyl vinyl sulfone and nitazoxanide and Caspase-3 expression. This disparity may be because of the rabbit polyclonal anti Caspase-3 used in contrast to the monoclonal antibody used in our study, which is more specific, precise and showed the characteristic apoptotic nuclear changes (28).

It is worth noting that Caspase-3 was evaluated in tumors as a marker of tumorigenesis by Noble et al. (29), who found diffuse Caspase-3 expression in cases of colorectal carcinoma and declared the higher the caspase-3 levels, the higher the apoptotic rate.

CDX2 is essential for intestinal development and differentiation as it is a marker normally expressed in colonic mucosa and decreased in cases of tumorigenesis (30). In the present study, CDX2 of negative control group showed remarkable nuclear positivity for CDX2 and following infection with cryptosporidium then treatment with coconut, the result was focal negative staining by CDX2, this could be due to the oncogenic effect of cryptosporidiosis, that could not be -at least- partially antagonized by the coconut extract.

Previously, CDX2 was evaluated in all stages of colorectal cancer. It's absence was found in advanced tumor grade and is related to poor outcome and metastasis (31-33).

CONCLUSION

It could be concluded that our study has opened fresh avenues and paves the way for development of natural therapy like coconut oil extracts which have potential therapeutic efficacy against Cryptosporidiosis confirmed by parasitological, histopathological, and immunohistochemical assays that exemplify for being a promising anti-parasitic agent against *Cryptosporidium*. However, further studies are still required to give more knowledge about different coconut extracts in order to reach the best treatment efficacy.

* Ethics

Ethics Committee Approval: According to the valid International Guidelines for animal experimentation, animal protocols were conducted and approved by the Ethical Committee at Theodor Bilharz Research Institute (TBRI).

Informed Consent: N/A.

Peer-review: Internally peer-reviewed.

* Authorship Contributions

Surgical and Medical Practices: B.O.M., H.M.E.N., T.A., H.F.A., Concept: B.O.M., H.M.E.N., T.A., H.F.A., Design: B.O.M., H.M.E.N., T.A., H.F.A., Data Collection or Processing: B.O.M., H.M.E.N., T.A., H.F.A., Analysis or Interpretation: B.O.M., H.M.E.N., T.A., H.F.A., Writing: B.O.M., H.M.E.N., T.A., H.F.A.

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Global Bibliometric Analysis of Leishmaniasis Literature for the Last 20 Years and Investigating the Contribution of Türkiye

Leishmaniasis Literatürünün Son 20 Yıllık Küresel Bibliyometrik Analizi ve Türkiye'nin Katkısının Araştırılması

Sevil Alkan[1], Oğuz Evlice[2], Mustafa Serhat Şahinoğlu[3]

[1]Çanakkale Onsekiz Mart University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Çanakkale, Türkiye
[2]Republic of Türkiye Ministry of Health, General Directorate of Public Health, Ankara, Türkiye
[3]Manisa City Hospital, Clinic of Infectious Diseases and Clinical Microbiology, Manisa, Türkiye

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ABSTRACT

Objective: Leishmaniasis is a global health problem seen in more than 98 countries. The aim of this study is to conduct a bibliometric analysis of worldwide scientific outputs related to leishmaniasis and to provide a perspective for researchers on this topic. It also aimed to investigate the contribution of Türkiye to the leishmaniasis literature.
Methods: This study was conducted using scientometric methodologies on leishmaniasis in the Web of Science database between 2003 and 2022. The visualizations were made with Vosviewer program. The most published institutions and organizations, countries, authors, trends in the number of publications and citations by year, H-indexes of the mostly publishing countries, the most popular keywords, scientific collaborations between countries, and many other bibliometric parameters were analyzed.
Results: In the last 20 years, research on *Leishmania* has been conducted in 143 different countries/regions. Brazil is the leading country with 4.463 articles (29.071%). The United States of America, India, Iran, and Spain published more than 1.000 articles, followed by European countries (Spain, United Kingdom, France, Germany, and Italy).
Conclusion: The number of publications, especially in endemic areas, was found to be limited other than Brazil. Studies in this area should be supported to ensure the eradication of the disease.
Keywords: Leishmaniasis, bibliometric analysis, network analysis, Web of Science

ÖZ

Amaç: Leishmaniasis 98'den fazla ülkede görülen küresel bir sağlık sorunudur. Bu çalışmanın amacı, leishmaniasis ile ilgili dünya çapındaki bilimsel çıktıların bibliyometrik bir analizini yapmak ve bu konuyla ilgili araştırmacılara bakış açısı sunmaktır. Ayrıca Türkiye'nin leishmaniasis literatürüne katkısının araştırılması amaçlanmıştır.
Yöntemler: Bu çalışma, 2003-2022 yılları arasında Web of Science veri tabanında leishmaniasis üzerine bibliyometrik metodolojiler kullanılarak gerçekleştirildi. Görselleştirmeler Vosviewer programı ile yapıldı. En çok yayın yapan kurum ve kuruluşlar, ülkeler, yazarlar, yıllara göre yayın ve atıf sayılarındaki eğilimler, en popüler anahtar kelimeler, ülkeler ve kurumlar arasındaki bilimsel iş birlikleri ve diğer birçok bibliyometrik parametre analiz edildi.
Bulgular: Son 20 yılda 143 farklı ülkede/bölgede *Leishmania* konusunda araştırma yapıldığı saptanmıştır. Brezilya 4,463 makale ile (%29,071) lider ülke konumundadır. Amerika Birleşik Devletleri, Hindistan, İran ve İspanya 1,000'den fazla makale yayınlamış olup, bu ülkeleri Avrupa ülkeleri (İspanya, İngiltere, Fransa, Almanya ve İtalya) takip etmektedir
Sonuç: Özellikle endemik bölgelerde yapılan yayın sayısının Brezilya dışında sınırlı olduğu görülmüştür. Hastalığın ortadan kaldırılmasını sağlamak için bu alanda yapılacak çalışmalar desteklenmelidir.
Anahtar Kelimeler: Leishmaniasis, bibliyometrik analiz, ağ analizi, Web of Science

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Address for Correspondence/Yazar Adresi: Sevil Alkan, Çanakkale Onsekiz Mart University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Çanakkale, Türkiye
Phone/Tel: +90 506 687 37 68 **E-mail/E-Posta:** sevil3910@gmail.com **ORCID ID:** orcid.org/0000-0003-1944-2477

Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Leishmaniasis is the general term for a group of zoonotic protozoan parasitic diseases caused by species of the obligate intracellular protozoan *Leishmania* genus. This genus consists of more than 20 *Leishmania* subspecies. There are various types of leishmaniasis in humans. Leishmaniasis is generally classified into three groups: Visceral leishmaniasis (VL) (also known as kala-azar, which is the most severe form of the illness), cutaneous leishmaniasis (CL) (the most common form), and mucocutaneous leishmaniasis. VL may affect multiple internal organs (usually spleen, liver, and bone marrow) (1-5). Leishmaniasis is transmitted to mammalian reservoirs (typically domestic dogs, rodents, marsupials, sloths, or wild dogs) through the bite of infected female sandflies. The people living in the endemic areas and the endemic area visitors become incidentally infected (2,3).

Leishmaniasis is one of the neglected tropical diseases diseases mentioned in the World Health Organization (WHO) report. According to WHO statistics, an estimated 700,000 to 1 million new cases are reported annually and approximately more than 95% of new cases reported from Brazil, China, Nepal, Ethiopia, Kenya, India, Iraq, Somalia and Sudan. Leishmaniasis is prevalent in more than 98 countries across five continents globally, especially in the tropics, subtropics, and southern Europe (5). Almost 80% of CL cases have been reported from seven countries: Afghanistan, Algeria, Brazil, Colombia, Iraq, Pakistan, and Syria Arab Republic (6). Climate and certain other environmental factors have the potential to expand the geographic range of sand fly vectors as well as the areas where leishmaniasis is present across the world (4,5). Except for Australia and Antarctica, humans are infected with leishmaniasis on every continent (4). The disease affects poor people more for the reasons such as malnutrition, forced migration, suppressed immune system and lack of financial sources (5).

Since leishmaniasis is a global health problem, an overview of studies on this topic is necessary. The aim of this study was to review the published literature on leishmaniasis worldwide (most contributing countries, institutions, journals with the highest number of publications, etc.) and to examine the publications from Türkiye.

METHODS

This study was performed by using the scientometric methodologies like previous studies (7,8-14). The selection of early research data is critical in scientometrics since these data have a direct influence on the findings and conclusions. The study covered all publications registered with the subject of *Leishmania* in the Web of Science (WOS) database between 2003-2022.

We used the following search strategy:

The search terms selected as title in the search bar of the WOS database were "leishmaniasis" OR "*Leishmania*" OR "*Leishmania* spp." OR "kala-azar" OR "Cutaneous Leishmaniasis".

Document Types: Research article

Timespan: January 1, 2003- December 31, 2022.

Indexes: WOS Core Collection.

Editions: Science Citation Index Expanded (SCI-EXPANDED) and Emerging Sources Citation Index (ESCI)

On March 17, 2023, all electronic searches were completed.

Inclusion and Exclusion Criteria: In this study, research articles published in the WOS database between 2003 and 2022 were analyzed. Publications outside the relevant date range, publications other than articles (such as letters, reviews, etc.) were not included. The search was made in the English language.

Bibliometric Analysis

The publications obtained after the literature search according to the search strategy were downloaded to the computer in plain text format. They were reviewed again for duplicate publications. The obtained data were transferred to vosViewer program (VOSviewer 1.6.18) for analysis. Graphics and visualizations of the WOS bibliometric database were also used. The data obtained were evaluated both at the global level and in terms of Türkiye. The most published institutions and organizations, countries, authors, trends in the number of publications and citations by year, H-indexes of the mostly publishing countries, the most popular keywords, scientific collaborations between countries and many other bibliometric parameters were analyzed.

Statistical Analysis

Statistical analysis was utilized in MS Office Excel 2016 to show the most effective organizations, authors, countries and funding sponsors. The resulting data are expressed in percentages and frequencies. In addition, the annual evolution of published papers was depicted, as well as a trend analysis of these articles.

RESULTS

Based on the search method utilized in this study, the findings revealed that 15,352 entries in the field of *Leishmania* were indexed in the WOS database between 2003-2022. According to WOS sub-indexes, it was found that the majority of the publications (93.708%) were published SCI-Expanded journals while 5.003% of them in ESCI. In our study, 56.931% of the articles were published as open-access publications.

Throughout the past 20 years, *Leishmania* research has been conducted in 143 different countries/regions. Figure 1 illustrates the global geographic distribution heat map that was created when the data was exported from the Excel software. Brazil was

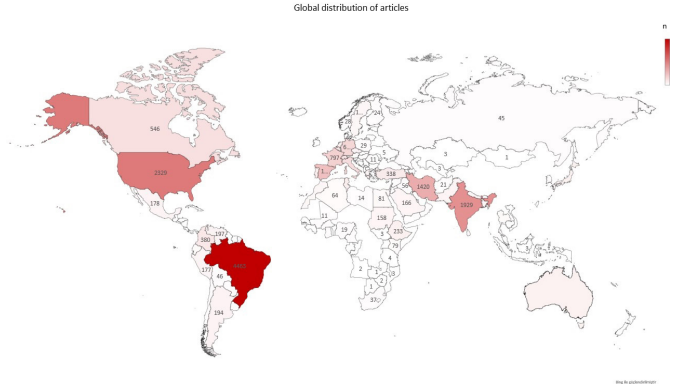

Figure 1. Global distribution of the articles

***Regional distribution of Leishmania research articles between 2003 and 2022. Map produced using Excel. Most publications are displayed in the reddest regions. Blank spaces indicate that no pertinent literature-related data was gathered*

the leading country with 4.463 articles (29.071%) on *Leishmania*. The United States, India, Iran, and Spain have published more than 1.000 articles in *Leishmania* research. And followed by European countries (Spain, England, France, Germany, and Italy). Türkiye ranked 14th in the number of publications about *Leishmania* (n=338, 2.202%).

Figure 2 displays the distribution of annual publications during the previous 20 years. In the analysis of the number of publications of Türkiye and the top 5 countries with the highest number of publications according to the time intervals (2003-2012 and 2013-2022), we found that the number of publications increased in all countries in the period 2013-2022, especially in Türkiye, Iran, and Brazil (Table 1).

In the last 20 years, Brazil published the most publications on *Leishmania*. In terms of the total number of citations, Brazilian publications received the highest number of citations. But the USA had both higher H-index and citations per publication (Table 2).

Figure 2. The distribution of annual publications during the previous 20 years

Worldwide, 7.781 organizations/institutions/universities have contributed to *Leishmania* research. The top 3 institutions/organizations/universities in *Leishmania* research between 2003 and 2022 were the Oswaldo Cruz Foundation (n=1.671), the University of São Paulo (n=844), and the Federal University of Minas Gerais (n=680) from Brazil. Top 10 of the most broadcasting organisations were from Iran, France and India (Table 3).

The publications on *Leishmania* were supported by 7.390 funding organisations and 36.862% of them did not have any funding sponsor. The organizations supporting the largest number of *Leishmania* publications were the National Council for Scientific and Technological Development from Brazil (n=1.950), the Coordination for the Improvement of Higher Education Personnel

Table 1. Comparison of the number of publications on *Leishmania* in Türkiye and the top 5 countries according to time periods (2003-2012 and 2013-2022)

Country	Time period		
	2003-2012	2013-2022	2003-2022
Brazil	1513	2950	4463
The USA	1058	1271	2329
India	759	1170	1929
Iran	385	1035	1420
Spain	402	676	1078
Türkiye	120	218	338

Table 2. Comparison of the number of publications, citations and H-indexes of the top leading 5 countries and Türkiye

Country	Number of publications	Number of total citations	Average number of citations	H-indexes
Brazil	4463*	78297*	17.54	85
The USA	2329	74320	31.91*	104*
India	1929	40477	20.98	77
Iran	1420	18290	12.88	51
Spain	1078	28639	26.57	66
Türkiye	338	4460	13.2	33

* Accessed from the WOS database

Table 3. The 10 most productive organizations/institutions/universities in *Leishmania* research between 2003-2022

Ranking	Organizations/institutions/universities and located country	n	%
1	Oswaldo Cruz Foundation, Brazil	1671	10.885
2	The University of São Paulo, Brazil	844	5.498
3	The Federal University of Minas Gerais, Brazil	680	4.429
4	The Pasteur Institute, France	620	4.039
5	Council of Scientific & Industrial Research, India	581	3.785
6	Tehran University of Medical Sciences, Iran	525	3.420
7	Udice French Research Universities, France	430	2.801
8	The Federal University of Rio de Janeiro or University of Brazil, Brazil	408	2.658
9	The Indian Council of Medical Research, India	344	2.241
10	Federal University of Bahia; University of Bahia, Brazil	336	2.189

from Brazil (n=1.177), and the United States Department of Health Human Services from the USA (n=1.153).

Most of the sponsors ranked in the top 10 were from Brazil. There were also organizations from Europe and India in the top 10 (Table 4).

The top 3 journals that published the most articles on *Leishmania* between 2003 and 2022 were PLOS Neglected Tropical Diseases (n=751), The American Journal of Tropical Medicine and Hygiene (n=492) and Acta Tropica (n=429). The list of the journals with the highest number of publications on this topic is summarised in Table 5.

Türkiye's Contribution to the Literature on *Leishmania* Between 2003-2022

A total of 338 articles on *Leishmania* from Türkiye were published between 2003 and 2022. The years with the highest number of articles published on *Leishmania* from Türkiye were 2020 (n=29), 2019 (n=26) and 2016 (n=25). Although the distrubition of publications according to years is irregular, 218 (64.496%) of them were published in 2013 and later. Since 2019, the number of publications has not fallen below 20 publications per year. Figure 3 shows the number of publications on *Leishmania* from Türkiye between 2003 and 2022.

Of these publications, 306 (90.533%) were published in SCIE-indexed journals and 32 (9.467%) in ESCI-indexed journals.

These publications were published by authors from 314 different institutions/affiliations/universities. Ege University (n=96, 28.402%), Manisa Celal Bayar University (n=44, 13.018%), and Çukurova University (n=41, 12.130%) were the institutions from Türkiye that published the most articles on *Leishmania* in the last 20 years. While the number of publications in Türkiye was limited to certain universities in previous years, other universities (Acıbadem University, University of Health Sciences Türkiye) have also contributed to the *Leishmania* literature in the last decade. Table 6 summarizes the institutions/organizations/universities from Türkiye that published most on *Leishmania* between 2003 and 2022.

TUBITAK (the Scientific and Technological Research Council of Türkiye) (n=37), the European Commission (n=12), Çukurova

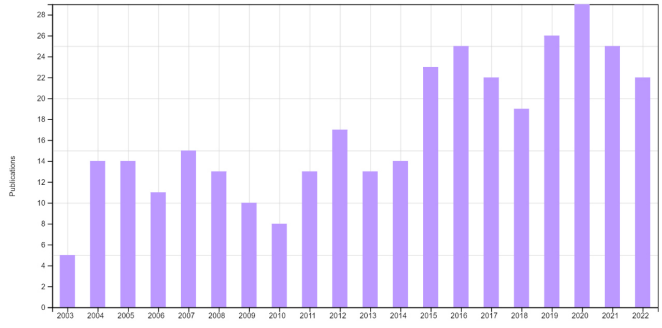

Figure 3. The number of publications from Türkiye on *Leishmania* between 2003-2022

Table 4. Leading funding sponsors/organisations of *Leishmania* publications

Funding agencies, country/region	n	%
National Council for Scientific and Technological Development, Brazil	1950	12.702
Coordination for the Improvement of Higher Education Personnel, Brazil	1177	7.667
United States Department of Health Human Services, the USA	1153	7.510
National Institutes Of Health, the USA	1127	7.341
The European Commission, Europe	684	4.455
São Paulo Research Foundation, Brazil	626	4.078
National Institute of Allergy Infectious Diseases, the USA	528	3.439
Minas Gerais State Agency for Research and Development – FAPEMIG, Brazil	520	3.387
Council of Scientific & Industrial Research, India	428	2.788
Rio de Janeiro State Research Foundation (FAPERJ), Brazil	412	2.684

Table 5. The list of journals with the highest number of articles on *Leishmania* between 2003 and 2022

Journals	n	%
PLOS Neglected Tropical Diseases	751	4.892
The American Journal of Tropical Medicine and Hygiene	492	3.205
Acta Tropica	429	2.794
PLOS ONE	411	2.677
Experimental Parasitology	382	2.488
Parasites & Vectors	347	2.260
Parasitology Research	293	1.909
Revista da Sociedade Brasileira de Medicina Tropical	262	1.707
Veterinary Parasitology	233	1.518
Memórias do Instituto Oswaldo Cruz	215	1.400
Transactions of the Royal Society of Tropical Medicine and Hygiene	202	1.316

University (n=9), Ege University (n=8), and Hacettepe University (n=5) were the leading organizations in Türkiye that supported the most publications on *Leishmania*. Only 37.574% were supported by the funding agency/organization.

The publications on *Leishmania* from Türkiye in the last 20 years were mostly published in the Bulletin of Microbiology (n=30), Acta Tropica (n=18), the American Journal of Tropical Medicine and Hygiene (n=10).

Figure 4 summarizes the most published journals on *Leishmania* from Türkiye in the last 20 years.

Yusuf Özbel (n=55) (Ege University Faculty of Medicine), Seray Toz (n=54) (Ege University Faculty of Medicine), Mehmet Karakuş (n=27) (İstanbul University Health Sciences Institution), Ahmet Özbilgin (n=26) (Manisa Celal Bayar University Faculty of Medicine) and Hatice Ertabaklar (n=24) (Adnan Menderes University Faculty of Medicine) were the authors who published most of the publications on *Leishmania* from Türkiye in the last 20 years.

Figure 4. Journals with the most publications on *Leishmania* from Türkiye in last 20 years

Table 6. Mostly publishing institutions/affiliations/universities on *Leishmania* from Türkiye between 2003-2022

Publishing institutions/affiliations/ universities	n	%
Ege University	96	28.402
Manisa Celal Bayar University	44	13.018
Çukurova University	41	12.130
Adnan Menderes University	39	11.538
Harran University	38	11.243
Hacettepe University	30	8.876
Mustafa Kemal University	26	7.692
Akdeniz University	18	5.325
Yıldız Technical University	17	5.030
University of Health Sciences	15	4.438
Fırat University	14	4.142
Dicle University	13	3.846
Mersin University	11	3.254
Acıbadem University	10	2.959
Başkent University	10	2.959
Gülhane Military Medical Academy	10	2.959
İstanbul University	10	2.959

A total of 23 countries were analyzed and a total of 6 clusters, 218 links and 32,450 total link strengths were found whenthe minimum number of publications of a country was set as at least three articles and bibliographic coupling analysis was performed with VosViewer. Türkiye (n=21,835), the USA (n=4.058), and France (n=6.165) have the highest total link strengths (Figure 5). According to our analysis of the overlay visualization of Türkiye's bibliographic coupling, Türkiye had the most publication collaboration with the USA (n=4.058) and France (n=6.165).

To learn more about the most used keywords, we obtained the visualization graph in Figure 6. We set the minimum number of occurrences of a keyword to at least three and performed keyword analysis with VosViewer. We reached 59 keywords. The size of each point on the map is determined by the number of items there. By default, this color is somewhere between red and blue. 2003 was expressed in shades of blue, 2010- 2015 in green-yellow, and 2015-2022 in yellow and red. The closer the color of a keyword was to a time period, the more it appeared in publications in that period.

DISCUSSION

Leishmaniasis is one of the neglected tropical disease and it is ranked second in terms of mortality and the loss of disability-adjusted life years (5,15). 6th World Health Assembly (A60/10)

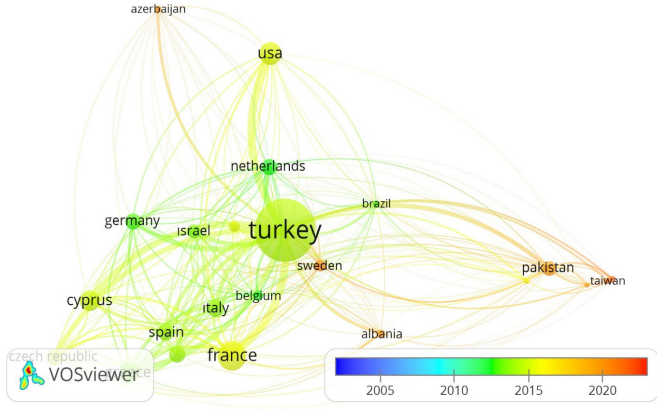

Figure 5. Overlay visualization of bibliographic coupling of Türkiye and other countries on *Leishmania* in the last 20 years

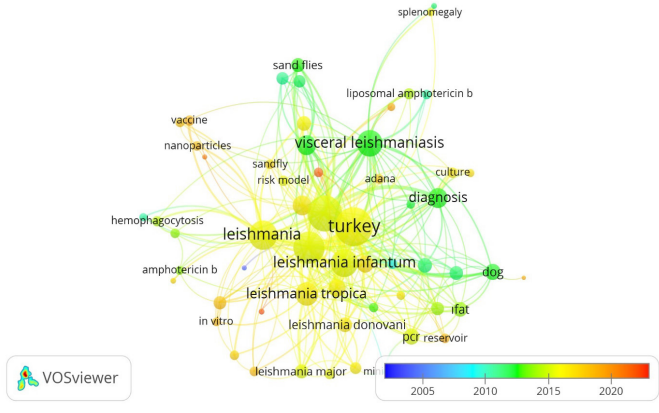

Figure 6. Overlay visualization of keywords

published in 2007, states that leishmaniasis patients should be able to receive leishmaniasis treatment for both VL and CL (18). Leishmaniasis has consequently received the attention of both the public health community and funders as a result of becoming a worldwide concern. The WHO has set goals and milestones for the management of leishmaniasis, including the eradication of VL, decreasing case fatality rate of primary VL under 1%, and giving the treatment to at least 90% of CL cases by 2030 (17).

There has been lots of interest in utilizing bibliographic data to analyze scientific research in recent years. This method is regarded as one of the most effective methods for determining the performance criteria of scientific research institutes. In medicine, the use of scientometric approaches for these bibliometric evaluations is constantly increasing (10-26). Although quick databases such as Scopus and WOS have been used in most of these bibliometric studies, sometimes these analyses can be carried out by examining the databases created by the researchers or the materials such as theses and books (8-22). In this study, the analyses were made by examining the WOS database, which is one of the most prestigious databases in the world.

The goal of this study was to conduct a bibliometric analysis of research output of 20 years on leishmaniasis using scientometric methodology. Our results can help to develop a clear picture of the scientific outputs of this field and also help planning and policy development about leishmaniasis. Although there are similar studies on leishmaniasis in the literature (15-24), the current study is the most comprehensive study on leishmaniasis as it examined the global situation of leishmaniasis between 2003-2022. We also aimed to investigate Türkiye's contribution to the leishmaniasis literature because there could not be found any study investigating Türkiye's contribution to the leishmaniasis in the literature.

In the literature although the USA ranked first in most of the global bibliometric studies (8-14), Brazil was ranked first in the current study with 4.463 articles. According to the results of our study, Brazil has always ranked first in both the 2003-2012 and 2013-2022 periods. This may be a result of scientific interest about *Leishmania* in Brazil or the disease's being endemic. In addition, it has been observed that the institutions that produce most of the articles on this subject are often located in Brazil. The USA, India, Iran and England were other productive countries on leishmaniasis. India and Iran, both of which have a significant prevalence of leishmaniasis (5,6), were ranked third and fourth in research output, respectively, and are Asia's leaders in leishmaniasis research. When we compare the results of González-Alcaide et al. (23) with our study, it has been determined that Iran was found to rise in the ranking with the number of publications. Although Türkiye ranked 14th, the number of publications and citations have increased over the years. Türkiye is among the endemic countries for this disease. CL is the most common form in Türkiye. And it is endemic in Şanlıurfa, Osmaniye, Adana, Kahramanmaraş, Hatay, and Mersin provinces (25). Within the scope of the "Parasitological, Molecular and Geographical Epidemiological Approach to the Control of Cutaneous Leishmaniasis in Türkiye" project, carried out in cooperation with the General Directorate of Public Health of the Turkish Ministry of Health, with the participation of the Turkish Dermatology Association, in 2018, in Şanlıurfa, "Diagnosis and Treatment of Oriental Boils" treatment training was carried out. The training was attended by people, from Şanlıurfa and surrounding provinces, as well as physicians who are members of the Turkish Dermatology Association. In addition, the geographical ınformation system based oriental sore notification system developed within the scope of the project was introduced (26). With this meeting, the importance given to the subject by the Turkish Ministry of Health was revealed. This may lead to an increase in the number of publications on *Leishmania* from Türkiye.

Ege University was the institution that published most of the articles on leishmaniasis. Other institutions were also located in regions where the disease was endemic. 37.574% of the studies were not funded from Türkiye and the articles were mainly published in the journals from Türkiye.

In the last 20 years, the advancement of technology has drastically changed the characteristics of research work in Türkiye. The use of high technology (polymerase chain reaction, sequencing, MALDI-TOF, etc.). The first reports of *Leishmania hybrid* isolates, *Leishmania* subtypes associated with Syrians and *Leishmania Virus* positive clinical isolates were reported in Türkiye after 2010 (1,27). While the number of publications in Türkiye was limited to certain universities in previous years, other universities (Acıbadem University, University of Health Sciences Türkiye) have also contributed to the *Leishmania* literature in the last decade due to the reasons mentioned above.

In conclusion, the number of publications, especially in endemic areas, is limited outside Brazil. There was an increase in publication trendin European countries. This may be due to the migration-related to the Syrian civil war in 2011. However, although the number of publications in our country has increased up to 2 times compared to the first 10 years, the number of publications in our country is limited. Ege University ranked first in Türkiye in terms of *Leishmania* publications. However, none of the other institutions have published more than 50 publications in total. Studies should be supported to ensure the eradication of the disease.

Study Limitations

A single database was used in the study. In addition, only research articles published in the last 20 years were included. English was used as the search language. Therefore, it may not reflect the entire scientific literature due to the WOS database not covering all medical literature. Data visualization was performed in our study.

CONCLUSION

However, content analysis was not performed and the most cited articles were not analyzed. The database is constantly updated with articles added every day. Although the study has some limitations it is the first study that makes comparisons between global and Turkish *Leishaminasis* literature, so it may give an idea to researchers in this field.

* Ethics

Ethics Committee Approval: Ethics committee approval was not obtained because there was no animal or human study and it was a document review study.

Informed Consent: Since the study was a document review study, the data of the patients were not used. Open data of websites were evaluated. Patient consent is not required.
Peer-review: Internally and externally peer-reviewed.

*** Authorship Contributions**

Concept: S.A., M.S.Ş., Design: S.A., O.E., M.S.Ş., Data Collection or Processing: S.A., M.S.Ş., Analysis or Interpretation: O.E., S.A., Literature Search: M.S.Ş., S.A., Writing: S.A., M.S.Ş., O.E.

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

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First Record, Distribution and Occurrence of A Protistan Entomopathogen, *Adelina mesnili* Perez (Coccidia: Adeleidae) in the Indian Meal Moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) Populations in Türkiye

Protistan Entomopatojen Adelina mesnili Perez'in (Coccidia: Adeleidae) Türkiye'deki Kuru Meyve Güvesi Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae) Popülasyonlarında İlk Kaydı, Dağılımı ve Enfeksiyon Oranları

Mustafa Yaman[1], Tuğba Sağlam[1], Ömer Ertürk[2]

[1]Bolu Abant İzzet Baysal University, Faculty of Arts and Science, Department of Biology, Bolu, Türkiye
[2]Ordu University Faculty of Arts and Science, Department of Molecular Biology and Genetic, Ordu, Türkiye

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ABSTRACT

Objective: *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) originating from South America is one of the important insect pests that damages storage products and is found on every continent. There is a new interest in using enthomopathogens for microbial control of *P. interpunctella* as well as other stored product pests. Coccidia as a group of protistan entomopathogens are host specific and their pathogenic effects on the hosts are more pronounced. Although this pathogenic effect results in increased host mortality or higher susceptibility to insecticides, the suppressive potential of coccidia in natural populations has not been adequately studied. In this study, characterization, distribution and occurrence of a coccidian entomopathogen was aim to show its natural suppressing potential in *P. interpunctella* populations.

Methods: During the three years (from 2019 to 2021), a total of 3.432 *P. interpunctella* samples (2.047 dead and 413 living larvae, 932 adults and 40 pupae) were collected from fourteen populations. After macroscopic examination, suspected samples were dissected in Ringer's solution and then prepared wet smears including host fat body were examined for presence of coccidian pathogens under a light microscope at a magnification of 400-1000X. The oocysts of the coccidian were measured and photographed using a microscope with a digital camera and soft imaging system.

Results: The pathogen was observed in the fat bodies of the larvae, pupae and adults. Oocysts measured as 29.52±3.32 (25.27-35.08) µm in diameter and they include 8 sporocysts. Sporocysts measured as 9.11±0.61 (8.90-9.85) µm. Forty-five of 3.432 *P. interpunctella* larvae, pupae and adults were found to be infected. Coccidian infections have also reached to the levels that can be considered high in some populations, as significant as 29.2%. The infection was observed in the three (21.4%) of the examined fourteen populations.

Conclusion: The coccidian entomopathogen presented in this study is the first Adeleid coccidian record from *P. interpunctella* populations in Türkiye. The detection of *Adelina mesnili* Perez (Coccidia: Adeleidae) in at least three populations and the infection rate reaching 29.2 percent, confirms that this pathogen has a considerable effect *P. interpunctella* populations that cannot be underestimated. Our results confirm that the coccidian pathogen is very effective in the larval stage.

Keywords: *Plodia interpunctella*, *Adelina mesnili,* stored product pest, distribution, biological control

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Address for Correspondence/Yazar Adresi: Mustafa Yaman, Bolu Abant İzzet Baysal University, Faculty of Arts and Science, Department of Biology, Bolu, Türkiye
Phone/Tel: +90 536 873 27 00 **E-mail/E-Posta:** muyaman@hotmail.com **ORCID ID:** orcid.org/000-0001-5656-7266

Türkiye PARAZİTOLOJİ Dergisi

ÖZ

Amaç: Güney Amerika kökenli *Plodia interpunctella* (Hübner) (Lepidoptera Pyralidae) her kıtada bulunan ve depolama ürünlerine zarar veren önemli zararlılardan biridir. *P. interpunctella* ve diğer depolanmış ürün zararlılarının mikrobiyal kontrolü için entomopatojenlerin kullanımına yönelik bir ilgi vardır. Bir protistan entomopatojen grubu olarak Coccidia, konakçıya özgü olup, konaklar üzerindeki patojenik etkileri daha belirgindir. Bu patojenik etki, artan konakçı ölümü veya insektisitlere karşı daha yüksek duyarlılık ile sonuçlanmasına rağmen, doğal popülasyonlarda coccidianların baskılayıcı potansiyeli yeterince araştırılmamıştır. Bu çalışmada, bir coccidian entomopatojenin karakterizasyonun yapılması, dağılımı ve enfeksiyon oranlarının belirlenerek *P. interpunctella* popülasyonlarındaki doğal baskılama potansiyelinin ortaya konulması amaçlanmıştır.

Yöntemler: Üç yıl boyunca (2019-2021), on dört popülasyondan toplam 3,432 *P. interpunctella* örneği (2,047 ölü, 413 canlı larva, 932 ergin ve 40 pupa) toplanmıştır. Makroskopik incelemeden sonra, şüpheli numuneler Ringer solüsyonunda disekte edilmiş ve daha sonra konak yağ dokusunu içeren preperatlar hazırlanarak Coccidian patojenlerin varlığı açısından ışık mikroskobunda 400-1000X büyütmede incelenmiştir.

Bulgular: Patojen larva, pupa ve erginlerin yağ dokusunda gözlenmiştir. 29,52±3,32 (25,27-35,08) µm çapında ölçülen ookistler, 8 sporokist içermektedir. Sporokistler 9,11±0,61 (8,90-9,85) µm olarak ölçülmüştür. İncelenen 3,432 *P. interpunctella* larva, pupa ve ergininin 45 tanesinin enfekte olduğu tespit edilmiştir. Coccidian enfeksiyonlarının bazı popülasyonlarda %29,2 gibi önemli sayılabilecek seviyelere ulaştığı tespit edilmiştir. Enfeksiyon, incelenen on dört popülasyonun üçünde (%21,4) gözlenmiştir.

Sonuç: Bu çalışmada sunulan coccidian entomopatojen, Türkiye'deki *P. interpunctella* popülasyonlarından elde edilen ilk Adeleid Coccidian kaydıdır. *Adelina mesnili* Perez'in (Coccidia: Adeleidae) en az üç popülasyonda saptanması ve enfeksiyon oranının %29,2'ye ulaşması, bu patojenin *P. interpunctella* popülasyonlarında küçümsenemeyecek kadar önemli bir etkiye sahip olduğunu doğrulamaktadır.

Anahtar Kelimeler: *Plodia interpunctella*, *Adelina mesnili,* depo zararlısı, dağılış, biyolojik mücadele

INTRODUCTION

Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae) originating from South America is one of the important insect pests that damages storage products and is found on every continent. It is a harmful species in a wide range of products such as dried figs, dried apricots, hazelnuts, raisins, oil seeds, cereals, flour and products, cocoa and spices. *P. interpunctella* larvae feed both inside and on the surface of the food. The infestation of *P. interpunctella* causes direct crop loss and economic cost.

There is a new interest in using enthomopathogens for microbial control of *P. interpunctella* as well as other stored product pests (1,2). Among the entomopathogens, protistan entomopathogens are often prevalent and persistent in natural populations of pest insects. Coccidia as a group of protistan entomopathogens are all intracellular parasites and multiply extensively in the host insect. Most species are host specific. Coccidia carry out extensive and more multiplication cycles in the insect host, so the pathogenic effects on the host are more pronounced (3). Although this pathogenic effect results in increased host mortality or higher susceptibility to insecticides, the suppressive potential of coccidia in natural populations has not been adequately studied. In this study, characterization, distribution and occurrence of a coccidian entomopathogen were studied to show its natural suppressing potential in *P. interpunctella* populations.

METHODS

Insect Samples

During the three years (from 2019 to 2021), a total of 3.312 *P. interpunctella* samples (2.032 dead and 413 living larvae, 830 adults and 37 pupae) were collected from warehouses, shops and houses in the fourteen provinces (Ankara, Aydın, Bolu, Denizli, Gaziantep, Isparta, İstanbul, İzmir, Kastamonu, Malatya, Ordu, Samsun, Siirt and Trabzon), widely dispersed geographically in Türkiye (Table 1).

Macroscopic-microscopic Examinations

Insect specimens suspected of having disease were subjected to macroscopic examination. The most common symptoms in larvae

Table 1. Sampling localities and dates

Sampling localities	Sampling date
Ankara	08.07.2021
Aydın	12.06.2019, 02.07.2019, 22.07.2019, 18.06.2020, 30.06.2020
Bolu	22.05.2019, 28.06.2019, 08.07.2019, 20.08.2019, 05.09.2019, 05.09.2019, 12.09.2019, 20.01.2020, 18.02.2020, 11.03.2020, 23.03.2020, 30.04.2020, 01.06.2020, 13.07.2020, 17.06.2021, 12.07.2021
Denizli	01.06.2019, 28.06.2019
Gaziantep	05.07.2019, 05.08.2019, 11.09.2019, 22.07.2020, 27.07.2020, 25.07.2021
Isparta	02.05.2019, 13.07.2019, 06.08.2020, 31.08.2020, 26.05.2021
İstanbul	20.12.2019, 16.03.2020, 02.04.2020
İzmir	12.06.2019
Kastamonu	08.12.2021
Malatya	13.06.2019, 21.06.2019, 12.09.2019, 16.07.2020, 20.08.2020
Ordu	18.06.2019, 21.06.2020
Samsun	10.06.2019, 10.07.2020, 28.07.2021
Siirt	28.06.2019
Trabzon	15.06.2019, 10.07.2020

were discolored death, slow movement, loss of appetite. After macroscopic examination, suspected samples were dissected in Ringer's solution and then prepared wet smears including host fat body were examined for presence of coccidian pathogens under a light microscope at a magnification of 400-1000X. When an infection was found, the slides were air-dried and fixed with methanol, then stained with freshly prepared 5% solution of Giemsa stain. They were then washed in running tap water, air-dried and examined under a microscope (4). The oocysts of the coccidian pathogen detected by the light microscopy were measured and photographed using a microscope with a digital camera and soft imaging system.

Statistical Analysis

A chi-square test was used to compare observed results. A p-value less than 0.05 was considered significant.

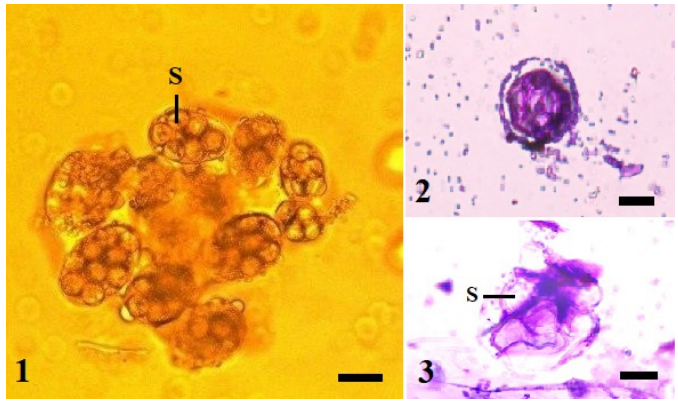
RESULTS

During the microscopic observations, a coccidian entomopathogen were found in the populations of *P. interpunctella* in Türkiye. The pathogen was observed in the fat bodies of the larvae, pupae and adults. Polysporocystic oocysts of the pathogen were the evidence of the infection (Figures 1-3). Oocysts measured as 29.52±3.32 (25.27-35.08) µm in diameter and they include 8 sporocysts. Sporocysts measured as 9.11±0.61 (8.90-9.85) µm.

During the study, 3.432 samples of *P. interpunctella* samples including larvae, adults and pupa were dissected and searched for the coccidian infections in the fourteen localities of Türkiye between the years 2019-2021. Totally, 2.047 dead and 413 living larvae, 932 adults and 40 pupae were examined for the presence of the coccidian pathogen, 45 of 3.432 *P. interpunctella* larvae, pupae and adults were found to be infected. Total infection occurred as 1.3% for the pathogen (Table 2). On the other hand, the average of coccidian infections for all populations was found as 0.2% for dead larvae, 0.2% for living larvae and 4.2% for adults. Coccidian infections have also reached to the levels that can be considered high in some populations, as significant as 29.2% (Tables 2, 3). The infection was observed in the three (21.4%) of the examined fourteen populations (Figure 4).

DISCUSSION

Light microscopic observations of the fresh and Giemsa-stained preperations indicate that the coccidian pathogen found in *P. interpunctella* populations belongs to the genus *Adelina*. The recorded parasite has typical characters of the genus *Adelina* such as shape and size of oocysts (Figures 1-3), number (3-30) of sporocyst per oocyst and number of sporozoites per sporocyst (3). In the literature there is no coccidian record from *P. interpunctella* populations in Türkiye. The Coccidian entomopathogen presented in this study is the first *Adeleid* coccidian record from *P. interpunctella* populations in Türkiye. Number of sporocyst per oocyst and the host affinity is generally recognized as a valid taxonomic character to discriminate the pathogen at the species level. According to Yaman et al. (2), up to now fifteen species belonging to the genus *Adelina* have been described from host insects; their distinctive characteristics are



Figures 1-3. Adeleid polysporocystic oocysts of coccidian pathogen. (1) A group of oocysts in fat body, (2) Giemsa stained oocyst, (3) Giemsa stained sporocysts releasing from the damaged oocyst. Bars, 10 µm

Table 2. Occurrence of *Adelina mesnili* in *P. interpunctella* populations

Locality	Examined sample	Infected sample	Infection rate (%)
Ankara	51	-	-
Aydın	101	3	2.9
Bolu	1.115	-	-
Denizli	9	-	-
Gaziantep	494	-	-
Isparta	120	-	-
İstanbul	121	-	-
İzmir	45	-	-
Kastamonu	120	35	29.2
Malatya	499	-	-
Ordu	193	7	3.6
Samsun	299	-	-
Siirt	145	-	-
Trabzon	120	-	-
Total	**3.432**	**45**	**1.3**

shown in Table 4. The oocyst dimension and sporocyst number per oocyst is a good feature for comparison of the fifteen *Adelina* species from host insects. As seen in Table 4, our coccidian differs from thirteen *Adelina* species in oocyst size and similar with *Adelina mesnili* (8 sporocysts per oocysts). The number of sporocyst in an oocyst varies from 6 to 8 with 8 being the most common. Pérez (5) recorded the number of sporocysts in each of *A. mesnili* as generally 6 to 8, rarely 9 in the original description. The morphological features of the pathogen show similarities

Figure 4. Distribution of *Adelina mesnili* infections in *Plodia interpunctella* populations in Türkiye

ANK: Ankara, AYD: Aydın, BOL: Bolu, DNZ: Denizli, GZP: Gaziantep, ISP: Isparta, İST: İstanbul, İZM: İzmir, KST: Kastamonu MLT: Malatya, ORD: Ordu, SAM: Samsun, ST: Siirt, TRB: Trabzon

with other species of the genus *Adelina* (Coccidia: Adeleidae) and especially resembles *A. mesnili*, described in lepidopteran hosts by Pérez (5) and observed in the artificial cultures of *P. interpunctella* and *Ephestia kühniella* by Steinhaus (6). Therefore, the coccidian pathogen was identified as the Turkish strain of *A. mesnili*. *A. mesnili* found in the present study was observed first in the larvae, pupae and adults of *P. interpunctella* in Türkiye.

No statistical difference between both dead and living larvae was found. As seen in Table 3, the infection rates in the adults are higher than those in pupae. There is statistically significant difference in the infection levels of adult and pupa of *P. interpunctella* (Pearson chi-square, p=0.000 <0.05). Coccidians occur naturally in Lepidoptera. So, have been recognized as potential biocontrol agents against Lepidoptera. However, the use of pathogenic protist species as a control agent should be in the early stages of development. At the same time, extensive research is required to be used as a protective agent (7).

There have been several studies on pathogens and parasites of stored-product pests, mainly focused on isolation and characterization of pathogenic microorganisms. A few of them were carried out on the protistan entomopathogens of *P. inteerpunctella*. Until now, microsporidian pathogens, *Nosema plodiae* (8,9), *Vairimorpha plodia* (1,10-12), neogregarine pathogen, *Mattesia dispora* (13), gregarine pathogen, *Leidyana* sp. (14), have been studied as microbial pathogen in *P. interpunctella*. However, there is only one study on the distribution, occurrence

Table 3. Occurrence of *Adelina mesnili* in the different life stages of *Plodia interpunctella*

Life stage	Number of examined sample	Number of infected sample	Infection rate (%)
Larva (living)	413	1	0.2
Larva (dead)	2.047	4	0.2
Adult	932	39	4.2
Pupae	40	1	-
Total	**3.432**	**45**	**1.3**

Table 4. *Adelina* species that infect insects and their morphological features [improved from (15)]

Adelina species	Tissues infected	Num. sporocyst per oocyst	Oocyst diam. (µm)	Sporocyst diam. (µm)	Reference
A. akidium	Fat body	12-20	30-40	10	(16)
A. collembolae	Fat body	24	40	7.5-8	(17)
A. eryptocerci	Various	5-21	24-51	10-12	(18)
A. mesnili	Fat body	6-8	-	15	(5)
A. riouxi	-	8-18	30-40	7-10	(19)
A. sericesthis	Fat body	4-8	30-40	10.8-11.9	(20)
A. simplex	Gut	8-16	25-40	-	(21)
A. tenebrionis	Fat body	2-12	20-35	10-12	(22)
A. tenebrionis	Fat body	3-13	29.2-45	12.3-14	(15)
A. tipulae	Gut	4-10	35-40	-	(21)
A. transita	Various	6-20	30-40	10-11	(23)
A. grylli		4-22	-	-	(24)
A. triboli	Fat body	4-16	40	10-13	(25)
A. melolonthae	Fat body	4-12	35.62±4.04 (23.97-44.56)	11.70±0.42 (11.02-12.52)	(26)
A. mesnili	Fat body	6-8	29.52±3.32 (25.27-35.08)	9.11±0.61 (8.90-9.85)	In this study

and potential of microsporidian entomopathogen, *V. plodia* in *P. interpunctella* under natural conditions (1). There is no any study on distribution, occurrence and potential of microbial pathogens about *A. mesnili* (Coccidia: Adeleidae) in *P. interpunctella* under the natural conditions. In this study, characterization, distribution and occurrence of the coccidian entomopathogen of *P. interpunctella* from 14 localities representing all Türkiye between the years 2019-2021 is given in an extensive field study for the first time by confirming its first record from Türkiye and effectiveness on natural populations. *A. mesnili* was detected in three populations (21.4%).

CONCLUSION

Coccidians occurring naturally in insect pest populations are highly pathogenic for them, therefore they have been considered as potential natural suppressing factor in insect pest populations. The detection of *A. mesnili* in at least three populations and the infection rate reaching 29.2 percent, confirms that this pathogen has a considerable effect in *P. interpuctella* populations that cannot be underestimated.

* Ethics

Ethics Committee Approval: Ethics committee approval is not required as it is studied on harmful insects.

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

* Authorship Contributions

Concept: M.Y., Design: M.Y., Data Collection or Processing: T.S., Ö.E., Analysis or Interpretation: M.Y., T.S., Ö.E., Literature Search: M.Y., T.S., Ö.E., Writing: M.Y., T.S.

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Ağrı İlinde Kesilen Sığırlarda Fascioliasis Yaygınlığı

Prevalence of Fascioliasis in Slaughtered Cattle in Ağrı Province

Maksut Şahin[1], Milad Torkamanian Afshar[1], Rahmi Yıldız[2], Selahattin Aydemir[1], Hasan Yılmaz[1], Zeynep Taş Cengiz[1]

[1]Van Yüzüncü Yıl Üniversitesi Tıp Fakültesi, Parazitoloji Anabilim Dalı, Van, Türkiye
[2]Van Yüzüncü Yıl Üniversitesi, Gevaş Meslek Yüksekokulu, Veterinerlik Bölümü, Van, Türkiye

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ÖZ

Amaç: Bu çalışmada, Ağrı ili mezbahalarında kesimi yapılan sığırlarda fascioliasis sıklığının ortaya çıkarılması amaçlanmıştır.
Yöntemler: Araştırma 230 sığır üzerinde yürütüldü. Her bir sığırdan alınan dışkı ve safra sıvısı örneklerinde, sedimentasyon-çinko sülfat flotasyon yöntemi ile *Fasciola hepatica* yumurtalarının varlığı, karaciğer ve safra kanallarında ise postmortem muayene ile erişkin parazit varlığı araştırılmıştır.
Bulgular: Çalışma 230 sığır numunesi üzerinde yapılmıştır. İncelenen dışkıların 43'ünde (%18,7) *Fasciola* spp. yumurtası görülmüştür. Karaciğer ve safra kesesinin postmortem incelenmesi sonucunda ise 52 (%22,6) sığırda *F. hepatica* erişkini saptanmıştır. Dışkı incelemesi ile pozitif olan tüm sığırlar karaciğer ve safra kesesi incelemesinde de pozitif bulunmuştur. Sığırların hiçbirinde *Fasciola gigantica* erişkini saptanmamış, istatistiksel olarak cinsiyet ve ırk açısından anlamlı bir farklılık tespit edilmemiştir.
Sonuç: Ağrı yöresindeki sığırlar üzerinde yapılan bu çalışmada *F. hepatica*'nın önemli oranda yaygın olduğu tespit edilmiştir. Elde edilen veriler yöre halkının önemli geçim kaynağı olan hayvancılıktaki ekonomik kayıpların önlenmesi için etkili bir korunma ve kontrol programlarının planlanması, uygulanması ve gerek küçükbaş gerekse büyükbaş hayvan yetiştiricilerinin bu enfeksiyonun önlenmesi hususunda bilinçlendirilmesi gerektiği sonucunu ortaya koymaktadır.
Anahtar Kelimeler: Ağrı, sığır, fascioliasis, sedimentasyon

ABSTRACT

Objective: In this study, it was aimed to reveal the frequency of fascioliasis in cattle slaughtered in the slaughterhouses of Ağrı province.
Methods: The study was carried out on 230 cattle. The presence of *Fasciola hepatica* eggs in stool and bile fluid samples taken from each cattle was investigated by sedimentation-zinc sulfate flotation method, and the presence of adult parasites in the liver and bile ducts by postmortem examination.
Results: The study was conducted on 230 cattle samples. *Fasciola* spp. eggs were observed in 43 (18.7%) of the stools examined. As a result of the postmortem examination of the liver and gall bladder, *F. hepatica* adults were found in 52 (22.6%) cattle. All cattle positive in stool examination were also positive in liver and gallbladder examination. Fasciola gigantica adults were not detected in any of the cattle, and there was no statistically significant difference in terms of gender and race.
Conclusion: In this study conducted on cattle in the Ağrı region, *F. hepatica* was found to be significantly common. The data obtained show that in order to prevent economic losses in animal husbandry, which is an important source of livelihood of the local people, effective prevention and control programs should be planned and implemented, and both sheep and cattle breeders should be made aware of the prevention of this infection.
Keywords: Ağrı, cattle, fascioliasis, sedimentation

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Yazar Adresi/Address for Correspondence: Maksut Şahin, Van Yüzüncü Yıl Üniversitesi Tıp Fakültesi, Parazitoloji Anabilim Dalı, Van, Türkiye
Tel/Phone: +90 533 269 25 12 **E-Posta/E-mail:** maksutsahin@gmail.com **ORCID ID:** orcid.org/0000-0002-0875-4654

Türkiye PARAZİTOLOJİ Dergisi

GİRİŞ

Fascioliasis; ılıman iklimlere sahip ülkeler başta olmak üzere dünya çapında yaygın olan bir trematod hastalığıdır. Zoonotik karaktere sahip olan bu hastalık başta sığır, keçi, koyun, manda ile deve olmak üzere farklı evcil ve yabani ruminantlar, domuz, köpek, fil, at, eşek, tavşan, kedi gibi hayvanlarda görülebilmektedir (1-3). Fascioliasis normalde bir sığır ve koyun enfeksiyonu olsa da dünyanın çeşitli bölgelerinde önemli bir halk sağlığı problemi olarak da karşımıza çıkmaktadır. Enfeksiyonun 70'ten fazla ülkede en az 2,4 milyon insanı etkilediği ve 180 milyondan fazlasının risk altında olduğu tahmin edilmektedir (1,3,4).

Enfeksiyon oluşturan en yaygın türler *Fasciola hepatica* ve *Fasciola gigantica*'dır (5). Son konaklar tarafından enfektif metaserkerlerin kontamine yeşil su bitkileri aracılığıyla oral olarak alınmasını takiben genç parazitler ince bağırsaklarda kistlerden çıkar ve bağırsak duvarını delerek peritona geçer. Karaciğer kapsülasını delerek peritondan parankim dokuya girer ve safra kanallarına ulaşır. Safra kanallarına yerleşerek eşeysel olarak olgun parazit olurlar. Enfektif formların konaklar tarafından alınmasını takiben parazitin türüne göre değişmekle birlikte erişkinliğe ulaşıp yumurtlama kabiliyeti kazanabilmesi için yaklaşık 10-12 hafta kadarlık bir süreç geçmesi gereklidir (6).

Fascioliasiste patojenite; parazitin parankimal göç safhasında oluşan karaciğer tahribatı ve hemoraji ile, göç dönemi sonunda parazitlerin safra kanallarına ulaşmasıyla birlikte mukozayı tahrip etmesi, kanama, tıkanma, safra akımında bozukluk, tromboz, kolanjit, mekanik hasar ve hematofajik aktiviteleri ile ortaya çıkan safra dönemi olmak üzere iki dönemden oluşur. Oluşan hasarda parazitin dikenleri önemli faktördür (7). Fascioliasise bağlı olarak düşük gebelik oranı, et ve süt veriminde azalma, pubertanın gecikmesi, düşük doğum ağırlığı oluşmaktadır (8,9). Bunların yanı sıra enfeksiyona bağlı olarak anemi, hipoalbüminemi, immünoglobulin sentezinde artma ve eozinofili oluşumu da gözlenebilir (10).

Ayrıca protein, vitamin ve mineral açısından zengin bir besin kaynağı olan karaciğerin kısmen veya tamamen imhası önemli ekonomik kayıplara da yol açmaktadır (8,9). Fascioliasis nedeniyle oluşan ekonomik kaybın Brezilya'da (11) yıllık 210 milyon ABD doları, İran'da (12) ise 3 yılda 7,948,332 ABD doları civarında olduğu bildirilmiştir. Elazığ'da (10) yapılan çalışmada *F. hepatica* enfeksiyonu nedeniyle oluşan ekonomik kaybın 417.089 TL olduğu tespit edilmiştir. Çarşamba Belediye Mezbahası'nda (13) yapılan çalışmada ise 7480 büyükbaş ve 340 küçükbaş hayvan karaciğerinin %29,34'ünün fasciolosis nedeniyle imha edildiği, meydana gelen karaciğer kaybının 7,669 kg olduğunu kaydedilmiştir.

Bu çalışma, Ağrı yöresinde kesilen sığırlarda fascioliasis yaygınlığının belirlenmesi amacıyla yapıldı.

YÖNTEMLER

Etik Kurul Onayı

Bu çalışma için Van Yüzüncü Yıl Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'ndan 25/03/2021 tarih ve 2021/03-06 sayılı kararla etik kurul onayı gerekmediği kararı verilmiştir. Çalışmada insan numunesi kullanılmadığından hasta onamı gerekmemiştir.

Örneklerin Toplanması

Bu çalışma, 24.01.2021-20.07.2021 tarihleri arasında Ağrı Et ve Süt Kurumu Mezbahanesi'nde yürütüldü. Çalışmaya kesimi yapılan 230 sığır dahil edildi. Kesimi yapılan her sığırdan dışkı numunesi alındı. Ayrıca her sığırın karaciğer ve safra kanalları makroskobik olarak incelendi. Sığırların yaş, ırk ve cinsiyet bilgileri kaydedildi.

Dışkı Örneklerinin Parazitolojik İncelemesi

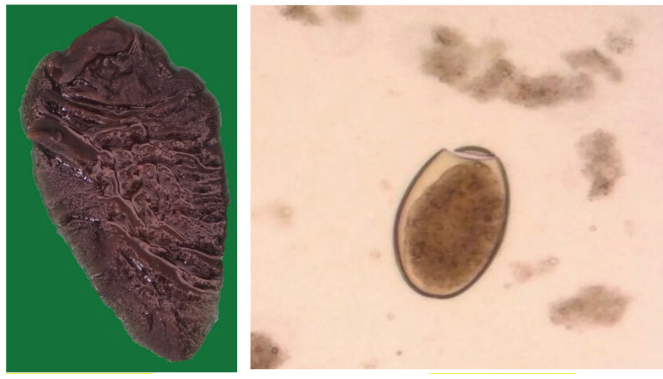
Kesim öncesi her bir sığırın rektumundan 30-50 gram dışkı örneği alındı. Dışkı örnekleri sedimentasyon-çinko sülfat flotasyon yöntemi uygulanarak incelendi (14).

Postmortem İnceleme

Kesimi yapılan tüm sığırların karaciğer ile safra kesesi ve kanalları bekletilmeden makroskobik olarak incelendi. Daha sonra yüzeysel olarak sıvazlamak ve basınç uygulamak suretiyle organın iç kısımlarında herhangi bir sertlik veya şişlik olup olmadığı araştırıldı. Şüpheli olan karaciğerlerin birkaç yerinden bıçakla kesit atılarak her iki tarafından el ile sıktırılmak suretiyle parazitlerin ortaya çıkması sağlandı. Sonra tespit edilen erişkin Fascioliasis etkenleri toplanarak %70'lik alkol içeren kaplar içerisine alındı ve tür tayini amacıyla incelendi. Muayeneler esnasında kesimi yapılan sığırların safra keseleri de toplanarak ayrı ayrı plastik poşetlere alındı, etiketlendi ve Van Yüzüncü Yıl Üniversitesi Tıp Fakültesi, Parazitoloji Anabilim Dalı Laboratuvarı'na getirildi. Laboratuvarda her bir safra kesesi bistüri ile açılarak safra sıvısı cam kaplara boşaltıldı ve üzerine çeşme suyu eklenerek 60 dakika bekletildi. Dipteki tortuya dokunulmadan üst kısım dökülerek üzerine tekrar çeşme suyu ilave edildi ve bu işlem dipteki tortu şeffaflaşıncaya kadar tekrarlandı. Son olarak dipteki tortu üzerine %10'luk formaldehit ilave edilerek makroskobik (erişkin form tespiti için) ve mikroskobik (sedimentasyon-çinko sülfat flotasyon yöntemi ile yumurta tespiti için) olarak incelendi.

İstatistiksel Analiz

Üzerinde durulan özelliklerden kategorik değişkenler sayı ve yüzde olarak ifade edilmiştir. Kategorik değişkenler için oranların karşılaştırmasında Z (t) testi kullanılmıştır. Ayrıca kategorik değişkenler arasındaki ilişkiyi belirlemede ki-kare testi yapılmıştır. Hesaplamalarda istatistik anlamlılık düzeyi %5 olarak alınmış ve hesaplamalar için SPSS (ver:13) ve MINITAB (ver:14) istatistik paket programları kullanılmıştır.



Şekil 1. *Fasciola hepatica* erişkini (formolde bekletilmiş) - Dışkı örneğinde *Fasciola hepatica* yumurtası (Lugol boyama)

Tablo 1. Kullanılan yöntem ve değişkenlere göre *F. hepatica* görülme oranı

Değişken		Toplam sayı %	*F. hepatica*		p-değeri
			Pozitif sayı %	Negatif sayı %	
Cinsiyet	Dişi	18 (7,8)	4 (22,2)	14 (77,7)	0,967
	Erkek	212 (92,1)	48 (22,6)	164 (77,3)	
Irk	Doğu Anadolu Kırmızısı	201 (87,3)	44 (21,9)	157 (78,1)	0,736
	Yerlikara	23 (10,0)	6 (26,0)	17 (74,0)	
	Holştayn	6 (2,6)	2 (33,3)	4 (66,6)	
İnceleme yöntemi	Dışkı incelemesi	230 (100)	43 (18,7)	187 (81,3)	0,299
	Postmortem inceleme	230(100)	52 (22,6)	178 (77,4)	

BULGULAR

Çalışmaya dahil edilen tüm sığırların dört yaş üstü olduğu belirlendi. İncelenen 230 sığır dışkısının 43'ünde (%18,7) *Fasciola* spp. yumurtası saptandı. Karaciğer ve safra kesesi incelemesinde ise 52 (%22,6) sığırda *F. hepatica* erişkini saptandı (Şekil 1). Dışkı incelemesi ile pozitif olan tüm sığırlar karaciğer ve safra kesesi incelemesinde de pozitif bulundu. Sığırların hiçbirinde *F. gigantica* erişkini saptanmadı. Bu nedenle dışkıda saptanan tüm yumurtalar *F. hepatica* yumurtası olarak değerlendirildi (Tablo 1). Irk, cinsiyet ve inceleme yöntemleri açısından istatistiksel olarak bir anlamlılık tespit edilmedi.

TARTIŞMA

Gerek büyükbaş gerekse küçükbaş hayvancılığın yaygın olarak yapıldığı ülkelerde karaciğer trematodları ciddi bir problem olarak karşımıza çıkmaya devam etmektedir. İnsan sağlığına olan direkt etkileri yanında, dünya nüfusundaki artışa paralel düzeyde gıda üretiminde artışın yapılamaması da dikkate alındığında, hayvansal üretimi kısıtlayan bu ve benzeri hastalıklar günümüz koşullarında daha da önemli hale gelmektedir (10).

Önemli ekonomik kayıplara neden olan fascioliasis ile ilgili pek çok çalışma yapılmış olup birbirinden farklı sonuçlara ulaşılmıştır. Yapılan çalışmalarda; Danimarka'da %25-29,3 (15), Güney Afrika'da %2-14,4 (16), Tanzanya'da %14,5 (17), Zambiya'da %64,4 (18), İran'da %8,48 (19), Etiyopya'da %16,8 (20), oranlarında *F. hepatica* tespit edilmiştir. Ülkeler arasında görülen bu fark iklim koşullarındaki farklar, bitki örtüsü, bataklıkta otlatma, mera, akarsulara veya göletlere erişim ve bölgenin endemik olup olmamasıyla bağlantılı olabilir (5).

Türkiye'de farklı zamanlarda yapılan mezbaha çalışmalarına bakıldığında, farklı yöntemlerle çalışmaların yapıldığı görülmektedir. Postmortem inceleme ile yapılan çalışmalarda sığırların; Van'da %50,3'ünde (21), Samsun'da %25,3'ünde (22), Afyon'da %4,6'sında (23), Malatya'da %4,42'sinde (24), Isparta'da %0,9'unda (13) *F. hepatica* pozitifliği saptanmıştır. Karaciğer incelemesinde Erzurum'da %21,21 ve %5'inde (9,25) *F. hepatica* pozitifliği saptanmıştır. Dışkı bakısı ve ELISA testlerine göre Kayseri'de %15,8 ve 69,2'sinde (26), Nevşehir'de %2,02 ve %3,03'ünde (27) *F. hepatica* saptanmıştır. Elazığ'da yapılan retrospektif çalışmada ise sığırların %7,94'ünde *F. hepatica* saptandığı belirtilmiştir (10). Bu çalışmanın yapıldığı Ağrı ilinde yapılan bir çalışmada ise fasciolosisin yaygınlığı dışkı muayenesi ile %33,5 (63/188), kopro antijen ELISA testi ile %78,7 (148/188) oranında saptanmıştır (28).

Bu sonuçlara göre, çalışmamızda elde edilen *F. hepatica* yaygınlık oranları postmortem inceleme sonuçlarıyla karşılaştırıldığında Isparta (13), Afyon (23) ve Malatya (25) yöresinde yapılan çalışmalardan daha yüksek, Van (21) ve Samsun (22) yöresinde yapılan çalışmalardan ise daha düşük oranda çıkmıştır.

Dışkı inceleme sonuçlarıyla karşılaştırıldığında; Kayseri (26) ve Nevşehir (27) yöresinde yapılan çalışmalardan daha yüksek, Ağrı (28) yöresinde yapılan çalışmadan ise daha düşük oranda *F. hepatica* pozitifliği saptanmıştır. Sadece karaciğer muayenesiyle Elazığ (10), Erzurum (9,24) bölgesinde yapılan çalışma sonuçlarına göre çalışmamızda daha yüksek oranda *F. hepatica* erişkini tespit edilmiştir.

Bu çalışmada postmortem incelemede (%22,6), dışkı incelemesine (%18,7) göre daha yüksek oranda pozitiflik bulunmuştur. Karaciğer ve safra yollarında yumurtlama erginliğine ulaşmamış erişkin parazitlerin bulunması ve yumurtlamanın dönemsel olması nedeniyle dışkıda daha düşük bir oranın tespiti beklenen bir durumdur.

Ağrı, sosyo-ekonomik gelişmişlik açısından Türkiye ortalamasının altında bulunan bir ilimiz olup ekonomik faaliyetleri çoğunlukla büyükbaş hayvancılık ve tarım sektöründe yoğunlaşmaktadır (29). Bu durumun en başta gelen nedenleri olarak bölgede mevcut olan mera ve geniş çayırlarla sağlanan rekabet avantajı, kırsal alan nüfusunun ülke ortalamasına göre yüksek olması ve gelişmiş bir sanayinin olmaması gösterilebilir (30). Bölge, Türkiye sığır varlığının en başta gelen merkezleri arasında yer almaktadır (31). Ayrıca bölgede bulunan Doğubayazıt Sazlığı sulak alanı, Doğubayazıt Havzası'nda Balık Gölü, Saz Gölü ve Gölyüzü Gölü olmak üzere üç büyük göl yanında, Sarısu Çayı, Sarısu Ovası ve sazlığı, Murat Nehri ve diğer bazı dere ve çayların oluşturduğu gölet ve sulak alanlar, akarsuların taşıdığı suların birikmesiyle oluşan göller, sazlık ve bataklık alanlar *Fasciola* biyolojisinde ve arakonak popülasyonunun yaşamını sürdürmesinde etkin olan iklim şartlarının oluşumunda önemli etkenlerdir (32,33).

SONUÇ

Çalışmada elde ettiğimiz veriler oransal olarak yüksek olup, önemli ekonomik kayıplara yol açmaktadır. Bu nedenle prevalansın gidişatının takip edilmesi için daha kapsamlı ve süreklilik arz eden çalışmaların yapılması, kayıpların önlenmesi adına hayvan sahiplerinin bilinçlendirilmesi ve tedavi amaçlı programların geliştirilmesine ihtiyaç olduğu kanaatindeyiz.

*** Etik**

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Hasta Onayı: Çalışmada insan numunesi kullanılmadığından hasta onamı gerekmemiştir.

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A Parasitological Examination on Aquarium Fish Sold in Petshops in Kırıkkale

Kırıkkale'deki Petshoplarda Satışa Sunulan Akvaryum Balıklarında Parazitolojik İncelemeler

Sami Gökpınar[1], Gözde Nur Akkuş[2], Sinem Akdeniz[2]

[1]Kırıkkale University Faculty of Veterinary Medicine, Department of Parasitology, Kırıkkale, Türkiye
[2]Kırıkkale University Health Sciences Institute, Department of Veterinary Parasitology, Kırıkkale, Türkiye

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ABSTRACT

Objective: The aim of the present study was to determine the prevalence of parasitic infections in aquarium fish sold in local pet shops.
Methods: Totally 502 fish samples from 8 species were obtained from 7 pet shops. Native preparations were prepared from the gills, fins, skin, intestines, and internal organs of fish and examined under a light microscope.
Results: It was detected at the end of the study that 62.7% of the fishes examined were infected with one or more parasite species. Among the fish examined, 28.9% were infected with a single parasite, whereas 33.9% were infected with mixed parasites. *Chilodonella* sp. identified as the most common species. Other species detected included *Trichodina* sp., *Piscinoodinium* sp., *Ichthyobodo* sp., *Ichthyophthirius multifiliis*, *Apiosoma* sp., *Epistylis* sp., *Vorticella* sp., *Gyrodactylus* sp., *Dactylogyrus* sp., *Capillaria* sp., *Camallanus* sp., metacercariae, and nematode larvae, *Argulus* sp., *Philodina* sp., *Euchlanis* sp., *Aelosoma* sp., and *Tetrahymena*.
Conclusion: The importance of the present study is that highest number of parasite species were detected in aquarium fish sold in pet shops in Türkiye. The aquarium owners should pay attention to the cleanliness of the aquarium water in order to reduce the rate of parasitic infection in fish; when new fish are purchased, they should be taken from reliable sources, and the quarantine process should be observed.
Keywords: Aquarium, arthropods, fish, helminths, protozoa

ÖZ

Amaç: Bu çalışmanın amacı yerel petshoplarda satışa sunulan akvaryum balıklarında paraziter enfeksiyonların yaygınlığının belirlenmesidir.
Yöntemler: Yedi petshoptan, 8 türe ait 502 adet balık örneği alınmıştır. Balıkların solungaç, yüzgeç, deri, bağırsak ve iç organlarından natif preparatlar hazırlanarak ışık mikroskobu altında incelenmiştir.
Bulgular: Çalışma sonunda incelenen balıkların %62,7'si bir veya birden fazla parazit türü ile enfekte bulunmuştur. Balıkların %28,9'unda tek, %33,9'unda birden fazla paraziter etken tespit edilmiştir. *Chilodonella* sp. en yaygın tür olarak belirlenmiştir. Tespit edilen diğer türler *Trichodina* sp., *Piscinoodinium* sp., *Ichthyobodo* sp., *Ichthyophthirius multifiliis*, *Apiosoma* sp., *Epistylis* sp., *Vorticella* sp., *Gyrodactylus* sp., *Dactylogyrus* sp., *Capillaria* sp., *Camallanus* sp., metaserkerler ve nematod larvaları, *Argulus* sp., *Philodina* sp., *Euchlanis* sp., *Aelosoma* sp., *Tetrahymena* sp.'dir.
Sonuç: Bu çalışma Türkiye'de petshoplarda satılan akvaryum balıklarında en fazla parazit türünün tespit edildiği çalışma olması bakımından önemlidir. Balıklarda paraziter enfeksiyon oranını azaltmak için akvaryum sahipleri akvaryum suyunun temizliğine özen göstermeli, yeni balık alındığında güvenilir kaynaklardan alınmalı ve karantina sürecine dikkat edilmelidir.
Anahtar Kelimeler: Akvaryum, arthropod, balık, helmint, protozoon

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Address for Correspondence/Yazar Adresi: Sami Gökpınar, Kırıkkale University Faculty of Veterinary Medicine, Department of Parasitology, Kırıkkale, Türkiye
Phone/Tel: +90 536 764 90 45 **E-mail/E-Posta:** samigokpinar@hotmail.com **ORCID ID:** orcid.org/0000-0001-7071-869X

Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

In recent years, there has been a rapid increase in aquarium fish farming in Türkiye, and it has become an important business line. There are many enterprises that breed or sell aquarium fish in each province of Türkiye. However, people raise aquarium fish as ornamental animals in their homes and workplaces.

Parasitic diseases may spread rapidly among aquarium fish and cause significant economic losses if necessary precautions are not taken (1). There are limited number of studies on determination of parasites in aquarium fish in Türkiye. *Chilodonella* sp., *Trichodina* sp., *Ichthyophthirius multifiliis, Ichthyobodo* sp., *Hexamita* sp., *Myxosporidia* sp., *Dactylogyrus* sp., *Gyrodactylus* sp., *Thaparocleidus* sp., *Centrocestus metaserkeri*, *Lernaea* sp., *Argulus* sp., *Camallanus* sp., *Capillaria* sp., *Ambiphyra* sp., *Epistylis* sp., *Tetrahymena*, *Vorticella*, *Philodina*, *Chaetonotus* sp., *Euchlanis* sp., (2), *Acanthocephala* sp., *Sciadicleithrum variabilum* (3) species were detected in aforesaid studies (4-11).

The aim of the present study was to determine the prevalence of parasitic infections in aquarium fish sold in local pet shops.

METHODS

The fish samples used in the study were collected from pet shops located in Kırıkkale. Approvals for the collection of samples and the carrying out the study were obtained from Kırıkkale University Animal Experiments Local Ethics Committee (E-608221397-010.99-74220 letter). Fish samples were taken with the approval of the pet shop owner. Petshops were visited on a daily basis, and the dead fish in the aquariums were duly delivered to Kırıkkale University Faculty of Veterinary Medicine, Routine and Epidemiology Laboratory in separate containers. Totally 502 dead fish were collected from 7 pet shops during the study period. Such dead fish belonged to goldfish (*Carassius auratus*), Lepistes (*Poecilia reticulata*), angelfish (*Pterophyllum scalare*), Beta fish (*Betta splendens*), stringray fish (*Hypostomus* sp.), molly fish (*Poecilia sphenops*), neon tetra fish (*Paracheirodon innesi*) and cichlid fish (Cichlidae) species.

The preparations were prepared by taking scrapings from the gills, fins, skin, intestines, and internal organs, separately through a slide from the fish delivered to the laboratory on the same day. The preparations were examined under the light microscope and identified at the genus level using the relevant literature, which was positive for parasites (12,13).

Statistical Analysis

All data were analyzed with frequency table. SPSS (IBM SPSS for Windows ver. 22) statistical package program was used for analysis. Infection rates are calculated as a percentage.

RESULTS

Among 502 fish examined during the study, 62.7% of these fish were found infected at least with one parasite types. Totally 19 parasite species were detected in these fish. Other species detected were protozoans including *Chilodonella* sp., *Trichodina* sp., *Piscinoodinium* sp., *Ichthyobodo* sp., *I. multifiliis*, *Apiosoma* sp., *Epistylis* sp., *Vorticella* sp.; helminths including *Gyrodactylus* sp., *Dactylogyrus* sp., *Capillaria* sp., *Camallanus* sp.; metacercariae, and nematode larvae; arthropods including *Argulus* sp., Rotifera including *Philodina* sp., *Euchlanis* sp., annelidae including *Aelosoma* sp., and a free-living ciliata, *Tetrahymena* sp. (Table 1).

The fish which were infected with a single parasite was 28.9% of the fish examined and 46% of the fish that were positive for

Table 1. Parasites detected in aquarium fish and their rates

Parasite	Number of infected fish	Ratio of positive samples to the number of fish examined (%)	Ratio of parasite species in positive fish (%)
Chilodonella sp.	192	38.2	61.0
Trichodina sp.	83	16.5	26.3
Gyrodactylus sp.	73	14.5	23.2
Dactylogyrus sp.	69	13.7	21.9
Piscinoodinium sp.	44	8.8	14.0
Ichthyobodo sp.	42	8.4	13.3
I. multifiliis	4	0.8	1.3
Capillaria sp.	2	0.4	0.6
Camallanus sp.	1	0.2	0.3
Apiosoma sp.	5	1	1.6
Vorticella sp.	1	0.2	0.3
Philodina sp.	64	12.7	20.3
Euchlanis sp.	23	4.6	7.3
Argulus sp.	1	0.2	0.3
Tetrahymena sp.	5	1	1.6
Metacercaria	4	0.8	1.3
Nematode larvae	7	1.4	2.2
Aelosoma sp.	2	0.4	0.6
Epistylis sp.	1	0.2	0.3

parasites. Among the fish samples, 18.3% were found infected with two parasite types, 7.0% were found infected with three parasite types, 5.4% were found infected with four parasites, 2.8% were found infected with five parasites, and 0.4% were found infected with six parasites. Majority of the fish that were positive for the parasite were infected with a single agent (Table 2).

Chilodonella sp., *Trichodina* sp., *Piscinoodinium* sp., *Ichthyobodo* sp., *Ichthyophthirius multifiliis*, *Gyrodactylus* sp., *Dactylogyrus* sp., Nematoda larvae, metacercaria, *Vorticella* sp., *Philodina* sp., *Euchlanis* sp., and *Aelosoma* sp. were detected from gill samples; *Trichodina* sp., *Piscinoodinium* sp., *Chilodonella* sp., *Capillaria* sp., *Camallanus* sp., and nematod larvae from viscera and intestinal samples; *Chilodonella* sp., *Trichodina* sp., *Piscinoodinium* sp., *Ichthyobodo* sp., *I. multifiliis*, *Apiosoma* sp., *Epistylis* sp., *Gyrodactylus* sp., *Dactylogyrus* sp., *Argulus* sp., *Philodina* sp., *Euchlanis* sp., *Tetrahymena* sp. from skin scratces were detected (Figure 1).

DISCUSSION

Aquarium and aquarium fishing has become a hobby for people today and a sector where significant gains are made commercially. Therefore, the number of studies on aquarium fish breeding and diseases is increasing in many parts of the world as well as Türkiye. Parasitic diseases are the diseases that are emphasized a lot because they may spread in a short time in aquarium fish and cause symptoms that may lead to the death of the fish.

Studies have been carried out on parasites of aquarium fish in different parts of the world in recent years. The presence of parasites at different rates was determined in those studies. The rate of parasitic infection was found between 43.3 and 90.90% in studies conducted on aquarium fish in Türkiye (4,9). The rate of parasitic infection in ornamental fish was found between 69.1% and 100% (14,15) in Pakistan, 22.5% (16) in Brazil, and between 26.33% and 95.0% in Iran (17,18). At least one parasite species was found in 62.7% of the aquarium fish examined in our study. This rate is between the rates in studies conducted in Türkiye and in different regions of the world. Different results between studies may depend on many factors, including the number and type of fish examined, the maintenance conditions of aquariums and ornamental ponds, the administration of antiparasitic treatment, and the cleanliness of the aquarium water.

The rate of *Chilodonella* sp. was found between 26% and 51% in studies conducted in different regions (19-21). *Chilodonella* sp. was found in scratched skin samples collected from the gills, fins and scales of all fish species examined in this study. The rate of

Table 2. Parasite infection rates detected in aquarium fish

	Positive						**Negative**
	Only	**Mix**					
	One parasite	**Two parasite**	**Three parasite**	**Four parasite**	**Five parasite**	**Six parasite**	
No	145	92	35	27	14	2	187
In positive fish (%)	46.0%	29.2%	11.1%	8.6%	4.4%	0.6%	0%
In all fish (%)	28.9%	18.3%	7.0%	5.4%	2.8%	0.4%	37.3%
Total	145 (28.9%)	170 (33.9%)					187 (37.3%)
	315 (62.7%)						187 (37.3%)

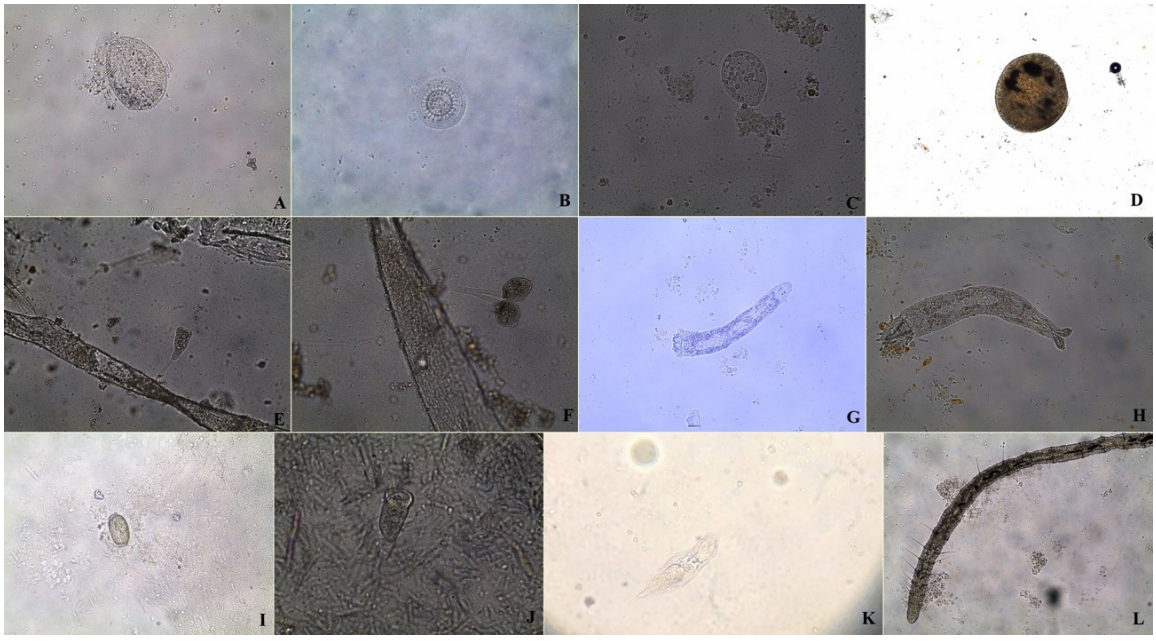

Figure 1. Some parasites species detected in examined fish, A: *Chilodonella* sp., B:*Trichodina* sp., C: *Piscinoodinium* sp., D: *I. multifiliis* E: *Apiosoma* sp., F: *Epistylis* sp., G: *Dactylogyrus* sp., H: *Gyrodactylus* sp., I: *Capillaria* sp. egg, J: *Philodina* sp., K: *Euchlanis* sp., L: *Aelosoma* sp.

Chilodonella sp. was determined as 38.2%. Such rate is similar to those obtained in previous studies. The most common parasite detected in this study was *Chilodonella* sp.

Trichodina sp. are protozoan parasites which are common in aquarium and ornamental fish. Studies conducted globally detected a rate between 3% and 26.6% (4,20,21). *Trichodina* sp. was found in gill, skin scraping, and visceral samples of our study. The rate of *Trichodina* sp. detected in this study was 16.5%. Such rate is similar to those obtained in previous studies conducted on ornamental fish.

Ichthyophthirius multifiliis is one of the protozoan parasites with the highest distribution in the world (18). In our study, the rate of *I. multifiliis* was determined as 0.8%. The rate of *I. multifiliis* was reported between 2.8% and 8% in previous studies in Türkiye and the world (4,21). The reason for lower rates detected in our study when compared to previous studies may be due to the fact that the factor is well known by the producers and that protective measures are taken for it.

The rate of *Piscinoodinium* sp. was reported as 8.8% in this study. The presence of this parasite including *Oodinium* and *Piscinoodinium* has been reported in aquarium and ornamental fish. It has been reported that 6% of *Piscinoodinium* is observed on the gills of guppy fish in the medan region of Indonesia (22). Kayıs et al. (9) reported that they detected *P. pillulare* on the skin of Beta fish for the first time in Türkiye. Florindo et al. (23) reported that the most common species in all farms was *P. pillulare* when they examined the ornamental fish from different breeding farms in Brazil. Furthermore, it has been reported with a rate of 16.7% in goldfish in Brazil (24). The reason for the higher prevalence of *Piscinoodinium* sp. in this study may be associated with the poor maintenance conditions of one of the pet shops and the higher rate of *Piscinoodinium* in the samples collected from this pet shop.

Ichthyobodosis which is caused by severe infections of the skin and gills of parasitic flagellates of the genus *Ichthyobodo* is an important parasitic disease that causes serious losses among ornamental and farmed fish worldwide (25). The rate of *Ichthyobodo* (*Costia*) sp. was found between 1.7% and 27.6% in studies conducted to date (9,19-21,26); however, it was found 8.4% in this study. The rate in our study is similar to previous studies.

Apiosoma species are resident ciliates that cause infection when present in large numbers, are located on the gills, skin and fins of fish, and are commonly detected in fish grown in ponds (27). The rate was detected 1% in our study. It has been reported that it is detected in 6% of goldfish in Brazil (24). It has been reported on the skin of aquarium fish in the Rize region of Türkiye (28). In our study, the parasite was found in samples collected from both gills and skin, and fins.

The *Epistylis* is a pedicellate ciliate that attaches to the skin or fins of the host (27). It has been detected in 100% of skin scrapings of goldfish in Brazil. However, it has been reported that no causative agent was found in the gills (24). Kayıs et al. (9) reported that the agent was found in gill and skin scrapings; Iqbal and Haroon (14) reported that they were found only in gills. The rate of the parasite found in the scraping preparation taken from the skin and fins was 0.2%.

Vorticella sp., a ciliate from the Ciliophora branch, has been detected in several studies conducted in Türkiye (2,10,21). The rate of *Vorticella* sp. in a study conducted in Tetra and stingray fish in Konya was found 1% (21) in average, and 2% in tetra fish. Isık et al. (10) reported that *Vorticella* sp. was detected on only the gills of discus fish. Bulguroğlu (2) reported that *Vorticella* sp. was detected on the skin of yellow princess, blue princess, ahli cichlid, velifera and white mole fish. In this study, *Vorticella* sp. was found in a gill sample of a fish.

Tetrahymena sp. is considered an important pathogen of ornamental fish and causes significant death in these fish. These parasites are known as guppy disease because they cause infection, especially in guppy fish; however, their presence was detected in different ornamental fish species other than guppies. *Tetrahymena* sp. was detected on the gill and skin of 7 discus fish examined in Konya province of Türkiye (10). It was found at a rate of 11 (21) in tetra fish in another study conducted in the same shade. The rate of *Tetrahymena* was reported 1% in the aforesaid study. The cause for that may be due to the different fish species studied and the low rate of guppies.

It is noted that the most common monogenean trematodes are *Gyrodactylus* sp. and *Dactylogyrus* sp. species. In studies carried up to date, the rate of *Gyrodactylus* sp. was detected between 1% and 40% (4,19-22,29), and *Dactylogyrus* sp. was detected between 1% and 28% (4,19-22,29). Such rates were 14.5% and 13.7%, respectively in this study. *Dactylogyrus* and *Gyrodactylus* species may spread rapidly among aquarium fish under stressful situations. Therefore, it is expected to be more common in poor care conditions, transportation, and situations that may cause stress. The reason for the different rates between studies unequal conditions in all aquariums.

Capillaria sp. is one of the most common nematode parasites of aquarium fish. The rate was reported 0.9% (4) in previous studies conducted in Türkiye. Dewi et al. (22) detected in the abdominal cavities of guppy and goldfish as 8% and 4%, respectively, in Indonesia. In a study conducted in Iran, *Capillaria* sp. rate was reported as 0.33% in aquarium fish (18). Adult female parasite and *Capillaria* sp. egg were detected in 2 (0.4%) of 502 fish examined in our study. The rate of *Capillaria* sp. detected in our study is similar to the studies conducted in Ankara and Iran.

The rate of *Camallanus* sp. was detected as 0.4% (4) in Türkiye. However, it was reported that *Camallanus* was detected in a guppy fish in Afyon (30). It was reported in Brazil that adult *C. cotti* (31) parasites were found in guppy and beta fish, and adult *C. maculatus* parasite in plati fish (32). In this study, adult *Camallanus* sp. was found in the gut of one fish (0.2%).

Aeolosoma sp. is a freshwater annelid. The causative agent was found in the gill samples of two (0.4%) fish examined in this study. In a previous study conducted in the Konya region on total tetra and stingray fish, it was found only in one stingray fish with a rate of 1% (21). It was considered that the different number and species of fish examined may have caused these rates to differ from each other.

Whether rotifers are true parasites are debatable. It was reported in studies conducted on aquarium and ornamental fish that rotifera were rarely detected. In this study, the rate of *Euchlanis* sp. was 4.6%, whereas the rate of *Philodina* sp. was found 12.7%. *Euchlanis* sp. and *Philodina* sp. were found in gill and skin samples. Philodina species were previously found in the gills of discus fish in Türkiye (10). *Euchlanis* sp. was reported as 5% in the study conducted on tetra and stingray fish (21). The rate of *Euchlanis* sp. was found similar to previous studies. However, *Philodina* sp. was detected higher than previous studies. The reason for that was

thought to be the cleanliness of the aquarium water and the poor maintenance conditions.

Argulus sp. is known as fish lice. It parasitizes on both marine and freshwater fish, and it is an arthropod that may cause infestations with higher morbidity and mortality under severe infections. The presence of *A. japonicus* species was reported in three goldfish in Texas (33). It was reported that *A. foliaceus* was found in the same fish species in Iran (34) and Pakistan (14). It was reported that *A. foliaceus* species was found in the skin and gills of an astronaut fish (*Astronotus ocellatus*) (7), *A. japonicus* species was found in 33% (35) koi fish and 28% in telescope fish (36) in Türkiye. One adult *Argulus* sp. was detected in only one (0.2%) of 502 fish samples examined in our study. It was considered in our study that the reason for the low incidence of the agent was recognition of the agent by the aquarium owners because they could see it macroscopically and started to struggle as soon as they saw it.

In our study, at least one and at most 6 different parasite species were found on the infected fish. It was determined that most of the fish that were positive for parasites were infected with a single parasite species (28.9%). This was followed by the fish infected with two (18.3%), three (7.0%), four (5.4%), five (2.8%), and six (0.4%) different parasite species. Doganay et al. (4) reported in their study conducted in Ankara that there were fish infected with at most 5 species at the same time, and they also reported that the fish they examined were generally infected with a single species (21.4%). Moyses et al. (24) reported in their study on goldfish in Brazil that 30% (9/30) of the fish had 2 or 3 parasite species, 40% (12/30) had 4 parasite species, and 30% (9/30) 5 had multiple infestations in the gills and/or skin, with one or more parasite species.

CONCLUSION

Consequently, the rate of parasitic infection was found higher in the fish examined in our study. The aquarium owners should pay attention to the cleanliness of the aquarium water in order to reduce the rate of parasitic infection in fish; when new fish are purchased, they should be taken from reliable sources, and the quarantine process should be observed.

* Ethics

Ethics Committee Approval: Approvals for the collection of samples and the carrying out the study were obtained from Kırıkkale University Animal Experiments Local Ethics Committee (E-608221397-010.99-74220 letter).

Informed Consent: A patient consent form is not required as the study was conducted on dead or dying fish.

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* Authorship Contributions

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

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COVID-19 Pandemisinin Kistik Ekinokokkoz İndirekt Hemaglütinasyon Test Dinamikleri Üzerindeki Etkisinin Değerlendirilmesi: Tek Merkez Deneyimi

Evaluation of the Impact of the COVID-19 Pandemic on Cystic Echinococcosis Indirect Hemagglutination Test Dynamics: A Single-center Experience

Özlem Ulusan Bağcı

İzmir Katip Çelebi Üniversitesi, Atatürk Eğitim ve Araştırma Hastanesi, Tıbbi Mikrobiyoloji Laboratuvarı, İzmir, Türkiye

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ÖZ

Amaç: Kistik ekinokokkoz (KE) Dünya Sağlık Örgütü tarafından açıklanan ihmal edilmiş tropikal hastalıklardan biridir. Koronavirüs hastalığı-2019 pandemisiyle birlikte girilen süreçte bu tür hastalıklarla mücadele daha da zorlaşmıştır. Çalışmamızda pandemi öncesinde ve sürecinde KE indirekt hemaglütinasyon testi (IHA) sayılarını ve sonuçlarını değerlendirerek, pandeminin hastalık tanısı üzerindeki etkileri hakkında çıkarımlarda bulunmayı amaçladık.

Yöntemler: Ülkemizde ilk olgunun görüldüğü 11 Mart 2020 tarihinden önceki ve sonraki 30 aylık süreçte IHA test istem sayıları ve pozitiflik oranları geriye dönük olarak değerlendirildi. İstatistiksel analizler SPSS versiyon 23 (SPSS, Chicago, IL, ABD) programı kullanılarak yapıldı.

Bulgular: Pandemi öncesinde 1444 ve pandemi döneminde 870 hastaya ait sonuç incelenmiştir. Pandemiden önce %18,49, pandemi sürecinde 14,6 olarak saptanan IHA pozitiflik oranları arasındaki fark istatistiksel olarak anlamlı bulunmuştur (p=0,016). Kadın ve erkeklerde pozitiflik oranları her iki dönemde de istatistiksel olarak benzer saptanmıştır ($p_{önce}$=0,621, p_{sonra}=0,238). IHA pozitiflik oranının en fazla saptandığı yaş grubu her iki dönemde de 20-39 olup, yaş gruplarının pozitiflik oranları arasındaki fark istatistiksel olarak anlamlı bulunmuştur (p<0,001).

Sonuç: Pandemi döneminde IHA pozitiflik oranında anlamlı bir azalma görülmüştür. IHA test istem sayısında belirgin bir düşüş gözlenmesine rağmen, pozitiflik oranlarında artış saptanmaması; bazı hastalarda tanının atlanmış olabileceğini veya hastaların takiplerinde aksamalar yaşanmış olabileceğini düşündürmektedir. Sonuç olarak ülkemiz için önemli bir halk sağlığı problemi olan KE mücadelesine başarıyla devam edebilmek adına farkındalık eğitimleriyle birlikte erken tanı ve düzenli takiplerin önemi vurgulanmalı; laboratuvar-klinik hekimleri arası iletişim güçlendirilerek testlerin daha etkili kullanılması sağlanmalıdır.

Anahtar Kelimeler: COVID-19, indirekt hemaglütinasyon testi (IHA), kistik ekinokokkoz (KE), pandemi

ABSTRACT

Objective: Cystic echinococcosis (CE) is one of the neglected tropical diseases announced by the World Health Organization. In the period entered with the Coronavirus disease-2019 pandemic, the fight against such diseases has become even more difficult. In our study, we aimed to make inferences about the effects of the pandemic on the diagnosis of the disease by evaluating the number and results of CE indirect hemagglutination test (IHA) before and during the pandemic.

Methods: The number of IHA test requests and positivity rates in the 30-month periods before and after March 11, 2020, when the first case was seen in our country, were evaluated retrospectively. Statistical analysis was made with SPSS version 23 (SPSS, Chicago, IL, USA) program.

Results: The results of 1444 patients before the pandemic and 870 patients during the pandemic period were examined. The difference between IHA positivity rates, which was found to be 18.49% before the pandemic and 14.6% during the pandemic, was statistically significant (p=0.016). The positivity rates of women and men were found to be statistically similar in both periods

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Yazar Adresi/Address for Correspondence: Özlem Ulusan Bağcı, İzmir Katip Çelebi Üniversitesi, Atatürk Eğitim ve Araştırma Hastanesi, Tıbbi Mikrobiyoloji Laboratuvarı, İzmir, Türkiye
Tel/Phone: +90 539 860 03 31 **E-Posta/E-mail:** drozlemulusan@gmail.com **ORCID ID:** orcid.org/0000-0002-9695-5703

Türkiye PARAZİTOLOJİ Dergisi

(p_{before}=0.621, p_{after}=0.238). The age group with the highest IHA positivity rate was 20-39 in both periods, and the difference between the positivity rates of the age groups was statistically significant (p<0.001).

Conclusion: A significant decrease was observed in the rate of IHA positivity during the pandemic period. The status of no increase in positivity rates despite a significant decrease in IHA tests makes us think that the diagnosis may be missed in some patients or that there could be disruptions in their follow-up. For this reason, in order to continue the fight successfully against CE, which is an important public health problem for our country, early diagnosis and regular follow-ups should be emphasized with educations, and the laboratory-clinician communication should be strengthened in order to use tests more efficiently.

Keywords: COVID-19, indirect hemagglutination test (IHA), cystic echinococcosis (CE), pandemic

GİRİŞ

Ekinokokkoz, insanlarda etken *Echinococcus* spp.'nin larval (metasestod) formlarının neden olduğu; karaciğer ve akciğerler başta olmak üzere, kemik, böbrek, dalak, kalp, beyin gibi birçok organın kan yoluyla tutulabildiği; kronik ve sakatlayıcı bir hastalıktır. Etkenin dört türü olmasına rağmen, insanlardaki enfeksiyondan daha çok *Echinococcus granulosus* ve *Echinococcus multilocularis* türleri sorumludur (1). Diğer türler insan için daha az patojeniktir ve insanlarda enfeksiyona nadiren neden olmaktadır (2). Daha yaygın görülen *E. granulosus*'un kesin konağı köpekler iken, ara konağı koyunlar başta olmak üzere otçul hayvanlardır. İnsanlara bulaş tesadüfi olarak, parazit yumurtası ile kontamine olmuş yiyeceklerin iyi yıkanmadan tüketilmesi veya köpek kıllarında bulunan yumurtaların alınması sonucu gerçekleşmektedir. Hastalığın tanısında sıklıkla radyolojik yöntemlerden faydalanılmaktadır. Serolojik yöntemlerden indirekt hemaglütinasyon testi (IHA), ELISA ve indirekt immünofloresan antikor testine genellikle tanıya yardımcı olarak veya takip amacıyla başvurulmaktadır (3).

Ekinokokkoz genellikle yoksul toplulukları etkilemekte ve diğer enfeksiyon etkenleri ile birlikte görüldüğünde toplumları sağlık ve sosyo-ekonomik açıdan olumsuz etkilemektedir (4). Hastalığın görülme sıklığı, ülkeden ülkeye, bölgeden bölgeye belirgin farklılık gösterse de ortalama olarak her 100.000 kişiden 1-200'ünün hayatının bir döneminde etkenle karşılaştığı bilinmektedir (5). Hastalığın endemik olarak seyrettiği Orta Asya, Çin, Doğu Afrika, Peru, Arjantin gibi ülkelerde, %5-10'a varan prevalans oranlarından bahsedilmektedir. Dünya Sağlık Örgütü (DSÖ) dünyada bir milyondan fazla olgu olduğunu ve her yıl 19.300 kişinin kistik ekinokokkoz (KE) nedeniyle hayatını kaybettiğini bildirmektedir. Güney Amerika'daki mezbahalarda hayvanların %20-95'inin hastalıktan etkilendiği raporlanmıştır (6). Ülkemiz de ekinokokkoz için endemik ülkelerden olmasına rağmen, hastalığın ülkemizdeki prevalansı ile ilgili bilgilerimiz geriye dönük olarak yapılan çalışmalarla sınırlıdır. Bildirimi zorunlu bir hastalık olmasına rağmen, bakanlığa bildirimlerin gerçek sayının oldukça altında olduğu düşünülmektedir (7). Gerek dünyada gerekse ülkemizde bu kadar sık görülen, ciddi morbiditeler, ölüm ve ekonomik kayıplara yol açan ekinokokkoz DSÖ tarafından ilan edilmiş 20 ihmal edilmiş tropikal hastalıktan bir tanesidir (8,9).

Aralık 2019'da Çin'de başlayan Koronavirüs hastalığı-2019 (COVID-19) salgını, ülkemizde de ilk olgunun görüldüğü 11 Mart 2020 tarihinde küresel pandemi olarak ilan edilmiştir. Bu süreçten itibaren sağlık sisteminin tüm ilgisinin pandemiyle mücadeleye aktarılması, mevcut diğer hastalıkların ihmal edilmesine neden olmuştur. DSÖ Nisan 2020'de ihmal edilmiş tropikal hastalıklar için uygulanan kitlesel ilaç profilaksisi, aktif olgu sürveyansı ve toplum taramalarında oluşabilecek COVID-19 bulaşının önüne geçebilmek için bu uygulamalara ara verme kararı almıştır (10,11). Takip eden süreçte bu hastalıkların insidansında ve mortalite oranlarında artış saptanmıştır (12,13). Önümüzdeki beş yıllık süreçte insan bağışıklık yetmezliği virüsü, tüberküloz ve sıtma nedeniyle ölümlerin sırasıyla %10, %20 ve %36 oranlarında artacağı tahmin edilmektedir (14). Ancak literatür tarandığında COVID-19'un KE üzerindeki etkisini araştıran bir çalışmaya rastlanılamamıştır. Pandemi sürecinde hastanelerde COVID-19 tanısında kullanılan testlere öncelik verilmesi nedeniyle parazitolojik tanısal testler dahil COVID-19 dışı laboratuvar test istem sayılarında azalma olduğu göze çarpmaktadır (15,16). Bu durumun hastalıkların tanısının atlanmasına veya takiplerinde aksamalar yaşanmasına neden olacağı düşünülmektedir. Biz de benzer bir gözlemden yola çıkarak pandemiden önce ve pandemi döneminde laboratuvarımıza gelen KE IHA test sayılarını, pozitiflik oranlarını değerlendirmeyi ve pandeminin KE tanısı üzerindeki olası etkileri hakkında çıkarımlarda bulunmayı hedefledik.

YÖNTEMLER

Çalışmamızda ülkemizdeki ilk COVID-19 olgusunun görüldüğü 11 Mart 2020 tarihinden önceki ve sonraki süreç, IHA sayıları ve pozitiflik yüzdeleri açısından geriye dönük olarak değerlendirildi. Aynı hastaya ait birden çok örneğin gelmesi durumunda sadece ilk örnek değerlendirmeye alındı. Hastanın negatif ve pozitif sonuçlarının olması durumunda ise pozitif olan ilk örnek çalışmaya dahil edildi.

Hastanemizde KE tanısı için serumda antikor saptayan ticari bir kit (Hydatidose, Fumouze laboratoires, France) kullanılmaktadır. Test 1/80'den 1/2500'e kadar dilüe edilen serum dilüsyonlarında çalışılmaktadır. U tabanlı mikroplaklarda dilüe edilmiş serumlar üzerine antijenli eritrosit süspansiyonu eklendiğinde belirli bir inkübasyon dönemi sonunda eritrositlerin yer çekimi etkisiyle kendiliğinden çökmesi durumunda kuyucuk negatif, çökelti olmaması veya dantela gibi düzensiz çökelti olması durumunda ise pozitif olarak değerlendirilmektedir. 1/2500'de pozitif sonuç alınması durumunda serum dilüsyonu 1/10000'e kadar ilerletilmektedir. Testin prospektüsüne göre; 1/320 ve üzerindeki değerler pozitif, 1/160 şüpheli sonuç, 1/80 ve altındaki değerler negatif olarak raporlanmaktadır. Antikor titresi 1/160 saptandığında 2-3 hafta sonra istenen yeni bir serum örneğiyle antikor titresindeki değişim incelenmektedir. Ancak çalışmamızda karşılaştırmaların kolay yapılabilmesi için 1/160 ve altındaki değerler negatif kategorisine dahil edilmiştir.

İstatistiksel Analiz

Çalışmada nicel veriler için ortalama ± standart sapma, nitel veriler için sayı ve yüzdeler verildi. İstatistiksel analizler SPSS versiyon 23 (SPSS, Chicago, IL, ABD) programı kullanılarak yapıldı. Nicel verilerin karşılaştırılması için verilerin normal dağılıma uygunluğuna göre Student's t-test veya Mann-Whitney

U testleri; nitel verilerin karşılaştırılması için ki-kare ve Fisher'in kesin testleri kullanıldı. Sonuçlar %95'lik güven aralığında, p<0,05 anlamlılık düzeyinde değerlendirildi.

Etik Kurul Onayı

Çalışma için İzmir Katip Çelebi Üniversitesi Girişimsel Olmayan Klinik Araştırmalar Etik Kurulu'ndan 20/10/2022 tarihli ve 0438 numaralı etik kurul onayı alınmıştır.

BULGULAR

Laboratuvarımızda ülkemizde ilk COVID-19 olgusunun görüldüğü 11 Mart 2020 tarihinden önceki 30 aylık süreç olan 10 Eylül 2017-10 Mart 2020 tarihleri arasında 840 (%58,16) tanesi kadınlara, 604 (%41,84) tanesi erkeklere ait 1444 IHA örneği değerlendirilmiştir. Erkeklerin yaş ortalaması 52,76±17,3 iken, kadınlarınki 52,10±15,5 olup; hastalar yaş ortalamaları açısından benzer bulunmuştur (p=0,442). Pozitiflik oranı kadınlarda, erkeklerde ve tüm hastalarda sırasıyla %18,93, %17,88 ve %18,49 olarak saptanmıştır. Kadınlar ve erkeklerin pozitiflik oranları arasındaki fark istatistiksel olarak anlamlı değildir (p=0,631). Hastalar 0-19, 20-39, 40-59, 60-79, 80 ve üzeri olmak üzere beş ayrı yaş kategorisine ayrılarak değerlendirilmiştir. Pozitiflik oranı en yüksek %31,96 ile 20-39 yaş grubunda iken, en düşük %9,8 ile 80 ve üzeri yaş grubunda olup; yaş grupları arasındaki fark istatistiksel olarak anlamlı bulunmuştur (p<0,001).

Pandemi süreci olan 11 Mart 2020-11 Eylül 2022 tarihleri arasındaki 30 aylık dönemde ise 530'u (%60,92) kadın, 340'ı (%39,08) erkek olmak üzere toplam 870 hastaya ait örnek değerlendirilmiştir. Erkeklerin ve kadınların yaş ortalaması sırasıyla 51,66±16,65 ve 51,54±15,21 olup, istatistiksel açıdan fark bulunmamıştır (p=0,914). Kadın hastalarda IHA pozitiflik oranı %13,40 iken, erkek hastalarda %16,47'dir. Kadın ve erkeklerin IHA pozitiflik oranları arasındaki fark istatistiksel olarak anlamlı değildir (p=0,238). Tüm hastalardaki pozitiflik oranı %14,6'dır. Yaş gruplarının IHA pozitiflik oranları karşılaştırıldığında en yüksek pozitiflik oranı %30,16 ile 20-39 yaş grubundayken, en düşük pozitiflik oranı %8,42 ile 60-79 yaş grubundadır. Yaş gruplarının pozitiflik yüzdeleri arasındaki fark istatistiksel olarak anlamlı bulunmuştur (p<0,001).

Eylül 2017 ile 2022 tarihleri arasındaki beş yıllık süreçte 1370'i (%59,2) kadın ve 944'ü (%40,8) erkek olmak üzere 2314 hastaya ait örnek değerlendirilmiştir. Yaş ortalaması erkeklerde 52,37±17,7, kadınlarda 51,88±15,39 olup; istatistiksel olarak benzer bulunmuştur (p=0,478). Kadın hastalarda pozitiflik oranı %16,79, erkek hastalarda %17,37'dir. Cinsiyet grupları arasında pozitiflik oranları açısından istatistiksel olarak anlamlı fark yoktur (p=0,736). En yüksek pozitiflik oranı %31,25 ile 20-39 yaş grubunda, en düşük pozitiflik oranı ise %10,14 ile pandemi öncesi döneme benzer olarak 80 yaş ve üzeri hastalardadır (p<0,001). IHA pozitiflik oranlarının cinsiyetlere göre değişimi Tablo 1'de, yaş gruplarına göre değişimi ise Tablo 2'de verilmiştir.

TARTIŞMA

İhmal edilmiş tropikal hastalıklar; çoğunluğu Afrika, Asya ve Amerika'da 149 ülkede yaşayan bir milyardan fazla insanı etkileyen ve her yıl 500.000'den fazla insanın ölümüne neden olan 20 hastalığı kapsamaktadır (8). Bu hastalıklar özellikle toplumdaki yoksul insanlarda görülen, eski çağlardan beri var olan ve her yıl milyarlarca dolar tutarında ekonomik kayıplara neden olan hastalıkları temsil eder (17). Bu grupta toplam 12 adet paraziter hastalık bulunmaktadır: Chagas hastalığı, drakunkuliyaz, ekinokokkoz, gıdalarla bulaşan trematod enfeksiyonları, Afrika uyku hastalığı, leyşmanyoz, lenfatik filaryoz, onkoserkiyaz, skabiyez, şistozomiyaz, topraktan bulaşan helmintler, tenya ve

Tablo 1. IHA pozitiflik oranlarının cinsiyetlere göre dağılımı

Yıllar	Cinsiyet				Toplam n (%)	
	Kadın n (%)		Erkek n (%)			
	Pozitif	Toplam	Pozitif	Toplam	Pozitif	Toplam
2017-2020	159 (18,93)	840 (58,17)	108 (17,88)	604 (41,83)	267 (18,49)	1444 (100)
2020-2022	71 (13,40)	530 (60,92)	56 (16,47)	340 (39,08)	127 (14,60)	870 (100)
Toplam	230 (16,79)	1370 (59,20)	164 (17,37)	944 (40,80)	394 (17,03)	2314 (100)

IHA: İndirekt hemaglütinasyon testi

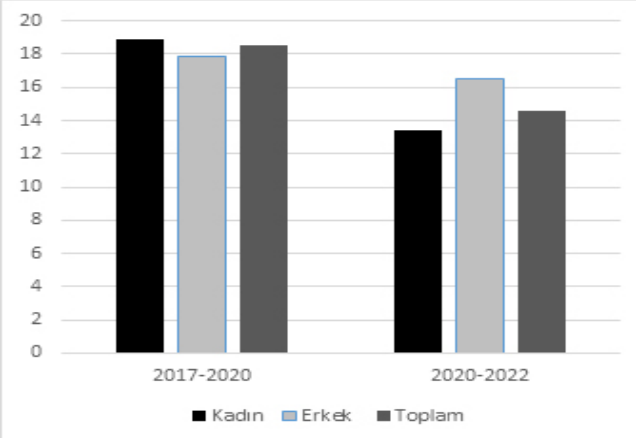
Tablo 2. IHA pozitiflik oranlarının yaş gruplarına göre dağılımı

Yaş aralıkları	2017-2020		2020-2022	
	Pozitif n (%)	Toplam n (%)	Pozitif n (%)	Toplam n (%)
0-19	8 (24,24)	33 (2,29)	2 (16,67)	12 (1,38)
20-39	93 (31,96)	291 (20,15)	57 (30,16)	189 (21,72)
40-59	92 (15,44)	596 (41,27)	42 (11,48)	366 (42,07)
60-79	69 (14,59)	473 (32,76)	24 (8,42)	285 (32,76)
80 ve üzeri	5 (9,8)	51 (3,53)	2 (11,11)	18 (2,07)
Toplam	267 (18,49)	1444 (100)	127 (14,60)	870 (100)

IHA: İndirekt hemaglütinasyon testi

sistiserkozlar (8). Son 20 yıldır DSÖ bu hastalıklarla mücadele etmek için kitlesel ilaç profilaksisi ve toplum taramaları gibi kampanyalar düzenlemektedir. 30 Ocak 2020'de gerçekleştirilen 1. Dünya İhmal Edilmiş Tropikal Hastalıklar Günü'nde, uygulanan politikaların tüm dünyayı kapsaması gerektiği vurgusu yapılmıştır. Ayrıca DSÖ önümüzdeki 10 yıllık süreçte paraziter hastalıkların kontrol altına alınmasını, hatta mümkünse eliminasyonunu hedeflemiş olup, bunun için tanısal testlerin önemini vurgulamıştır (11). Ancak COVID-19 pandemisinin başlamasıyla birlikte DSÖ, pandemi bitene kadar ihmal edilmiş paraziter hastalıklarla ilgili uygulamaları bir kenara bırakarak, tüm ilgisini pandemiyle mücadeleye yöneltmiştir (10). Bu durumun paraziter hastalıkların görülme sıklığı ve mortalite oranları üzerinde olumsuz etkileri olacağı açıktır. Sıtma çalışmalarında mücadeledeki bu duraksamanın sıtma mücadelesinde elde edilen başarılarda gerilemeye yol açtığı gösterilmiştir. Sıtma çalışmalarında, bu duraksamanın sıtma mücadelesinde elde edilen başarılarda gerilemeye yol açtığı gösterilmiştir (12). Yine benzer olarak kitlesel ilaç profilaksi uygulamasına ara verilmesinin, şistozomiyaz için konulan hedeflere ulaşmada ortalama iki yıllık bir gecikmeye neden olacağı hesaplanmıştır (13). Literatür tarandığında pandeminin ekinokokkoz üzerindeki etkilerini araştıran bir çalışmaya rastlanmamıştır. Biz de çalışmamızda buradan yola çıkarak ihmal edilmiş tropikal hastalıklardan biri olan ekinokokkoz tanısında kullanılan IHA testinin pandemiden önce ve pandemi dönemindeki sonuçlarını değerlendirmeyi amaçladık.

Pandemi sürecinde KE IHA testi istem sayılarında belirgin bir azalma görülmüştür. Pandemi öncesi dönemde %18,49 saptanan IHA pozitiflik oranı, pandemi döneminde %14,6'ya gerilemiştir ve aradaki fark istatistiksel olarak anlamlı bulunmuştur (p=0,016) (Grafik 1). IHA test istem sayısında belirgin bir düşüş gözlenmesine rağmen, pozitiflik oranlarında artış saptanmaması; bazı hastalarda tanının atlanmış olabileceğini veya hastaların takiplerinde aksamalar yaşanmış olabileceğini düşündürmektedir. Bu durum özellikle kadın hastalarda daha belirgin olarak göze çarpmaktadır. Pandemi öncesi dönemde genel literatürle uyumlu olarak pozitiflik oranı kadınlarda yüksek iken (18,19), pandemi sürecinde erkeklerde daha yüksek saptanmıştır. Kadınlarda pozitiflik oranı %18,93'ten, %13,4'e gerilemiş olup (Grafik 1), aradaki fark istatistiksel olarak anlamlı bulunmuştur (p=0,008). Bu durum bize hastalıkla mücadele etmek için yol



Grafik 1. IHA pozitiflik oranlarının yıllara göre değişimi
IHA: İndirekt hemaglütinasyon testi

haritası çizerken hedef popülasyonda kadınların ön planda yer alması gerektiğini düşündürmektedir. Çalışmamızda pandemi döneminin IHA pozitiflik oranının en fazla görüldüğü yaş grubuna ise etkisi olmadığı görülmektedir. En yüksek pozitiflik oranına sahip yaş grubu hem pandemi öncesinde ve hem de pandemi sürecinde 20-39 olarak saptanmıştır. Literatür tarandığında çalışmalarda pozitifliğin en fazla saptandığı yaş grubunun değişkenlik gösterdiği görülmektedir. Bazı çalışmalar pozitifliğin 40 yaş üzerinde daha fazla görüldüğünü belirtirken (20-22), bazı çalışmalar da 20-40 yaş aralığını işaret etmektedir (19,23). Çalışmamızda IHA pozitiflik oranının 20-39 yaş grubunda daha yüksek saptanması, hedef popülasyon belirlenirken yol gösterici olacaktır. KE'nin uzun olan inkübasyon süresi düşünüldüğünde, hastalığın 20-39 yaş grubunda ortaya çıkması için, etkenin vücuda çocukluk/gençlik çağında alınmış olma ihtimali yüksektir. Bu durumda okullardaki derslere entegre edilecek el yıkama ve gıda hijyeni alışkanlığını kazandırmaya yönelik eğitimlerle hastalıktan korunmada başarılı sonuçlar alınabileceği düşünülmektedir. Ayrıca yine toplum taramaları planlanırken genç popülasyonun hedeflenmesi erken tanı konması ve sağkalımın artırılması açısından faydalı olacaktır. IHA pozitiflik oranının en düşük saptandığı yaş grubu pandemi öncesinde 80 yaş ve üzeri hastalar iken, pandemi sürecinde değişerek 60-79 yaş aralığındaki hastalar olmuştur.

SONUÇ

Çalışmamızda COVID-19 sürecinde pek çok hastalıkta olduğu gibi, KE hastalarının tanı ve takiplerinde de gecikmeler yaşandığı görülmektedir. Hastalarda tanının gecikmesi veya takiplerin aksaması ekinokokkoz kaynaklı morbidite ve mortalitenin artışına yol açacaktır. Bu nedenle ülkemiz için önemli bir halk sağlığı problemi olan KE mücadelesine başarıyla devam edebilmek için çocuklar ve gençler başta olmak üzere topluma yönelik düzenlenen eğitimler ile halk bilinçlendirilmelidir. COVID-19 nedeniyle tanı ve takipte görülen aksaklıkların telafi edilebilmesi adına hastalıkla ilgili broşürler hazırlanmalı, kampanyalar düzenlenmeli ve hastalıkla mücadele toplumsal bir harekete dönüşmelidir. Ayrıca laboratuvar-klinik hekimleri arası iletişim güçlendirilerek hastalığın ayırıcı tanıda akla gelmesi sağlanmalı ve testin istem-yorumlama-tekrarlama algoritmaları oluşturularak testin akılcı kullanımına katkıda bulunulmalıdır.

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Scientometric Evaluation of the Itch Mite, *Sarcoptes scabiei* (Acari: Sarcoptidae): The Last Four Decades of Global Academic Output on Scabies

Sarcoptes scabiei (Acari: Sarcoptidae), Uyuzunun Scientometrik Değerlendirmesi: Uyuz Üzerine Küresel Akademik Çıktının Son Kırk Yılı

Kosta Y Mumcuoğlu[1], Engin Şenel[2], Ayşegül Taylan Özkan[3]

[1]Parasitology Unit, Department of Microbiology and Molecular Genetics, The Kuvin Center for the Study of Infectious and Tropical Diseases, The Hebrew University-Hadassah Medical School, Jerusalem, Israel
[2]Department of Dermatology, Hitit University Faculty of Medicine, Çorum, Türkiye
[3]Department of Medical Microbiology, TOBB University of Economics and Technology Faculty of Medicine, Ankara, Türkiye

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ABSTRACT

Objective: Scabiosis, the infestation of the skin with *Sarcoptes scabiei*, is a neglected tropical disease, with at least 200 million people being infested with the parasite at any time. It is estimated that scabies is responsible for 0.07% of the total burden of disease worldwide. Objective of this study is to perform a scientometric analysis of *S. scabiei* literature using the Web of Science Core Collection database for the years 1981-2020.
Methods: All documents indexed between 1981 and 2020 in scabies literature were analyzed by using a search string including keywords of "scabies", "*Sarcoptes scabiei*" and "*S. scabiei*" in Web of Science Core Collection database. We excluded all materials including data on the bacterium species named Streptomyces scabies and *Streptomyces scabiei*.
Results: Overall, 2,933 articles were retrieved on scabies, 66.3% of which were original article. With 663 publications the USA was the most productive country, while The International Journal of Dermatology was the journal with the highest number of publications on scabies. Half of the most productive institutions and seven of the top ten prolific authors were also from Australia. The National Health and Medical Research Council of Australia was the most supportive funding agency. With 4,706 citations, 2020 was the year with most references on scabies. The most cited publication was "The Global Burden of Skin Disease in 2010: An Analysis of the Prevalence and Impact of Skin Conditions" by Hay et al. in the Journal of Investigative Dermatology with a total of 565 citations. The most collaborative country was Australia and the most cooperative institution was the University of Melbourne.
Conclusion: The majority of the studies were done in a given country while multicenter studies are very rare. It is recommended that more studies should be conducted on scabiosis in developing countries where the problem of scabies is the biggest.
Keywords: *Sarcoptes scabiei*, scabies, scientometric evaluation, bibliometrics

ÖZ

Amaç: Uyuz, derinin *Sarcoptes scabiei* ile enfestasyonudur. Herhangi bir zaman diliminde en az 200 milyon insan bu parazit tarafından enfeste edilmekte olup, küresel öneme sahip ihmal edilmiş bir tropikal hastalıktır. Uyuzun dünya çapındaki toplam hastalık yükünün %0,07'sinden sorumlu olduğu tahmin edilmektedir. Bu çalışmanın amacı; 1981-2020 yılları için Web of Science Core Collection veritabanını kullanarak uyuz etkeni *S. scabiei* ile ilgili literatürünün scientometrik analizini yapmaktır.
Yöntemler: Web of Science Core Collection veritabanında "scabies", "*Sarcoptes scabiei*" ve "*S. scabiei*" anahtar kelimelerini içeren bir arama dizisi kullanarak, uyuz literatüründe 1981-2020 yılları arasında indekslenen tüm belgeler analiz edilmiştir. *Streptomyces scabiei* adlı bakteri türleri hakkındaki veriler araştırmanın kapsamına alınmamıştır.

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Address for Correspondence/Yazar Adresi: Kosta Y Mumcuoğlu, Parasitology Unit, Department of Microbiology and Molecular Genetics, The Kuvin Center for the Study of Infectious and Tropical Diseases, The Hebrew University-Hadassah Medical School, Jerusalem, Israel
Phone/Tel: +972542559374 **E-mail/E-Posta:** kostasm@ekmd.huji.ac.il **ORCID ID:** orcid.org/0000-0001-8125-6099

Türkiye PARAZİTOLOJİ Dergisi

Bulgular: Genel olarak, uyuzla ilgili 2,933 makale işleme alındı ve bunların %66,3'ü orijinal makaleydi. Altı yüz altmış üç yayın ile ABD en üretken ülke olurken, The International Journal of Dermatology uyuz konusunda en fazla yayına sahip dergi oldu. En üretken kurumların yarısı ve en üretken on yazarın yedisi de Avustralya'dandı. En çok atıf alan yayın, Journal of Investigative Dermatology'de toplam 565 atıf ile Hay ve ark.'nın yayınladığı "The Global Burden of Skin Disease in 2010: An Analysis of Prevalence and Impact of Skin Conditions" olmuştur. En işbirlikçi ülke Avustralya ve en işbirlikçi kurum Melbourne Üniversitesi idi.

Sonuç: Çalışmaların çoğu belirli bir ülkede yapılmış olup çok merkezli çalışmalar oldukça nadirdir. Uyuz sorununun en çok görüldüğü gelişmekte olan ülkelerde bu konuda daha fazla çalışma yapılması önerilir.

Anahtar Kelimeler: *Sarcoptes scabiei*, skabies, uyuz, scientometrik değerlendirme, bibliometric

INTRODUCTION

Scabiosis is the infestation of the skin with the itch mite, *Sarcoptes scabiei*. It is a neglected tropical disease of global significance, with at least 200 million people being infested with the parasite at any time. It has been estimated that scabies is responsible for 0.07% of the total burden of disease worldwide. This ectoparasite was held responsible for >0.21% of disability-adjusted life years from all conditions studied by Global Burden Disease in 2015 and ranked in the 101st place (1).

Depending on the country or population examined, the prevalence of scabies ranges between 0.2% and 71%, while it mostly affects people living in humid and hot areas such as in Central and South America (2-8).

The prevalence of scabies among children from low-income countries ranges between 0.2% and 24% while in endemic tropical regions the prevalence is estimated between 10-25% and in the Pacific region as high as 50% (9). Scabies prevalence in Aboriginal and Torres Strait Islander children in Australia is estimated to be as high as 33%, while this population group also have very high rates of secondary complications such as impetigo, rheumatic heart disease and post-streptococcal glomerulonephritis (10). A total of 34% of Aboriginal and Torres Strait Islander Australians are under the age of 18 years, compared with just 18% of non-Aboriginal Australians. This younger age profile likely contributes to the high prevalence of scabies among those children as there is a higher risk of transmission due to poor hygienic conditions and lack of social distancing (11).

In American Samoa, located in the South Pacific Ocean, 1,139 children with scabies (incidence 29.3/1.000 children aged ≤14 years) were identified during the years 2011-2012. Overall, 53% of the children has a bacterial superinfection while 15.3% had one or more re-infestations (9).

Scabies may also cause emotional and social problems. In the Fortaleza slams, capital of Ceara State, Brazil, a modified dermatology life quality index was used to assess the quality of life in 58 children with scabies. Overall, 46.6% of the children had the feeling of shame, 29.3% dressed differently, 36.8% restricted their leisure activities, 17.9% felt social exclusion, 25% stigmatization and 26.3% teasing by other children, when girls perceived more restrictions than boys (12).

Scabies is however also a public health issue in developed countries, especially among immunocompromised, disabled individuals and residential and nursing care home residents. In Europe, outbreaks occurred in a further education college for persons with learning disabilities, in a school for children with learning disabilities, in a workshop for handicapped children, in a day care center, and in a kindergarten (13,14).

In humans, the variety *var. hominis* usually burrows in the interdigital areas of the hands, wrists, feet and ankles, around the nipples in females and on the penile shaft in males. The antigens of the mites elicit a pruritic hypersensitivity reaction 3-6 weeks after the initial infestation. A polymorphic papular rash can be seen, especially in areas such as the waist, thighs, lower buttocks, lower legs, ankles and wrists. The damage done to the skin through scratching could lead to superinfections with pathogenic bacteria such as *Streptococcus pyogenes* and *Staphylococcus aureus*, as a result of which sepsis, indirect effects on renal and cardiovascular complications can develop, leading sometimes even to death (7). Ordinary scabies is characterized by a low number of mites (usually less than 15 per patient), while the crusted scabies is a rather rare form of the disease characterized by the presence of thousands of mites. The latter is a hyperkeratotic skin condition with formation of thick and scaly crust and involves face, eyelids, neck, and scalp, when mites can also be found under the hand nails of the affected subjects, and secondary infections are common (2,3,15,16).

Sarcoptic mange is known from over 100 domestic and wild mammal species, and also in these cases the clinical symptoms depend on the immune status of the host animal. Immunocompetent animals usually develop strong type I and IV hypersensitivity reactions while they are infested with a low number of mites. However, with the time the skin becomes thickened, greyish in color and it is characterized by a marked eosinophilia in epidermis and dermis accompanied with an extensive alopecia. Immunocompromised animals develop a general hyperkeratosis with a large number of mites and an underlying chronic dermal inflammation (17).

Scientometrics also known as bibliometrics and as "science of science" is a popular statistical branch providing information on publication trends and patterns in a certain academic area (18). Scientometric studies reveal holistic evaluation on productivities of the authors, institutions and countries producing academic documents. Although scabies is a common disease and there has been an increasing interest in scientometrics in the last decades, only few publications exists on the scientometric assessment on scabies literature (19,20). This study aims to perform a holistic analysis of scabies literature.

METHODS

We analyzed all documents indexed between 1981 and 2020 in scabies literature by using a search string including keywords of "*scabies*", "*Sarcoptes scabiei*" and "*S. scabiei*" in Web of Science (WoS) Core Collection database. We chose WoS database since it was reported that it was the most reliable source in academic literature evaluation (21). We excluded all materials including data on the bacterium species named *Streptomyces scabies* and *Streptomyces scabiei*.

Statistical Analysis

For statistical analysis of data for percentages, frequencies and trend analysis MS Office Excel 2020 were used. The info map revealing countries producing scabies publications was generated in a free web source titled Paintmaps (22). All literature data of the indexed documents were downloaded from WoS database using the export option for scientometric network analysis and text files were created to be processed in VOSviewer literature analyze section. Scientometric network images were created by using VOSviewer version 1.6.11 (www.vosviewer.com, Centre for Science and Technology Studies, Leiden University, The Netherlands) (23).

Ethical approval and patients' consents are not needed for this research since neither human nor animal included.

RESULTS

General Features of the Literature

Our main search with the keyword string retrieved 2,933 articles, 806 of which were open access documents. The most indexed article type was original article (66.3%) followed by letter, review, and editorial material (9.3, 8.4 and 6.7%, respectively; Table 1). The most studied areas were Dermatology, Infectious Diseases, and Internal Medicine (34.7, 12.1 and 11.9%, respectively; Table 1). English was the main language of the literature covering 87.6% of all publications followed by German, French and Spanish (4.5, 4.4 and 1.6%, respectively).

Only one item was indexed under the topic "Scabies" in the WoS Core Collection between 1975 and 1979, which was a poetry titled "Scabies" and published in 1977 in Malahat Review. In 1981 there were 24 documents in WoS database and the year in which the most papers were published was recorded as 2020 with 233 items (Figure 1). We chose 1981 as the starting year for our study since we examined four decades of medical literature on the topic of scabies.

Productivities of Countries, Source Titles, Authors and Institutions

The USA was the most productive country with 663 documents (22.6%) followed by Australia, France, Germany, the United Kingdom (UK) and India (11.9, 8.2, 6.7 and 6.4%, respectively; Figure 2). Scabies articles were produced all over the world except in the majority of countries in Africa (Figure 3). The most contributor source titles were International Journal of Dermatology, Journal of The American Academy of Dermatology and British Journal of Dermatology (n=101, 97 and 70 items, respectively: Table 1). The most prolific author was Currie BJ from Australia with 59 papers (Table 1). Half of the most productive institutions were from Australia and seven of the top ten prolific authors were also from Australia (Table 1).

National Health and Medical Research Council of Australia, United States Department of Health Human Services and National Institutes of Health (USA) were the most supportive funding agencies (n=77, 68 and 58 studies, respectively).

Citation Analysis

H-index of scabies literature between 1981 and 2020 was calculated as 77 and total number of citations was 43,873 (25,830 without self-citations). Average citations per item were noted as 14.96. Top year according to citation number by year was 2020 with 4,706 records. The most cited document of scabies literature was an original article titled "The Global Burden of Skin Disease in 2010: An Analysis of the Prevalence and Impact of Skin Conditions" by Hay et al. (24) published in 2014 in the Journal of Investigative Dermatology with a total of 565 citations (Table 2) (1-3,24-30).

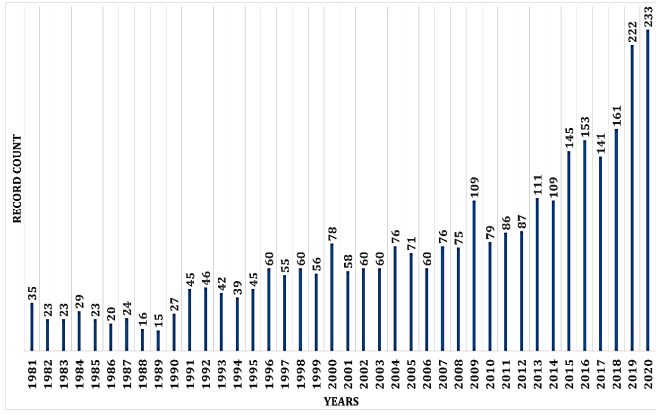

Figure 1. Number of publications on scabies between the years 1981 and 2020

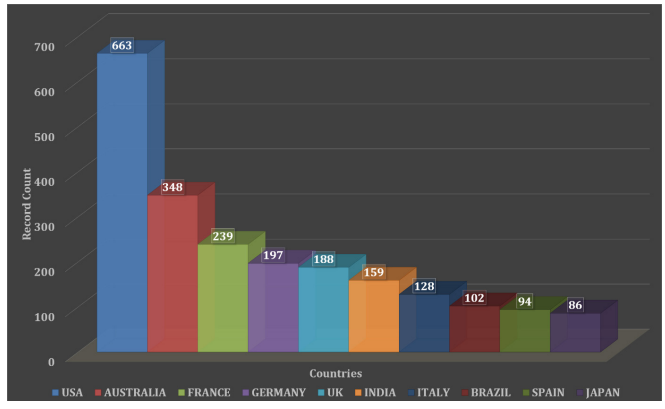

Figure 2. Countries with the highest number of publications on scabies

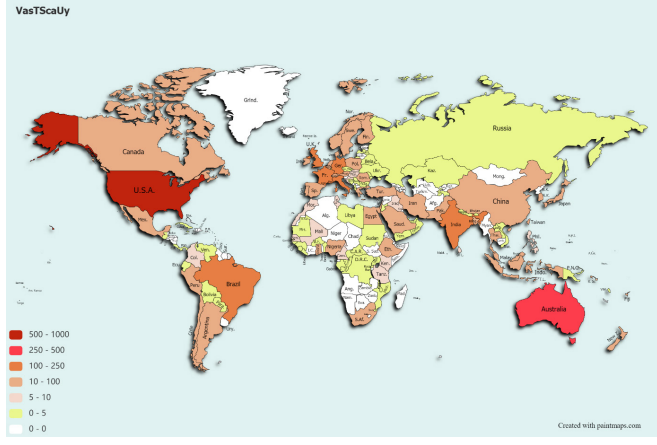

Figure 3. Distribution of scabies articles according to the country in which they were written

Table 1. Top ten document types, research areas, source titles, authors and organizations in scabies literature between 1981 and 2010

Document type		**Number**	**%[a]**	
Original article		1,944	66.280	
Letter		272	9.274	
Review		247	8.421	
Editorial material		197	6.717	
Meeting abstract		166	5.660	
Note		57	1.943	
Proceeding paper		57	1.943	
Book chapter		37	1.262	
News		7	0.239	
Biographical item		3	0.102	
Research area				
Dermatology		1,019	34.743	
Infectious diseases		356	12.138	
Internal medicine		349	11.899	
Occupational health		270	9.206	
Parasitology		235	8.012	
Veterinary sciences		223	7.603	
Tropical medicine		220	7.501	
Pediatrics		172	5.864	
Pharmacology		113	3.853	
Immunology		112	3.819	
Journal name				
International Journal of Dermatology		101	3.444	
Journal of The American Academy of Dermatology		97	3.307	
British Journal of Dermatology		70	2.387	
PLOS Neglected Tropical Diseases		65	2.216	
Annales de Dermatologie et de Venereologie		59	2.012	
Hautarzt		56	1.909	
Pediatric Dermatology		55	1.875	
American Journal of Tropical Medicine and Hygiene		49	1.671	
Archives of Dermatology		39	1.330	
Journal of Dermatology		37	1.262	
Author	**Institution**	**Country**	**Number**	**%**
Currie BJ	Royal Darwin Hospital	Australia	59	2.012
Fischer K	Queensland Institute of Medical Research	Australia	56	1.909
Chosidow O	Paris-Est Créteil University	France	47	1.602
Steer AC	Murdoch Children's Research Institute	Australia	44	1.500
Walton SF	University of the Sunshine Coast	Australia	44	1.500
Arlian LG	Wright State University	USA	39	1.330
Morgan MS	Wright State University	USA	33	1.125
Romani L	University of New South Wales	Australia	30	1.023
Engelman D	University of Melbourne	Australia	29	0.989
Kemp DJ	Queensland Institute of Medical Research	Australia	28	0.955
Organizations		**Country**	**Number**	**%**
Charles Darwin University		Australia	119	4.057
Menzies - School of Health Research		Australia	119	4.057
QIMR Berghofer Medical Research Institute		Australia	114	3.887
Assistance Publique - Hôpitaux de Paris		France	91	3.103
University of Melbourne		Australia	77	2.625
University of London		UK	65	2.216
University of California System		USA	64	2.182
University of Queensland		Australia	59	2.012
Murdoch Children's Research Institute		Australia	58	1.977
Wright State University		USA	50	1.705
Total		**2.933**	**100**	

[a]Total percentage may exceed 100% because certain items were included in more than one category, UK: United Kingdom, USA: United States of America

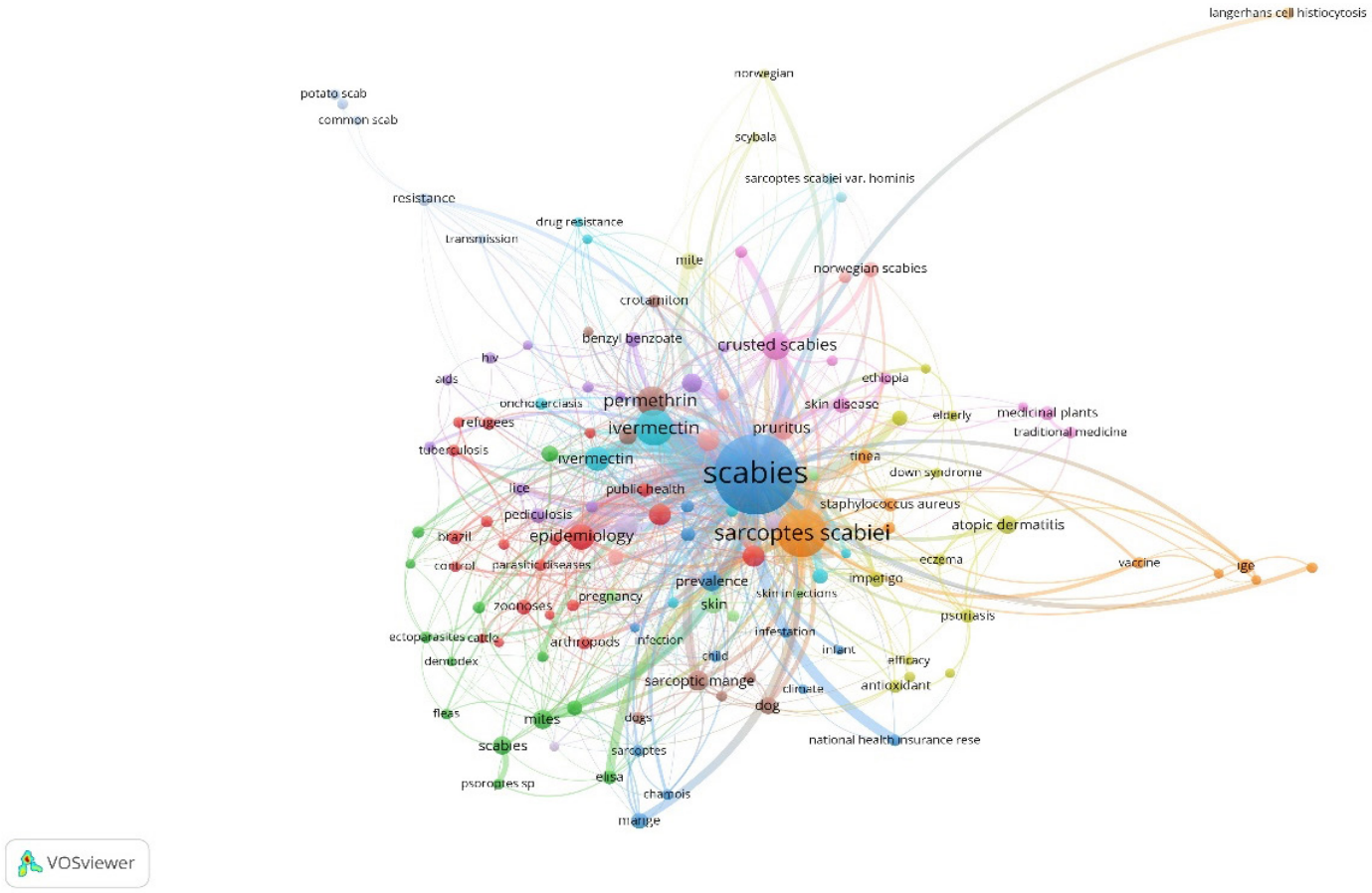

Figure 4. Scientometric network analysis of keywords in a starburst pattern where the keyword "scabies" is centered in

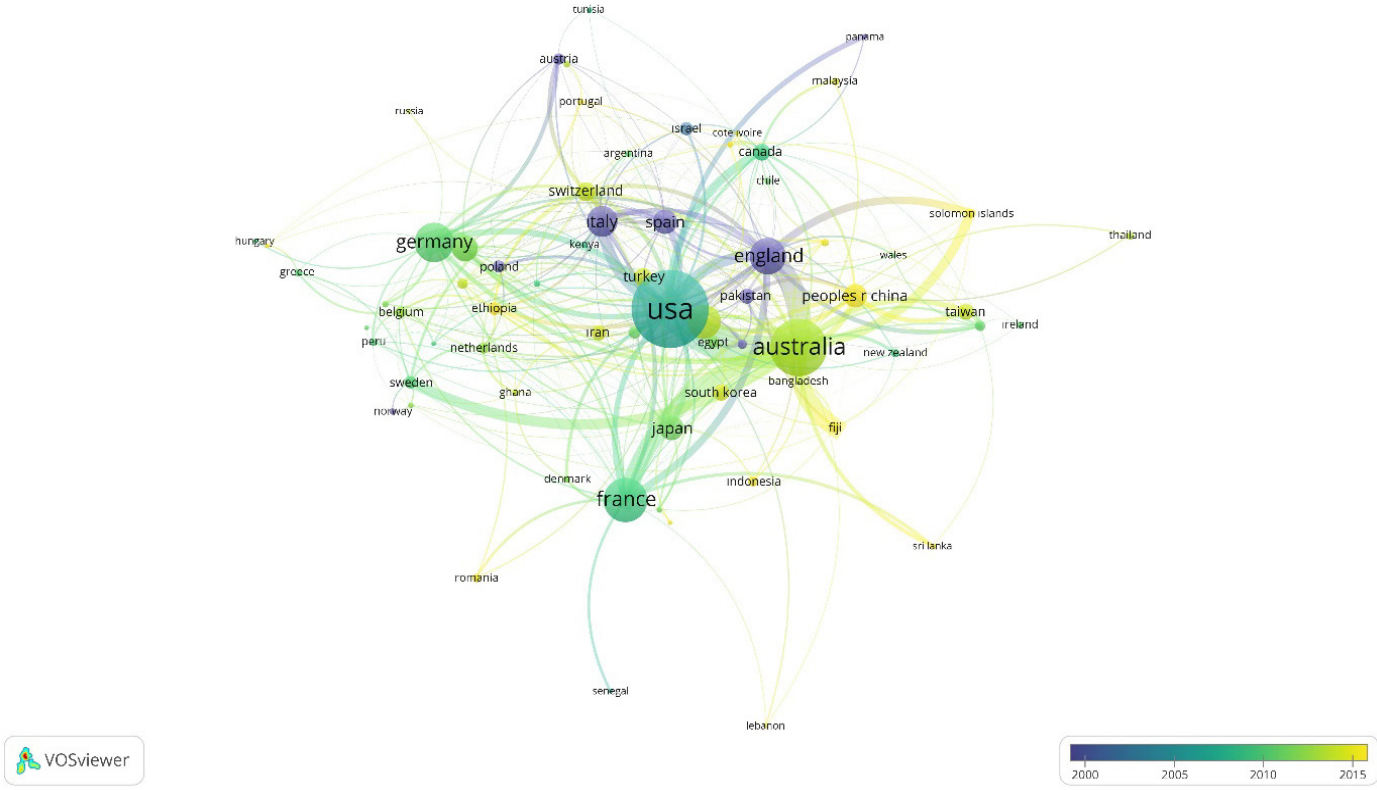

Figure 5. The most collaborative countries which published articles on scabies

Scientometric Networks

The most indexed keywords were "scabies", "*Sarcoptes scabiei*", "ivermectin", "permethrin", "crusted scabies" and "epidemiology" (Table 3). Scientometric network analysis of keywords revealed a starburst pattern that the keyword of "scabies" centered in (Figure 4). The most collaborative countries were Australia, the USA, the UK, France, and Germany (Figure 5). It was noted that top five cooperative institutions were from Australia, i.e., University of Melbourne, Charles Darwin University, University of Queensland, Menzies - School of Health Research, and Murdoch Children's Research Institute (Figure 6).

DISCUSSION

In the present study using the Web of Science Core Collection database 2,933 articles were detected. A search by Google Scholar using the words "*Sarcoptes scabiei*" gave 16,900 hits (17,300 when not in brackets), while the number of publications on this subject in PubMed was 1,218 and 1,220 when used in brackets and without, respectively.

Romani et al. (30) searched Medline, Embase, and LILACS for the years 1985-2014 regarding the prevalence of scabies and impetigo, and found 2.409 articles, 48 of which were relevant for their analyses. With the exception of North America to which no data were available, scabies prevalence was between 0.2-71.4%. With the exception of Europe and Middle East the prevalence of scabies was >10%, while it was highest in the Pacific and Latin American regions. More children than adolescents and adults were infested with scabies, who also showed the highest percentages of impetigo, when the highest prevalence (49.0%) was seen in Australian aboriginal communities.

Bansal (19) using the PubMed retrieved 1,460 articles relevant to scabies between the years 2001 and 2015. The number of publications on scabies increased from 95 in 2001 to 137 in 2015.

Table 2. The ten most cited manuscripts in scabies literature between 1981 and 2020 (1-3,24-30)

Article	Author	Journal	Total citation	Average citations per year
The global burden of skin disease in 2010: An analysis of the prevalence and impact of skin conditions	Hay et al. 2014 (24)	Journal of Investigative Dermatology	565	70.63
Scabies and pediculosis	Chosidow, 2000 (25)	Lancet	265	12.05
Scabies	Chosidow, 2006 (2)	New England Journal of Medicine	252	15.75
The treatment of scabies with ivermectin	Meinking et al. 1995 (26)	New England Journal of Medicine	230	8.52
Scabies: A ubiquitous neglected skin disease	Hengge et al. 2006 (27)	Lancet Infectious Diseases	215	13.44
Problems in diagnosing scabies, a global disease in human and animal populations	Walton & Currie, 2007 (28)	Clinical Microbiology Reviews	200	13.33
Scabies	Heukelbach & Feldmeier, 2006 (3)	Lancet	199	12.44
Global skin disease morbidity and mortality an update from the Global Burden of Disease Study 2013	Karimkhani et al. 2017 (1)	JAMA Dermatology	192	38.4
Crusted scabies: clinical and immunological findings in seventy-eight patients and a review of the literature	Roberts et al. 2005 (29)	Journal of Infection	191	11.24
Prevalence of scabies and impetigo worldwide: a systematic review	Romani et al. 2019 (30)	Lancet Infectious Diseases	177	25.29

Table 3. The 20 most used keywords in scabies literature. Keyword (total link strength)

1.	Scabies (372)	11.	Dermoscopy (21)
2.	*Sarcoptes scabiei* (116)	12.	Mite(s) (19)
3.	Ivermectin (73)	13.	Diagnosis (17)
4.	Permethrin (51)	14.	Skin (14)
5.	Crusted scabies (39)	15.	Lindane (13)
6.	Epidemiology (35)	16.	Dog (12)
7.	Children (23)	17.	Impetigo (12)
8.	Pruritus (23)	18.	Atopic dermatitis (11)
9.	Prevalence (22)	19.	Norwegian scabies (11)
10.	Sarcoptic mange (22)	20.	Outbreak (11)

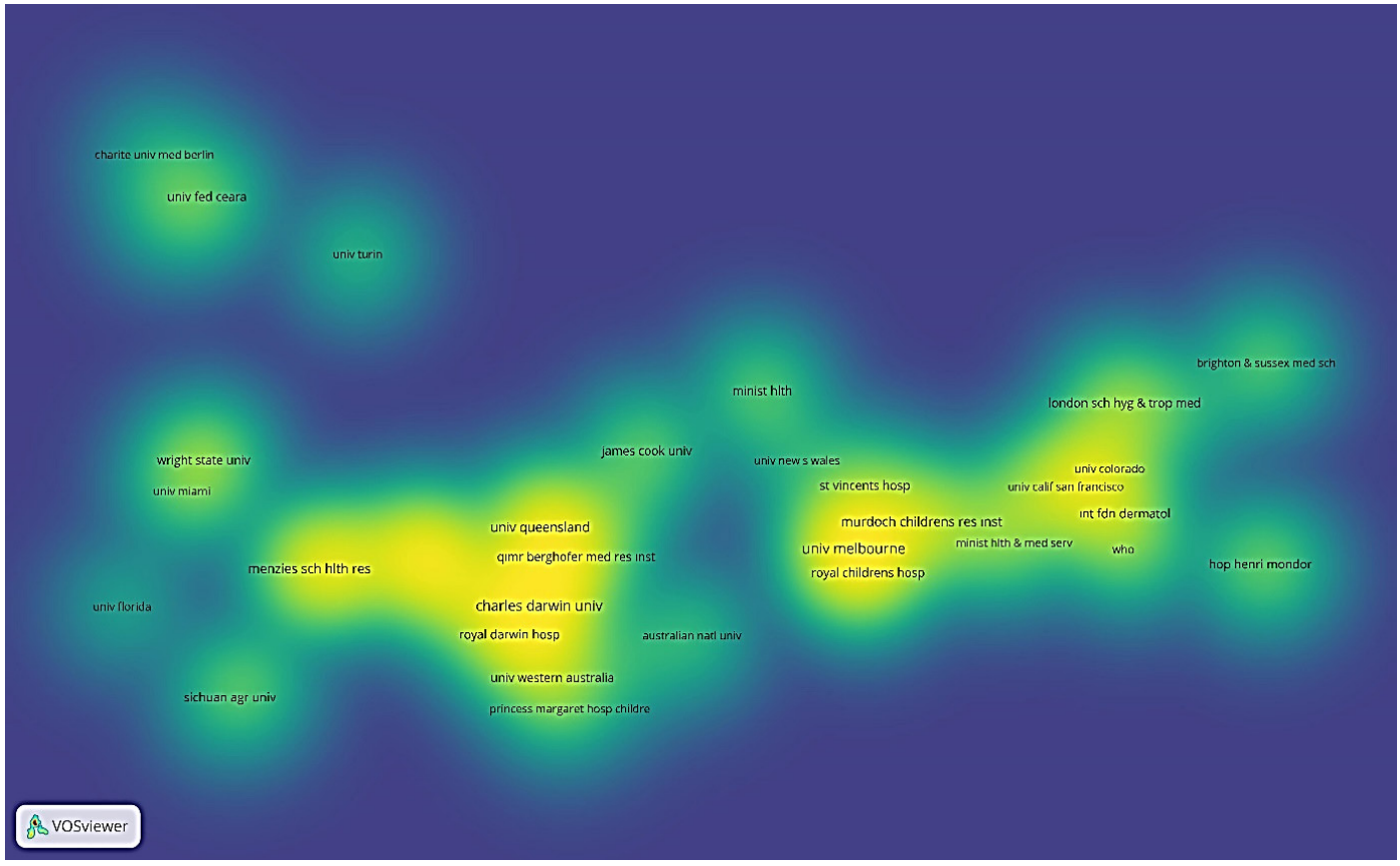

Figure 6. The institutions worldwide were the scabies articles were created

With 46 articles, Veterinary Parasitology topped the list of journals with the highest number of publications on scabies, followed by International Journal of Dermatology (27 articles) and Pediatric Dermatology (25 articles), while the first six most prolific authors on scabies were from Australia (B.J. Currie, S.F. Walton, K. Fischer, JS McCarthy, D.C. Hold and D.J. Kemp).

Singh et al. (20) using the Scopus Database for the years 2009-2018, found 2,268 publications on scabies, most of them coming from USA, followed by India, Australia, UK and France. The most cited articles were published in the Lancet and Journal of Investigative Dermatology. The top 5 organizations were from Australia. Top authors contributing in this field include O. Chosidow with 28 publications, followed by K. Fischer B.J. Currie and A.C. Stier. Among the top scorer countries, the US and the UK has maximum collaboration of six and five countries each, towards scabies research.

Using Medline, Embase, WoS and Scopus, Rinaldi and Porter (31) found 353 publications on mass drug administration for endemic scabies, 12 of which was used for the evaluation of their study.

A systematic search of the databases CAB Direct, PubMed, Scopus, WoS, Embase, and Discovery revealed 2,205 publications on the treatment of sarcoptic mange in wildlife. Using Endnote X6 to remove the duplicates, 1.687 publications remained, 28 of which were relevant for the study (32).

To evaluate the cost-effectiveness of scabies interventions and using PubMed, Medline, Embase, CINAHL, and the Cochrane Library for the years 2000-2017, van der Linden et al. (7) identified 821 articles, 30 of which were include in their study.

Using Medline, Embase and Cochrane databases for the years 1946-2013, Thompson et al. (33) identified 239 articles regarding the diagnosis of scabies in therapeutic trials.

When the number of publications is evaluated by years, a linear increase from 1981 to 2020 can be seen, which in agreement with a scientometric analysis done with house dust mites and hirudotherapy (18,34).

With the exception of India and Brazil, the highest number of publications were done by scientists from developed countries such as USA, Australia, France, Germany, UK, Spain and Japan, which is in agreement with publications done on house dust mites (34).

The fact that Australia is leading in all aspects of scabies publications seems to be related to the fact that the infestation rate with scabies in the indigenous people of this continent is very high and accordingly a lot of attention has been paid to this ectoparasitosis (35,36).

Overall, it can be said that scientometric analysis using different databases, different years and keyworks can give different results, including the most prolific countries, authors and journals.

As limitations of the study, it could be indicated that publications from PubMed, Google Scholar, and Scopus were not included. In addition, WoS database uses only journals with high impact factors. It might be that some publications were included more than once, however it should be also noted that WoS is one of the most reliable databases (37,38).

CONCLUSION

The majority of the studies were done in a given country while multicenter studies are very rare. It is recommended that more studies should be conducted on scabiosis in developing countries where the problem of scabies is the biggest.

* Ethics

Ethics Committee Approval: Ethical approval and patients' consents are not needed for this research since neither human nor animal included.

Informed Consent: Ethical approval and patients' consents are not needed for this research since neither human nor animal included.

Peer-review: Internally and externally peer-reviewed.

* Authorship Contributions

Concept: K.Y.M., E.Ş., A.T.Ö., Design: K.Y.M., E.Ş., A.T.Ö., Data Collection or Processing: K.Y.M., E.Ş., A.T.Ö., Analysis or Interpretation: K.Y.M., E.Ş., A.T.Ö., Literature Search: K.Y.M., E.Ş., A.T.Ö., Writing: K.Y.M., E.Ş., A.T.Ö.

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Ordu, Giresun, Trabzon ve Rize İllerinde Ev Tozu Akarlarının Yaygınlığı ve Der p 1 ve Der f 1 Varlığı

Prevalence of House Dust Mites and Presence of Der p 1 and Der f 1 in Ordu, Giresun, Trabzon and Rize Provinces

Cihangir Akdemir[1], Ülkü Karaman[2], Nejla Cebeci Güler[1], Şahin Direkel[1], Emel Uzunoğlu[1], Hakan Şentürk[3], Uğur Ayhan[4]

[1]Giresun Üniversitesi Tıp Fakültesi, Tıbbi Mikrobiyoloji Anabilim Dalı, Giresun, Türkiye
[2]Ordu Üniversitesi Tıp Fakültesi, Tıbbi Parazitoloji Anabilim Dalı, Ordu, Türkiye
[3]Giresun Üniversitesi Şebinkarahisar Sağlık Hizmetleri Meslek Yüksek Okulu, Giresun, Türkiye
[4]Sağlık Bakanlığı, Trabzon Halk Sağlığı Laboratuvarı, Trabzon, Türkiye

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ÖZ

Amaç: Orta ve Doğu Karadeniz Bölgesi illerinden Ordu, Giresun, Trabzon ve Rize'de evlerde ev tozu akarlarının tespit edilmesi ve Dermatophagoid türlerden *D. pteronyssinus* ve *D. farinae*'ye ait antijenlerinin araştırılması amacıyla yürütülmüştür.
Yöntemler: Yataklardan temin edilen toz örnekleri hem mikroskobik hem de antijenik incelemeye tabi tutulmuştur. Mikroskobik inceleme için laktik asit yöntemiyle hazırlanmış örnekler ışık mikroskobunda (10x, 40x) değerlendirilmiştir. Antijenik inceleme *D. pteronyssinus* ve *D. farinae*'ye ait Der p 1 ve Der f 1'in ELISA testi ile araştırılmasıyla yapılmıştır.
Bulgular: Mikroskobik incelemede toz örneklerinin %90,3'ü pozitif değerlendirilmiş ve 149 adet akar tespit edilmiştir. *D. pteronyssinus* %74, *D. farinae* %13, *Dermatophagoides* spp. gelişim formları %5, *Cheyletus* spp. %1, *E. maynei* %1, *C. arcuatus* %1, *T. putrescentiae* %1, *L. destructor* %1 ve tanımlanamayanlar %3 oranında belirlenmiştir. Der p 1 antijeni %93, Der f 1 antijeni ise %84,7 oranında tespit edilmiştir. Bir gram yatak tozunda saptanan en yüksek antijen miktarı Der p 1 için 1,272 µg, Der f 1 için ise 0,482 µg belirlenmiştir.
Sonuç: Çalışmanın yürütüldüğü illerde akar türleri ve dağılımları arasında fark gözlenmemiştir (p<0,05). *Dermatophagoides* spp. popülasyonunun %93'ünü oluşturmuştur. Depo/gıda akarlarının düşük (%4) oranda bulunmasının zeminlerden örnek alınmamış olmasıyla ilgili olduğu, ılıman ve nemli bölgelerde akarların aktivitesi yıl boyunca seyrettiği için yataklarda antijen birikiminin önemli olabileceği, antijen testlerinin akar tespitinde kullanılan mikroskobik yöntemlere ek olarak, akar alerjen yüklerinin ayrıntılı değerlendirilmesinde alternatif bir yöntem olarak kullanılabileceği ve duyarlı kişilerin yaşadığı ortamlar açısından bu teşhis yönteminin dikkate alınabileceği düşünülmektedir
Anahtar Kelimeler: *D. pteronyssinus*, *D. farinae*, Der p 1, Der f 1, Karadeniz Bölgesi

ABSTRACT

Objective: This study was carried out to detect house dust mites in houses and to investigate group 1 antigens of Dermatophagoid species in Ordu, Giresun, Trabzon and Rize provinces of the Central and Eastern Black Sea Region.
Methods: Dust samples obtained from the beds were subjected to both microscopic and antigenic examination. Samples prepared by the lactic acid method for microscopic examination were evaluated under a light microscope. Antigenic analysis was performed by investigating Der p 1 and Der f 1 belonging to *D. pteronyssinus* and *D. farinae* by ELISA test.
Results: 90.3% of the dust samples were evaluated positive by microscopic examination (10x, 40x) and 149 mites were detected. *D. pteronyssinus* 74%, *D. farinae* 13%, *Dermatophagoides* spp. growth forms 5%, *Cheyletus* spp. 1%, *E. maynei* 1%, *C. arcuatus* 1%, *T. putrescentiae* 1%, *L. destructor* 1% and unidentified mites were detected at the rate of 3% respectively. Der p 1 antigen was detected in 93% and Der f 1 antigen in 84.7%. The highest amount of antigen detected in one gram of powder was 1,272 µg for Der p 1 and 0,482 µg for Der f 1.
Conclusion: No difference was observed between mite species and distribution in the provinces where the study was conducted (p<0.05). Dermatophagoides were found in 93% of the population. The low (4%) rate of storage/food mites is related to the fact

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Yazar Adresi/Address for Correspondence: Emel Uzunoğlu, Giresun Üniversitesi Tıp Fakültesi, Tıbbi Mikrobiyoloji Anabilim Dalı, Giresun, Türkiye
Tel/Phone: +90 505 778 55 29 **E-Posta/E-mail:** emeluzunoglu@yahoo.com **ORCID ID:** orcid.org/0000-0002-9523-0380

Türkiye PARAZİTOLOJİ Dergisi

that samples were not taken from the floors. Antigen accumulation may be important in the beds since the activity of the mites is observed throughout the year in temperate and humid regions. It is thought that this diagnosis method can be used and can be taken into account in terms of the environments in which sensitive people live.

Keywords: *D. pteronyssinus*, *D. farinae*, Der p 1, Der f 1, Black Sea Region

GİRİŞ

Ev tozu akarları, Acarina dizisinin Glycyphagoidea, Acaroidea ve Analgoidea üst ailelerinde yer alan, dört çift ekstremiteli mikroskobik eklem bacaklı canlılardır. Dermatophagoides ve Euroglyphus soyu dünya genelinde görülmekle beraber Blomia tropikal bölgelerde baskın olan soydur (1,2). Çoğunlukla yatak, halı, kilim ve kumaş kaplı mobilyalarda bulunmaları ve ev tozunda da tespit edilebilmeleri nedeniyle ev tozu akarı olarak isimlendirilirler. Hububat, un, kuru yemiş ve peynir gibi gıda maddelerinden kaynaklananlar gıda/depo akarı olarak tanımlanır ve bunlar da ev tozunda bulunabilir (1). Glycyphagus, Tyrophagus, Acarus, Suidasla, Lepidoglyphus, Chortoglyphus soyları bu grupta yer alır. Ayrıca *Cheyletiella malacensis* ve *C. eruditus* gibi türlere de rastlanılabilir (1,3-5).

Bu canlıların astım gibi alerjik hastalıkları tetiklediği fikri ilk kez 1922 yılında Giocomo R. Ancona'ya atfen Fernández-Caldas ve ark. (6) tarafından ifade edilmiştir. Antijenlerin ev tozundan kaynaklandığını ise 1968'de Pepys J'ye atfen Spieksma ve Dieges (7) bildirmiş ve sonrasında Voorhorst ve ark. (8) da ev tozundaki ana antijen kaynağının akarlar olduğunu doğrulamışlardır.

Akarlarda günümüze kadar dışkı, tükürük salgısı, kütikül partikülleri, dermis hücreleri ve yumurtalarından kaynaklı 22 önemli alerjen grubu tespit edilmiştir. İki önemli ev tozu akar türü olan *Dermatophagoides pteronyssinus* ve *Dermatophagoides farinae*'den kaynaklı Der p 1 ve Der f 1 antijenleri Grup I olarak kabul edilenler içinde yer almaktadır (9,10). Ortama karışıp havada bir süre asılı kalabilen bu yapılar kontak yolla, solunumla ve sindirim sistemiyle etkileşmekte, atopik dermatit, konjonktivit, alerjik rinit ve astım gibi hastalıkların gelişimini tetikleyebilmekte, duyarlı kişilerde anafilaksiye neden olabilmektedir (11,12).

Tovey ve ark. (13) bir akarın günde 20-40 parçacık salgıladığını, bunların %90'ının dışkılarından kaynaklandığını ve yaklaşık 0.1-10 ng'lik kısmının ise Grup I alerjeni olduğunu bildirmişlerdir. Arlian ve ark. (14) ise Gram-tozda bulunan 2 µg grup I antijenin predispoze kişiler için risk faktörü oluşturduğuna, bunun 10 µg'ye çıkması durumunda ise alerjik reaksiyonları tetiklendiğine dikkat çekmişlerdir. Yaşamın erken dönemlerinde yüksek oranda bu antijenlere maruz kalmanın da duyarlılık gelişimine neden olabileceği ifade edilmiştir (15,16).

Bu çalışmada, Orta ve Doğu Karadeniz Bölgesi illerinden Ordu, Giresun, Trabzon ve Rize'deki evlerin yataklarında ev tozu akar türlerinin araştırılması ve aynı zamanda *D. pteronyssinus* ve *D. farinae*'ye ait Der p 1 ve Der f 1 antijenlerinin tespit edilmesi amaçlanmıştır.

YÖNTEMLER

Numune Temini

2020 yılı Ocak, Şubat ve Mart aylarında Ordu'dan 26, Giresun'dan 24, Trabzon'dan 12 ve Rize'den 10 olmak üzere toplamda 72 adet toz örneği yetişkinlerin kullandığı yataklardan temin edilmiştir. Numuneler elektrikli süpürgelerin teleskopik hortumuna adapte olan bir kolektör (Dustream® collector) ve bunun hava kanalına yerleştirilen toz filtresi (Dustream® filters, 40 µm) ile yataklardan m^2/2 dk süreyle alınmış, her ev için ayrı filtre kullanılmıştır.

Toz örneğini kendileri almak isteyen ev sakinlerine (n=44) aparat teslim edilerek uygulaması anlatılmış ve laboratuvara ulaştırılıncaya kadar filtrenin kilitli naylon poşet içerisinde muhafaza edilmesi istenilmiştir. Öncelikle çarşaf daha sonra yatak/döşek zemini dahil bütün katmanlar vakum gücü en az 1200w olan süpürge ile süpürülmüştür. Ev sakinlerinin, bu imkana sahip olmamalarını bildirmeleri durumunda portatif bir elektrikli süpürge (Fakir® A120, 1200w, 220v) temin edilmiştir. Toz örnekleri tartım öncesinde saç, kıl, tüy, elyaf, lif vb. kaba partiküllerden arındırıldıktan sonra 100 mg'lik iki porsiyona ayrılmıştır.

Mikroskobik İnceleme

Porsiyonlanmış örnekler 25 mL'lik beherglas içerisinde yaklaşık 3-4 mL laktik asit ile karıştırılmış ve plastik pipet yardımıyla tamamı lam-lamel arasında geçici preparat haline getirilmiştir. Işık mikroskobunun 10x ve 40x objektiflerinde (Nikon® Eclipse Ni) her bir ev için yaklaşık 1½ -2 saat süreyle incelenmiş ve ilgili literatürler (17-19) ışığında sonuçlar kayıt altına alınmıştır. Toz örnekleri laktik asit ile geçici preparat haline getirildiği için herhangi bir daimi ortamda (Hoyer's medium vs.) sabitlenme işlemi uygulanmamıştır.

Antijenik İnceleme

Porsiyonlanmış ikinci örnekler 0,05 cm çaplı cam tüplere konulmuş ve 2cc PBS (pH 7,4 Tween 20 %0,5) ilave edilip 2 saat süreyle çalkalanarak (GFL® 3005) antijenlerin ayrışması sağlanmıştır. Hazırlanan süspansiyonlar +4 °C'de 2,500 rpm'de 10 dakika santrifüj (Nüve® NF 800R) edilmiş ve süpernatant kapaklı tüplere 0,5 mL'lik hacimlerde porsiyonlanarak -20 °C'de muhafaza edilmiştir. *D. pteronyssinus* ve *D. farinae*'den kaynaklı Der p 1 ve Der f 1 antijenlerini tespit etmek için Der p 1 Elisa 2.0 ve Der f 1 Elisa 2.0 testleri (Indoor Biotechnologies, Inc.) kullanılmıştır. Antijen solüsyonları tek seferde oda ısısında çözülerek üreticinin bildirdiği şekilde teste tabi tutulmuş ve Elisa okuyucuda (Biotek® ELX800) 450 nm dalga boyunda okunmuştur.

Test üreticisi antijen miktar tayini için en az 3 dilüsyon basamağının çalışılarak sonuçların değerlendirmesini bildirmiş olmasına karşın dilüsyon basamakları kullanılmadan sonuçlar pozitif veya negatif olacak şekilde değerlendirmeye alınmıştır.

İstatistiksel Analiz

Elde edilen veriler sayı ve yüzde olarak verilmiştir. Çalışmada ki-kare testi yapılmış, Likelihood ratio ki-kare değeri hesaplanmıştır. Tüm hesaplamalar SPSS 28 (IBM Inc., Chicago, IL, USA) istatistik paket programı ile yapılmıştır. $P<0,05$ anlamlı olarak kabul edilmiştir.

Çalışmada hasta materyali ve deney hayvanı kullanılmamış olması nedeniyle etik kurul onayı gerektirmemektedir.

BULGULAR

Mikroskobik bakısı yapılan örneklerin 65'i (%90,3) pozitif, 7'si ise (%9,7) negatif değerlendirilmiş ve bunlarda 149 adet akar tespit edilmiştir (Tablo 1). *D. pteronyssinus* %74, *D. farinae* %13, *Dermatophagoides* spp. gelişim formları %5, *Cheyletus* spp. %1, *E. maynei* %1, *C. arcuatus* %1, *T. putrescentiae* %1, *L. destructor* %1 ve tanımlanamayanlar %3 oranında belirlenmiştir (Grafik 1). Gelişim formları dahil olmak üzere Dermatophagoid akarlar popülasyonun %93'ünü oluşturmuştur. Gram-tozda ortalama 23 akar saptanmıştır.

Der p 1 %93, Der f 1 %84,7 oranında tespit edilmiştir. Yatak tozunda saptanan en yüksek antijen miktarı gram başına Der p 1 için 1,272 µg, Der f 1 için ise 0,482 µg olarak belirlenmiştir.

Mikroskobik yöntem ile en yüksek akar tespiti 65 ev ile *D. pteronyssinus*'da gözlenmişken en düşük bulunma ise birer evde olmak üzere *L. destructor*, *C. arcuatus*, *T. putrescentiae* ve *Cheyletus* spp.'de saptanmıştır (Grafik 2).

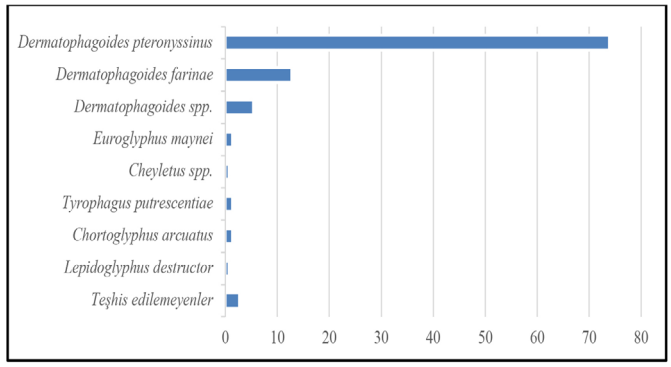
Akar türlerinin illere göre dağılımı ki-kare testi ile karşılaştırılmıştır (Tablo 2) ve dağılımı bakımından anlamlı farklılık olmadığı belirlenmiştir (p=0,786, χ^2=18,336).

Tablo 1. Akar sayısı ve % değerleri

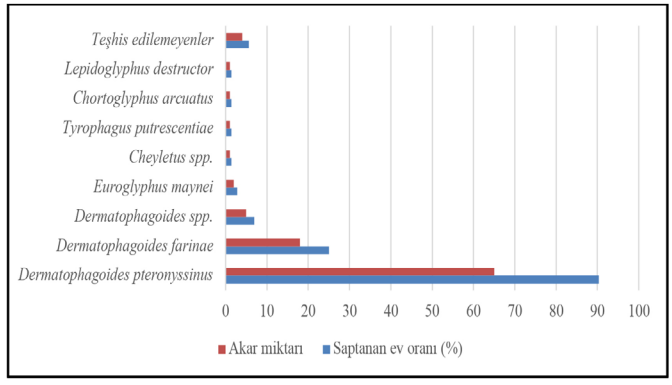
Tür	Sayı	%
Dermatophagoides pteronyssinus	110	74
Dermatophagoides farinae	19	13
Dermatophagoides spp. gelişim formu	8	5
Euroglyphus maynei	2	1
Cheyletus spp.	1	1
Tyrophagus putrescentiae	2	1
Chortoglyphus arcuatus	2	1
Lepidoglyphus destructor	1	1
Teşhis edilemeyenler	4	3
Toplam	149	100

TARTIŞMA

D. pteronyssinus, D. farinae, Euroglyphus maynei, Lepidoglyphus destructor, Tyrophagus putrescentiae, Chortoglyphus arcuatus ve *Glycyphagus domesticus* dünyada ve ülkemizde yaygın bulunan türlerdir (14,20-23). Türkiye'deki baskın pyroglyphid türün *D. pteronyssinus* olduğu, *D. farinae* kısmen olmakla birlikte *E. maynei* ve *D. evansi*'nin ise sınırlı sayıda bulunduğu ifade edilmiştir (23-25). Orta ve Doğu Karadeniz illerinde yürütülen bu çalışmada da



Grafik 1. Teşhis edilen akar tür ve dağılımları grafiği (%)



Grafik 2. Akar saptanan ev sayılarının dağılımları grafiği (%)

Tablo 2. İllere göre akar türlerinin dağılımı

Türler	İller								Toplam	
	Giresun		Ordu		Rize		Trabzon			
	n	%	n	%	n	%	n	%	n	%
D. pteronyssinus	38	79,2	47	72,3	9	69,2	16	69,6	110	73,8
D. farinae	5	10,4	9	13,8	2	15,4	3	13,0	19	12,8
Dermatophagoides spp. gelişim formu	2	4,2	4	6,2	0	0,0	2	8,7	8	5,4
E. maynei	1	2,1	1	1,5	0	0,0	0	0,0	2	1,3
Cheyletus spp.	0	0,0	0	0,0	1	7,7	0	0,0	1	0,7
T. putrescentiae	1	2,1	1	1,5	0	0,0	0	0,0	2	1,3
C. arcuatus	0	0,0	0	0,0	1	7,7	1	4,3	2	1,3
L. destructor	0	0,0	1	1,5	0	0,0	0	0,0	1	0,7
Teşhis edilemeyenler	1	2,1	2	3,1	0	0,0	1	4,3	4	2,7
Toplam	48	100,0	65	100,0	13	100,0	23	100,0	149	100,0
p	0,786 (χ^2=18,336)									

χ^2: Likelihood ratio chi-squared test

D. pteronyssinus baskın tür olarak (%74) belirlenmiş, *D. evansi*'ye ise rastlanılmamıştır.

Ev tozu akarının prevalansı Kayseri'de (26) %20, Kütahya'da (27) %18, Bitlis ve Muş'ta (28,29) %100, Erzincan'da (30) %94,4, Bursa'da (31) %34,4, İstanbul'da %66,7, Tekirdağ'da %38,5 ve Sivas'ta (32) %18 oranında bildirilmiş, aynı ilde yapılan bir başka çalışmada ise tespit edilememiştir (33). Samsun (25), Ordu (34), Giresun (22), Bitlis ve Muş'ta (28,29) evlerin tamamında (%100) toz akarı tespit edilmiş olmasına karşın Kalpaklioğlu ve ark. (35) Karadeniz Bölgesi genelindeki yaygınlığı %46 olarak ifade etmişlerdir. Bu durumun çalışma merkezleri, dönemleri ve kullanılan yöntemlerin farklılığından veya Kalpaklioğlu ve ark.'nın (35) prevalans, diğer araştırıcıların (22,25,28,36) ise popülasyon dinamiği çalışmaları yapmış olmalarından kaynaklanmış olabileceği düşünülmektedir.

Türkiye'de *D. pteronyssinus* ve *D. farinae* sırasıyla Samsun'da (25), %60,8 ve %3,8, Erzincan'da (24,30) %63,3, ve %5,1, Hatay'da (37) %72,2 ve %20 ve Giresun'da (22) %81,8 ve %0,5 oranında bildirilmiştir. Bitlis ve Muş'ta (28,29) %78,9-83,2 ve %0,24 oranında tespit edilmiş, sınırlı sayıda olmakla birlikte *Dermatophagoides evansi*, *Dermatophagoides siboney*, *Dermatophagoides aureliani* ve *E. maynei* de saptanmıştır. Gerçekleştirilen çalışmada *D. pteronyssinus* %74, *D. farinae* %13, *Dermatophagoides* spp. gelişim formları %5 ve *E. maynei* %1 oranında tespit edilmiştir. *D. pteronyssinus* evlerin %90,3'ünde, *D. farinae* ise %25'inde gözlenmiştir.

Ev tozundaki alerjen yoğunluğunun soğuk ve kuru bölgelerde düşük, deniz kıyısı gibi nemli ve sıcak bölgelerde ise yüksek seviyede olduğu bildirilmiştir (6,15,38). Demirtaş ve ark. (39) akar alerjenlerini evlerin %54,1'inde, Gulbahar ve ark. (40) ise %53,8'inde tespit etmişler, çalışmada ise %93 oranında belirlenmiştir. Araştırmamız dahil her üç çalışma da deniz seviyesinde gerçekleştirilmiş olmasına karşın tespit ettiğimiz yüksek oranın çalışma bölgesi, dönemi ve toz temin etme yöntemlerinin farklılığından kaynaklanmış olabileceği düşünülmektedir. Gulbahar ve ark. (40) Der p 1 ve Der f 1 antijenlerini miks %23 oranında olduğunu bildirmişlerdir. Çalışmamızda da benzer şekilde miks antijen evlerin %25'inde tespit edilmiştir.

Mikroskobik olarak *D. farinae* evlerin %25'inde, *D. pteronyssinus* ise %90,3'ünde tespit edilmesine karşın antijen pozitifliği açısından *D. farinae* %84,7, *D. pteronyssinus* ise %93 oranında saptanmıştır. Araştırmada akar mikroskobisi ve antijen varlığı arasında doğrudan bir ilişki beklenmekle beraber *D. farinae*'de antijen varlığı daha yüksek oranda gözlenmiştir. Bu durumun yataklarda bulunan az sayıdaki akarın vücut salgı ve partiküllerinin zamanla birikebileceğinden veya numunelerin yatakların bütün katmanlarından alınmasından kaynaklanmış olabileceği düşünülmektedir. Kort ve Kniest (11) akarların canlılıkları sonlandıktan sonra kalan rezidülerinin 4 yıl boyunca antijenik aktivitelerini sürdürdüğünü, bu nedenle yatakların alerjen rezervuarı olabileceğine dikkat çekmişlerdir.

Arlian ve ark. (14) gram-tozda 2 µg grup I antijeninin yaklaşık 100 adet akara denk geldiğini bildirmiştir. Çalışmada saptanan en yüksek antijen miktarı Der p 1 için 1,272 2 µg, Der f 1 için ise 0,482 µg olmasına karşın mikroskobik olarak tespit edilen akar sayısı araştırıcıların bildirdiğinden düşük gözlenmiş ve Gram-tozdaki akar sayısı 23 olarak belirlenmiştir. Bu farklılığın tozun elde edilmesinde kullanılan yöntemden, iklim ve mevsim farklılıklardan veya numunenin elde edilmesinde araştırıcıların müdahil olamadığı durumlardan kaynaklanmış olabileceği düşünülmektedir.

SONUÇ

Araştırmanın yürütüldüğü Ordu, Giresun, Trabzon ve Rize'de saptanan akar türleri ve dağılımları arasında bir fark gözlenmemiştir (p>0,05). *D. pteronyssinus*, *D. farinae*, *E. maynei* ve *Dermatophagoides* spp. gelişim formlarının genel toplamı popülasyonunun %93'ünü oluşturmuştur. Bu oranın yüksek olması örneklerin sadece yataklardan elde edilmiş olmasıyla açıklanabilmektedir. Benzer şekilde depo/gıda akarlarının düşük düzeyde (%4) bulunmasının da zeminlerden örnek alınmamış olmasıyla ilgili olabilir.

Antijen testlerinin, akar tespitinde kullanılan mikroskobik yöntemlere ek olarak, evlerin akar alerjen yüklerinin ayrıntılı değerlendirilmesinde alternatif bir yöntem olarak kullanılabileceği ve duyarlı kişilerin yaşadığı ortamlar açısından bu teşhis yönteminin dikkate alınması gerektiği düşünülmektedir.

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Etik Kurul Onayı: Çalışmada hasta materyali ve deney hayvanı kullanılmamış olması nedeniyle etik kurul onayı gerektirmemektedir.

Hasta Onayı: Çalışmada hasta materyali ve deney hayvanı kullanılmamış olması nedeniyle hasta onayı gerektirmemektedir.

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Subtype Distribution of *Blastocystis* in Türkiye

Türkiye'de Blastocystis Alt Tip Dağılımı

Erdoğan Malatyalı, Hatice Ertabaklar, Sema Ertuğ

Aydın Adnan Menderes University Faculty of Medicine, Department of Parasitology, Aydın, Türkiye

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ABSTRACT

Blastocystis is an anaerobic protozoan with global importance because of infecting a variety of hosts and having high prevalence in many countries. *Blastocystis* isolates display remarkable genetic differences, and many subtypes (STs) have currently been defined based on polymorphism in *SSU rRNA* coding gene. Each 25 subtype may have different characteristics such as pathogenicity, host specificity, and structural variations. Most current research on *Blastocystis* has focused on these differences and molecular epidemiology. This review aimed to provide a summary of *Blastocystis* subtype distribution in Türkiye. Regarding human samples, 16 manuscripts were found in the literature, which presented 783 *Blastocystis* isolates from 9 cities in Türkiye. The most common subtype was ST3 (47.9%), the others were ST1 30 (17.5%), ST2 (14.7%), ST4 (4%), and ST5-ST7 (15.9%). There were few studies on animal hosts and environmental samples. The faecal samples from rats, farm, and pet animals were examined for *Blastocystis* subtypes and ST1, ST3, ST4-ST7, ST10, and ST12-ST14 were reported. In addition, two studies reported *Blastocystis* ST1 and ST3 subtypes in environmental water samples. In conclusion, the review of available literature showed that a systematic understanding of the subtype distribution of 35 *Blastocystis* in Türkiye is still lacking. Most of the studies were performed in a limited number of cities, animal hosts, and environmental samples, therefore, more studies from different provinces are needed in forthcoming research. The majority studies were performed in a limited number of provinces, animal species and very few environmental samples, so in the future; there is a need of novel studies that evaluate more samples from different provinces

Keywords: *Blastocystis*, subtypes, Türkiye

ÖZ

Blastocystis, farklı konaklarda enfeksiyon oluşturması ve birçok ülkede yaygın görülmesi nedeniyle küresel öneme sahip anaerobik bir protozoondur. *Blastocystis* izolatları arasında yüksek derecede genetik farklılıklar gözlenmekte olup *SSU rRNA* gen bölgesindeki polimorfizmlere dayanan birçok alt tipi (ST) tanımlanmıştır. Bu alt tipler patojenite, konak özgüllüğü ve yapısal varyasyonlar gibi farklı fenotipik özellikler sergilemektedir. *Blastocystis* ile ilgili güncel araştırmaların çoğu, bu farklılıklara ve moleküler epidemiyolojiye odaklanmıştır. Bu derleme ile Türkiye'deki *Blastocystis* alt tipi dağılımını konu alan çalışmaların özetlenmesi amaçlanmıştır. İnsan örnekleri ile ilgili olarak Türkiye'nin 9 ilinden toplam 783 *Blastocystis* izolatının yer aldığı 16 makale literatürde yer almaktadır. En sık görülen alt tip ST3 (%47,9) olup bunu sırasıyla ST1 (%17,5), ST2 (%14,7), ST4 (%4) ve ST5-ST7 (%15,9) izlemektedir. Ülkemizde hayvanlar ve çevresel örnekler üzerine az sayıda çalışma yapılmıştır. Sıçan, çiftlik ve evcil hayvanlardan alınan dışkı örnekleri *Blastocystis* alt tipleri açısından incelenmiş olup bu araştırmalarda ST1, ST3, ST4-ST7, ST10 ve ST12-ST14 alt tipleri rapor edilmiştir. Ek olarak, iki çalışmada çevresel su örneklerinde *Blastocystis* ST1 ve ST3 alt tipleri bildirmiştir. Sonuç olarak, mevcut literatür incelendiğinde, *Blastocystis*'in Türkiye'deki alt tip dağılımına ilişkin sistematik ve kapsamlı çalışmaların halen eksik olduğu görülmektedir. Çalışmaların çoğu sınırlı sayıda ilde, sınırlı hayvan türünde ve çok az sayıda çevresel örneklerde yapılmıştır, bu nedenle ilerideki araştırmalarda farklı illerden daha fazla örneğin değerlendirileceği çalışmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: *Blastocystis*, alt tip, Türkiye

INTRODUCTION

Blastocystis is an intestinal, anaerobic protozoan that infects humans and many other species including primates, canids, swine, rodents, birds, reptiles, and cockroaches (1). It has recently been classified in stramenopiles (Heteroconta) but differs from others because of colonizing the human intestine and not having a flagellated form. It has a global distribution with an independency of climate and many epidemiological studies reported *Blastocystis* as the most common protozoan in human faecal

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Address for Correspondence/Yazar Adresi: Erdoğan Malatyalı, Aydın Adnan Menderes University Faculty of Medicine, Department of Parasitology, Aydın, Türkiye
Phone/Tel: +90 505 380 06 58 **E-mail/E-Posta:** erdogan.malatyali@adu.edu.tr **ORCID ID:** orcid.org/0000-0002-3943-467X

Türkiye PARAZİTOLOJİ Dergisi

samples (2). *Blastocystis* has a frequency rate of up to 100% in underdeveloped countries, because of poor hygiene habits and socio-economic factors (3). The studies in Türkiye revealed a prevalence of *Blastocystis* varying between 1.4% and 23.5% (4). These studies are highly different from each other in terms of diagnostic methods and also the study population.

Although it has been a long-time since it was revealed that *Blastocystis* infects humans, genetic diversity, life cycle, treatment, and pathogenesis of *Blastocystis* have been subjected to considerable discussions in the literature (5-7). It was defined as a pathogen, opportunistic pathogen, or non-pathogenic microorganism. The main problem encountered here was that *Blastocystis* was found in both healthy individuals and symptomatic groups (8-10). *Blastocystis* infections are asymptomatic in a high rate of infected individuals and gastrointestinal symptoms are rarely observed (2,5). It was suggested that *Blastocystis* may be an indicator of healthy intestinal flora (11,12). In addition to non-specific gastrointestinal symptoms such as diarrhoea, abdominal pain, and bloating, dermatological symptoms can be seen in symptomatic *Blastocystis* infections (2,10,13).

Blastocystis has high polymorphic variations in its genome. Following the molecular phylogenetic analysis of *Blastocystis* small subunit ribosomal *RNA* gene (SSU rRNA), many genotypes or subtypes (STs) have been defined in isolates from humans and non-human hosts (14). Currently, the number of proposed subtypes reached at least to 31; however, some of them are not validated yet and a strong argument is raised (15,16). Recently, many reviews reported *Blastocystis* subtypes in different countries, but not from Türkiye. In this paper, we reviewed the available studies on *Blastocystis* subtypes in humans, animal hosts, and environmental samples.

Blastocystis Subtypes in Human Samples

Türkiye is a transcontinental country that bridges Europe and Asia. It is considered to have suitable conditions for a high prevalence and genetic diversity of *Blastocystis* such as moderate climate, inadequate sanitation in some cities, and migration from other countries. The first available record on *Blastocystis* was published in 1969, a case report (17). There was a large paucity of *Blastocystis* research or papers until the 1990s. Afterwards, studies about the frequencies in renal transplant patients and the treatment of *Blastocystis* were published (18,19). Because of unfamiliar findings on *Blastocystis* pathogenicity, most studies have focused on the clinical properties of *Blastocystis*-infected people. Research on the subject has been mostly limited to the comparisons of patient and control groups before the identification of *Blastocystis* subtypes. The possible clinical consequences of *Blastocystis* infection were studied in different patient groups such as iron deficient, cancer, haemodialysis, irritable bowel syndrome, and inflammatory bowel disease patients (20-23).

Among the *Blastocystis* subtypes, ST1 to ST9 and ST12 have been isolated from human samples (14). The common subtypes in humans are ST1 to ST4, in addition, ST9 has not been reported other than in human samples (16). The studies on *Blastocystis* subtypes in different provinces of Türkiye mostly revealed similar findings in terms of subtype distribution; however, some differences were reported in terms of the number of subtypes. More recently, ST1-7 has been found in human samples in Türkiye. Most of them revealed the predominance of ST3, followed by either ST2 or ST1. In general, Europeans are mostly colonized by ST1–ST4, in contrast, ST4 is rarely found in other territories including Africa, America, and the Middle East (24). The studies from Türkiye used two methods to identify *Blastocystis* subtypes: Partial sequencing of SSU rDNA coding gene also known as barcoding and sequence-tagged-sites polymerase chain reaction (PCR) (STS PCR). The sequencing of amplified PCR products allows to determine allele differences and intra-subtype genetic variation of *Blastocystis*. In addition, with this method the comparison of other sequences from Genbank or other sources is possible. The detailed data on *Blastocystis* subtypes in human samples is presented in Table 1. *Blastocystis* subtypes from the following cities: Adana, Ankara, Aydın, Erzincan, Eskişehir, İstanbul, İzmir, Manisa, and Muğla. Most studies have been conducted in the Aegean region of Anatolia. Currently, we have no data for human samples in eastern Türkiye. The data from other regions are also very limited, for example, there are only two studies from south of Türkiye, the Mediterranean Region.

Blastocystis Subtypes in Animal Samples

Although relatively most research has been carried out on humans, a very limited number of studies exist that detected *Blastocystis* subtypes in animal samples. The animal hosts of *Blastocystis*, which are previously studied in Türkiye for *Blastocystis* subtypes, are livestock animals, pet animals, and laboratory rats (38-43). The details of these studies are given in Table 2. Host specificity and zoonotic potential of *Blastocystis* have been shown in these studies. The prevalence of *Blastocystis* in animal samples is an important topic for humans, because of the zoonotic potential of *Blastocystis*. It was reported that identified STs in humans are also found in domestic and wild animals (44). Many subtypes including ST1-ST7, ST10, ST12, and ST14 have been reported in cattle samples with higher frequencies of ST10 and ST14 (45,46). Similar to the studies in Türkiye , ST10 is the predominant *Blastocystis* subtype in cattle samples in several countries including the USA (47), China (48,49), the UK, Thailand (50), and Lebanon (51). A study in Türkiye reported that all laboratory animals were infected with ST4, this finding was also consistent with the others that showed the predominance of ST4 in rodents (39,52). A meta-analysis of *Blastocystis* ST found in the cats showed that ST4 (29.5%) was the most common, which was followed by ST10 (22.5%), ST1 (19.8%), and, ST3 (17.6%) (53). In accordance with this data, a study in Türkiye reported that all *Blastocystis* isolates in stray cats were ST4 (38). Finally, birds can be infected with zoonotic subtypes including ST1, ST2, and ST4-ST8, among them ST6 and ST7 are the most common (14,54). The predominant subtypes were ST6 and ST7 in chicken faecal samples in Diyarbakır (42). However, it should be noted that the study groups in animal studies in Türkiye were limited to specific animal groups mostly cattle, sheep, and chicken. *Blastocystis* subtypes in Türkiye are presented in Figure 1.

Blastocystis Subtypes in Environmental Samples

The previous studies reported the presence of *Blastocystis* in water samples, including rivers, recreational waters, and lakes (55,56).

Table 1. *Blastocystis* subtypes in human samples in Türkiye

Province	Study population	Method	ST1	ST2	ST3	Other STs	Ref.
			n (%)	n (%)	n (%)	n (%)	
Manisa	Diagnostic parasitology lab. (n=92)	STS-PCR	17 (18.4)	20 (21.7)	51 (55.4)	ST1+ST3: 2 (2.1) ST2+ST3: 2 (2.1)	(25)
İstanbul	Diagnostic parasitology lab. (n=87)	Sequencing*	8 (9.2)	12 (13.7)	66 (75.8)	ST4: 1 (1.1)	(26)
Ankara	IBD, Chron and diaphoretic patients (n=35)	STS-PCR	1 (2.8)	10 (28.6)	21 (60)	ST2:+ST1: 2 (5.7) ST2+ST3: 1 (2.8)	(20)
Adana	Symptomatic and non-symptomatic cases (n=32)	STS-PCR	20 (62.5)	3 (9.3)	9 (28.1)		(27)
İzmir	Symptomatic patients (n=94)	RT-PCR	13 (13.8)	11 (11.7)	42 (44.7)	ST6: 1 (1.1) ST7 1 (1.1) ST2+ST3: 2 (2.1) ND: 24 (25.5)	(28)
Aydın	Diagnostic parasitology lab. (n=61)	STS PCR	9 (20.5)	13 (29.5)	17 (38.6)	ST1+ST3: 4 (9.1) ST2+ST3: 1 (2.3) ND: 17 (27.9)	(29)
Aydın	Ulcerative colitis patients (n=12)	Sequencing*	2 (16.7)	1 (8.3)	8 (66.7)	ST7: 1 (8.3)	(30)
Aydın	Cancer patients (n=25)	STS PCR	5 (23)	4 (18)	13 (59)	ND: 3 (12)	(31)
İstanbul	Diagnostic parasitology lab. (n=50)	Sequencing*	3 (6)	2 (4)	34 (68)	ST1+ST3: 1 (2) ST2+ST3: 1 (2) ST3+ST5: 3 (6) ST4+ST5: 1 (2) ST1+ND: 1 (2) ST3+ND: 4 (8)	(32)
Muğla	Children (n=35)	Sequencing*	11 (31.4)	9 (25.7)	12 (34.2)	ST7: 1 (2.8)	(33)
Eskişehir	Children (n=46)	RT-PCR	12 (26.1)	4 (8.7)	20 (43.4)	ST4: 5 (10.9) ST1+ST3: 3 (6.5) ST1+ST2: 1 (2.1) ST2+ST3: 1 (2.1)	(34)
Aydın	Diagnostic parasitology lab. (n=95)	Sequencing* and STS PCR	17 (17.9)	21 (22.1)	50 (52.6)	ST7: 4 (4.2) ST2+ST3: 2 (2.1) ST1+ST3: 1 (1.1)	(4)
Erzincan	Urticarial patients and control group (n=18)	STS PCR	4 (22.2)	1 (5.5)	13 (72.2)		(35)
Muğla	Pregnant women (n=14)	Sequencing*	3 (21.4)	2 (14.2)	9 (64.4)		(36)
Adana	Haemodialysis patients and control group (n=44)	STS PCR	1 (12.5)	2 (25)	2 (25)	ST3+ST6: 2 (25) ND:1 (12.5)	(21)
İzmir	Cancer patients (n=43)	Sequencing*	11 (25.6)		8 (18.6)	ST4: 24 (55.8)	(37)

STS PCR: Sequence-tagged-sites PCR, RT-PCR: Real-time PCR, ND: Not detected, * Sanger sequencing, PCR: Polymerase chain reaction

Table 2. *Blastocystis* subtypes in animal samples in Türkiye

Province	Animal	Subtypes n (100)	Method	Ref
Aydın	Cattle (n=9)	ST14: 7 (77.7) ST10: 2 (22.3)	Sequencing*	(43)
İzmir	Stray cats (n=7)	ST 4: 7 (100)	Sequencing*	(38)
Kayseri	Cattle (n=32)	ST10: 32 (100)	Sequencing*	(40)
	Sheep (n=84)	ST10: 84 (100)		
	Chicken (n=32)	ST7: 32 (100)		
	Water buffaloes (n=35)	ST14: 35 (100)		
Aydın	Laboratory rats (n=33)	ST4: 33 (100)	Sequencing*	(39)
Kayseri	Cattle (n=88)	ST10: 88 (100)	Sequencing*	(41)
Diyarbakır	Sheep (n=4)	ST5: 4 (100)		
	Chicken (n=18)	ST6: 6 (33.3%) ST7: 11 (61.1%) ST6 and ST7: 1 (5.5%)	Sequencing* and STS PCR	(42)
İzmir	Cattle (n=13)	ST1, ST3, ST13: 1 (7.7%) ST5, ST12: 2 (15.4%) ST10: 6 (46.1%)	Sequencing**	(55)

*Sanger sequencing, ** Not available from the manuscript

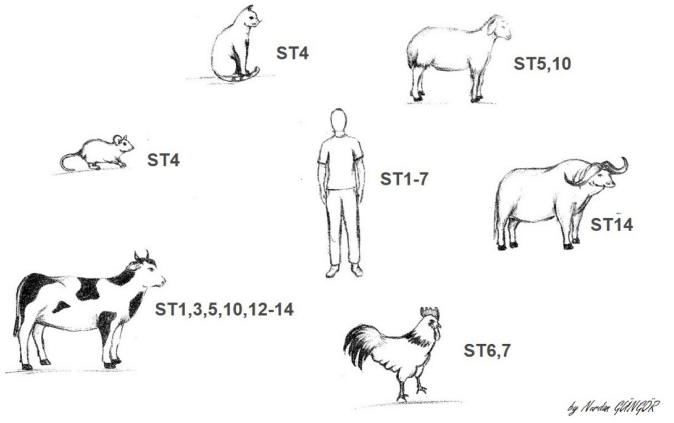

Figure 1. *Blastocystis* subtypes in human and animal hosts in Türkiye. The illustration was originally created by Nurdan Güngör

Lack of appropriate filtration or piped water supplies may be a significant risk factor for the spread of *Blastocystis* infection (57,58). In addition, the consumption of raw or untreated water sources in the environment may be associated with the infection (59,60). Currently, two studies in Türkiye investigated *Blastocystis* subtypes in environmental samples. The first study investigated the *Blastocystis* subtypes in different water samples from Middle Black Sea Region (Sinop, Amasya, and Samsun). They defined subtypes in ten samples and found an equal distribution of ST1 and ST3 (61). The subtypes in this study were as follows: Sinop (ST1, one seawater sample), Samsun (ST1 and ST3, two and one river samples), and Amasya (ST1 and ST3, two and four river samples). Another study in the same region included only river samples and subtyped four *Blastocystis* isolates. They reported that three were ST1 and one was ST3 (62).

CONCLUSION

In different provinces, *Blastocystis* subtypes were investigated in limited numbers and sample sizes. More data is required to have better information on the *Blastocystis* molecular epidemiology in Türkiye. In addition, there is a need for studies dealing with *Blastocystis* subtypes in animal samples, especially wild animal hosts.

* Ethics

Peer-review: Internally peer-reviewed.

* Authorship Contributions

Concept: E.M., H.E., S.E., Design: E.M., H.E., S.E., Data Collection or Processing: E.M., Analysis or Interpretation: E.M., H.E., S.E., Literature Search: E.M., H.E., S.E., Writing: E.M.

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The Parasites of Cats in Türkiye

Türkiye'de Görülen Kedi Parazitleri

Öykü Barılı, Tuğçe Tuygun, Elif Burcu Gençay Topçu, Şinasi Umur

Ondokuz Mayıs University Faculty of Veterinary Medicine, Department of Veterinary Parasitology, Samsun, Türkiye

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ABSTRACT

Cats have an important and different place due to their close relationships with humans. Since most of the parasites they carry are zoonotic, it is important to detect them. According to the research, *Dipylidium caninum, Joyeuxiella pasqualei, Toxocara* spp., *Toxascaris leonina, Giardia* spp., *Isospora* spp., and *Toxoplasma* sp. were found to be higher in cats compared to other parasites. It has been determined that scabies and flea infestations are common as ectoparasites. This review aims to present the reported parasites and their prevalence rates in cats in Türkiye.
Keywords: Cat, helminth, ectoparasite, protozoan, Türkiye

ÖZ

Kediler, insanlarla olan yakın ilişkileri nedeniyle önemli ve farklı bir yere sahiptir. Taşıdıkları parazitlerin birçoğunun zoonoz olması sebebiyle bu parazitlerin tespit edilmesi önemlidir. Yapılan çalışmalar incelendiğinde, kedilerde *Dipylidium caninum*, *Joyeuxiella pasqualei, Toxocara* spp., *Toxascaris leonina, Giardia* spp., *Isospora* spp. ve *Toxoplasma* sp.'nin, diğer parazitlere göre yüksek oranda bulunduğu görülmüştür. Ektoparazitler açısından değerlendirildiğinde, uyuz etkenleri ve pire enfestasyonlarının yaygın olduğu tespit edilmiştir. Bu derlemede, Türkiye'de kedilerde bugüne kadar bildirilmiş parazitler ve yaygınlık oranlarının verilmesi amaçlanmıştır.
Anahtar Kelimeler: Kedi, helmint, ektoparazit, protozoon, Türkiye

INTRODUCTION

Many historical findings regarding the domestication of cats have been recorded. Although it has yet to be determined periodically, it is estimated that it reached the period when agriculture started 9.500 years ago. Looking at the 5.300-year-old cat fossils found in China, it was seen that cats were more common in agricultural areas. Based on these findings, it is suggested that farmers cooperate with cats to protect their fields from pests such as mice (1,2).

According to the findings obtained in a recent study, a cat's bone was found next to a human skeleton in Cyprus and showed that these cats have adapted to human lives since ancient times (3).

Cats have become integral to human life and are considered family members. These animals, which have developed an emotional bond with us, are considered harmless and cute, even if they are looked after and fed on the streets. However, such coexistence paves the way for the transmission of many diseases. Parasitic diseases cover many of the conditions found in cats. They are essential for public health because some are zoonotic, and some parasites carry pathogenic agents with zoonotic properties.

Doğanay (4) made a similar review on cat parasites in Türkiye in the intervening 30 years, but many new studies have been conducted from that time to today, and new parasites have been recorded. Therefore, this compilation has been made to provide up-to-date information, and the parasites seen in cats in Türkiye are given in Tables (1-5).

METHODS

References used in this review article; were obtained by searching the archive data of various journals and publications in electronic media such as PubMed,

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Address for Correspondence/Yazar Adresi: Öykü Barılı, Ondokuz Mayıs University Faculty of Veterinary Medicine, Department of Veterinary Parasitology, Samsun, Türkiye
Phone/Tel: +90 534 300 59 09 **E-mail/E-Posta:** oykudoyran5@gmail.com **ORCID ID:** orcid.org/0000-0001-7692-5171

Türkiye PARAZİTOLOJİ Dergisi

Scopus, and Google Scholar. Communication was made with the relevant publishing houses for all the articles whose full text could not be reached.

While searching the literature, general terms such as cat, helminth, parasite, protozoan, and ectoparasite were used, and then the research was deepened by using more specific words.

This review was written using the articles cited in the references. In the tables prepared, helminths reported in cats are in Table 1, ectoparasites are in Table 2, and protozoans are in Table 3. While parasitic prevalences are indicated in the table, the number and percentages of animals written in the source articles are added.

Table 1. Trematode species in cats

Trematode species	City	Prevalence	Method	Reference
Dicrocoelium dentriticum	İstanbul	2.35%	Fecal examination	(5)
Dexiogonimus ciureanus (Sin.: *Metagonimus ciureanus*)	Bursa	+	Necropsy	(6)
Fasciola hepatica	İstanbul	1.76%	Fecal examination	(5)
*Metagonimus yokogawai**	Kars	5.8% (1/17) ***	"	(7)
*Metorchis albidus** (Sin.: *M. bilis*)	Bursa	14% (14/100)	"	(8)
Opisthorchis tenuicollis * (Sin.: *O. felineus*)	Ankara	0.66%	Necropsy	(9)
	"	+	"	(10)
	Elazığ	1%	"	(11)
	"	16.6%	"	(12)
	Van	+	"	(13)
Platynosomum fastosum	Bitlis	+	Fecal examination/eggs	(14)

Table 2. Cestode species in cats

Cestode species	City	Prevalence	Method	Reference
*Dipylidium caninum**	Bursa	65% (65/100)	Necropsy	(8)
	Elazığ	33%	"	(11)
	"	22.2%	"	(12)
	Konya	28%	Necropsy	(15)
	Ankara	+	Experimental infection	(16)
	"	46%	Necropsy	(17)
	Hatay	12.5%	"	(18)
	Van	5.94% (4/140)	Fecal examination	(19)
	İzmir	0.21% (1/465) ***	"	(20)
Diplopylidium noelleri	Bursa	12% (12/100)	Necropsy	(8)
	Elazığ	19%	"	(11)
	"	33.3%	"	(12)
	Konya	5%	"	(15)
	Ankara	6%	"	(17)
Echinococcus granulosus *	Ankara	+	"	(21)
	Van	+	"	(104)
Hymenolepis sp.*	İzmir	0.21% (1/465)***	Fecal examination	(20)
Joyeuxiella spp.	Bursa	33% (33/100)	Necropsy	(8)
	Elazığ	64%	"	(11)
	Van	11.9% (8/140)	"	(19)
	"	7.84% (4/51)	Fecal examination	(22)
	Kırıkkale	4.2%	"	(23)
Joyeuxiella echinorhynchus (Sin.: *J. echinorhynchoides*)	Elazığ	2.7%	Necropsy	(12)
	Ankara	1%	"	(17)

Table 2. continued

Cestode species	City	Prevalence	Method	Reference
Joyeuxiella pasqualei	Elazığ	36.1%	Necropsy	(12)
	Konya	58%	"	(15)
	Ankara	36%	"	(17)
	Hatay	50%	"	(18)
	Ankara	+	"	(24)
	İstanbul	7.6% (2/26)***	Fecal examination	(25)
Mesocestoides sp.*	Elazığ	20%	Necropsy	(11)
	Hatay	12.5%	"	(18)
	Ankara	+	Operation	(26)
*Mesocestoides lineatus**	Elazığ	19.4%	Necropsy	(12)
*Tetrathyridium elongatus**	"	8.3%	"	(12)
Taenia sp.	Van	7.84%	Fecal examination	(22)
	Ankara	5.3%	Necropsy	(27)
	Elazığ	5%	Fecal examination	(28)
Taenia taeniaeformis	Bursa	3% (3/100)	Necropsy	(8)
	Elazığ	59%	"	(11)
	"	44.4%	Necropsy	(12)
	Konya	10%	"	(15)
	Ankara	11%	"	(17)
	Hatay	25%	"	(18)
	Van	+	"	(22)

Table 3. Species of nematodes identified in cats

Nematode species	City	Prevalence	Method	Reference
Aelurostrongylus abstrusus	İstanbul	+	Fecal examination	(29)
	Kırıkkale	+	Necropsy	(30)
	Balıkesir	+	Fecal examination & radiography	(31)
Ancylostomidae*	Van	11.9% (8/140)	Fecal examination	(19)
	Kırıkkale	4.2%	"	(23)
Ancylostoma sp.*	Hatay	12.5%	Fecal examination	(18)
	Van	7.84% (4/51)	"	(22)
*A. tubaeforme**	Kırklareli	+	Necropsy	(32)
Ascarit sp.*	Ankara	2.7%	Necropsy	(27)
Capillaria sp.	"	4%	"	(17)
Capillaria aerophila	Elazığ	4%	"	(11)
	Ankara	3.3%	Necropsy	(33)
Ollulanus tricuspis	Bursa	9% (9/100)	Necropsy	(8)
	Elazığ	19.4%	"	(12)
	Ankara	17%	"	(17)
	Van	+	"	(22)
Physaloptera sp.	İstanbul	0.58%	Fecal examination	(33)
Physaloptera praeputialis	Bursa	3% (3/100)	Necropsy	(8)
	Elazığ	6%	"	(11)
	"	8.3%	"	(12)
	Konya	2%	"	(15)
	Ankara	3%	"	(17)

Table 3. continued

Nematode species	City	Prevalence	Method	Reference
Strongyloides sp.*	Antalya	+	Fecal examination	(33)
	Bursa	+	"	(33)
	İstanbul	0.58%	"	(33)
Toxocara spp.*	İzmir	3.01% (14/465)***	"	(20)
	Kırıkkale	48.9%	"	(23)
	Elazığ	43%	"	(28)
	Samsun	27.8%	"	(34)
	Ankara	13.3%	Egg control on the t hair	(35)
*Toxocara cati** (Sin.: *T. mystax*)	Bursa	54% (54/100)	Necropsy	(8)
	Elazığ	5%	"	(11)
	"	47.2%	"	(12)
	Konya	47%	"	(15)
	Ankara	47.6%	"	(17)
	Hatay	62.5%	"	(18)
	Van	37% (28/140)	Fecal examination	(19)
	"	+	Necropsy	(22)
	"	36.29% (18/51)	Fecal examination	(22)
	İstanbul	27.6%	"	(33)
	Ankara	93.76%	"	(36)
	"	95.6% (22/23)	Fecal examination & necropsy	(37)
	"	+	Fecal examination	(38)
	"	49.3%	Necropsy	(39)
*Toxocara canis**	Elazığ	2.7%	"	(12)
	Ankara	24.6%	"	(39)
*Toxascaris leonina**	Elazığ	5.5%	"	(12)
	Ankara	3%	"	(17)
	Van	7.46% (5/140)	Fecal examination	(19)
	"	25.53% (12/51)	"	(22)
	Elazığ	1%	"	(28)
	İstanbul	20.5%	"	(33)
	Samsun	1.8%	"	(34)
	Ankara	6.25%	"	(36)
	"	3.3%	Necropsy	(39)
Trichuris spp.*	Hatay	12.5%	Fecal examination	(18)
	İstanbul	0.58%	"	(33)
	Samsun	3.2%	"	(34)
Troglostrongylus brevior	Samsun	+	Necropsy	(40)
*Unciaria stenocephala**	Elazığ	1%	"	(11)
	Elazığ	2.7%	"	(12)
	Ankara	+	Experimental infection	(41)

Table 4. Ectoparasite speces in cats

Main groups	Parasite species	City	Number of examined cats	Prevalence	Reference
Mites	Scabies	Ankara	300	5%	(42)
	*Notoedres cati**	Elazığ	36	2.7%	(12)
		Ankara	150	2.6%	(39)
		Ankara	1	+	(43)
		Van	8	37.5%	(44)
		İstanbul	2.200	6.6%	(45)
		Aydın	1	+	(46)
	Otodectes cynotis	Elazığ	36	8.3%	(12)
		Ankara	150	6%	(39)
	*Cheyletiella blakei**	Ankara	1	+	(47)
		"	8	+	(48)
		Elazığ	100	14%	(49)
		İstanbul	1	+	(50)
		Kırıkkale	2	+	(51)
Ticks	*Haemaphysalis otophila**	**	**	+	(56)
	*Ixodes ricinus**	**	**	+	(56)
	*Rhipicephalus sanguineus**	Elazığ	100	3%	(49)
Lice	*Felicola subrostratus*	Kocaeli	1	+	(52)
Fleas	*Ctenocephalides canis*	Elazığ	36	5.5%	(12)
		"	5	10%	(53)
		Antalya	23	1.06%	(54)
		İstanbul and Hatay	15	12%	(25)
		Hatay	50	36%	(55)
	Ctenocephalides felis	Ankara	100	9%	(17)
		Antalya	23	98.94%	(54)
		Elazığ	36	8.3%	(12)
		Elazığ	100	41%	(49)
		"	5	10.4%	(53)
		Hatay	50	64%	(55)
		İstanbul and Hatay	15	88%	(25)
	*Pulex irritans**	Elazığ	5	12%	(53)
	Xenopsylla cheopis	"	5	8.3%	(53)
Diptera	*Lucilia sericata** (1st stage larvae)	Aydın	1	+	(57)
	Lucilia sericata (2nd and 3rd stage larvae)	Konya	1	+	(58)
	Lucilia sericata (3rd stage larvae)	Afyon	1	+	(59)
		Konya	1	+	(60)
	*Phormia regina**	Samsun	3	+	(61)
Pentastomida	*Linguatula serrata** (nimf)	Elazığ	100	1%	(49)

Table 5. Protozoon species in cats

Protozoan species	City	Number of examined cats	Prevalence	Method	Reference
*Anaplasma phagocytophilum**	Tekirdağ	167	7.2%	PCR	(62)
*Anaplasma platys**	"	"	30.5%	"	(62)
*Babesia microti**	"	"	2.4%	"	(62)
Babesia canis canis	"	"	24%	"	(62)
Babesia felis	Van	120	10.8%	Blood smear	(63)
Cryptosporidium sp.*	Kırıkkale	100	1%	Flotation, Giemsa stain	(23)
	"	140	10.44%	Flotation, sedimentation, carbol-fuchsin stain	(19)
	Van	46	13.0%	Formol-ether sedimentation method	(64)
	Van	100	2.1%	PCR	(65)
*Cryptosporidium felis**	Kırıkkale	1	+	PCR	(66)
Cytauxzoon felis	Tekirdağ	167	6.6%	PCR	(62)
	Van	120	7.5%	Blood smear	(67)
Ehrlichia canis	Burdur	1	+	IFAT	(68)
Giardia cati	Ankara	100	4%	Giemsa stain, flotation	(17)
*Giardia duodenalis** (Sin.: *G. intestinalis*)	Burdur	1	+	ZnS04 centrifuge flotation method, Giemsa stain	(69)
	Central Anatolia region	102	68.6%	PCR	(70)
	Kayseri, Samsun	100	8%	PCR	(71)
Hepatozoon canis	Aydın	1	+	PCR	(72)
Hepatozoon felis	Tekirdağ	167	10.8%	PCR	(62)
Isospora sp.	Van	140	43.28%	Flotation, sedimentation, carbol-fuchsin stain	(19)
	"	51	19.61%	Flotation	(22)
	Kırıkkale	100	31%	Fecal examination	(23)
	Samsun	187	1.8%	Flotation	(34)
Isospora felis (Sin.: *Cystoisospora felis*)	Ankara	100	43%	Giemsa stain, flotation	(17)
	"	5	40%	Flotation	(36)
	Elazığ	36	5.5%	Sporulation	(12)
	"	100	20%	Parasitological examination	(49)
	"	3	+	Flotation	(74)
	İstanbul	212	18.9%	Flotation, sedimentation	(73)
Isospora rivolta	Ankara	100	21%	Giemsa stain, flotation	(17)
	İstanbul	212	2.8%	Flotation, sedimentation	(73)
	Elazığ	36	16.6%	Sporulation	(12)
	"	3	+	Flotation	(74)
Isospora bigemina	İstanbul	212	2.3%	Flotation, sedimentation	(73)

Table 5. continued

Protozoan species	City	Number of examined cats	Prevalence	Method	Reference
*Leishmania infantum**	Adana, Mersin	22	4.5%	PCR	(75)
	İzmir, Aydın, Muğla, Manisa	147	8.84%	PCR	(76)
	İzmir	1101	10.8% ELISA 15.2% IFAT	IFAT, ELISA	(77)
	İzmir	19	5.2%	IFAT, PCR	(78)
	Aydın	1	+	PCR	(79)
	Aydın, Muğla, İzmir, Manisa	386	2.3% PCR 15.6% IFAT	IFAT, PCR	(80)
Sarcocystis sp.	Ankara	100	8%	Giemsa stain, flotation	(17)
*Tritrichomonas foetus**	Samsun	100	0%	PCR	(81)
	Ankara	45	8.8%	PCR	(82)
	İstanbul	1	+	Giemsa stain	(83)
*Toxoplasma gondii**	Ankara	77	23.4%	SFDT	(84)
	"	300	0.3%	Necropsy, histopathology	(27)
	"	248	0.4%	Flotation	(86)
	"	2	+	Necropsy, histopathology	(87)
	"	65	43%	SFDT	(90)
	"	99	40.3% SFDT 34.3% IFAT	SFDT IFAT	(93)
	"	14	100%	USG, PCR, ELISA	(98)
	"	129	66.6%	SFDT	(101)
	"	2	+	ELISA	(102)
	Sivas	50	78%	IHA	(88)
	Kırıkkale	53	69.8%	IHA	(89)
	Elazığ	36	55.5%	SFDT	(91)
	Van	140	16.41%	Flotation, sedimentation, carbol-fuchsin stain	(19)
	"	62	8.06%	IFAT	(92)
	İzmir	1.121	34.2% IFAT 35.6% ELISA	IFAT, ELISA	(94)
	"	465	0.43%	ELISA, SFDT	(103)
	Kars	102	44.1%	SFDT	(95)
	Niğde	72	76.4%	SFDT	(96)
	Kırıkkale, Ankara	102	48.03%	PCR	(97)
	Kars	100	65%	SFDT	(99)
	İstanbul	26	42.3%	ELISA	(100)

+: Only case records are given, *: Zoonotic parasites,**: There is no information about this section in the references, ***: Prevalence rates calculated, ELISA: Enzyme-linked immunosorbent assay, IFAT: Indirect fluorescent antibody test, IHA: Indirect hem agglutination, PCR: Polymerase chain reaction, SFDT: Sabin-Feldman dye test, USG: Ultrasonography

CONCLUSION

To date, 68 parasite species have been reported in cats, including 13 ectoparasites, 33 helminths, and 22 protozoan species in Türkiye. Parasites and the diseases they cause are a point to be considered for public health since some have zoonotic properties (marked with an asterisk).

The most common parasites are *Dipylidium caninum* in Bursa and Elazığ; *Joyeuxiella pasqualei* in Konya and Hatay; *Toxocara* spp. in Ankara, and Hatay; *Toxascaris leonina* in Van, and İstanbul; *Giardia* spp. in Central Anatolia; *Isospora* spp. in Van, and Ankara; *Toxoplasma* sp. in Ankara, Sivas, Kırıkkale, and Kars were found to be high in provinces. As ectoparasitic, scabies agents and flea infestations were more common in Van, Antalya, İstanbul, and Hatay provinces. This evaluation does not have a meta-analysis feature and is based on reporting existing data.

Since there are veterinary faculties in all of the provinces with parasitic density, the research may have been concentrated in these regions. For this purpose, if it is desired to create a table throughout Türkiye, conducting studies in the regions outside these provinces will be important.

As a result, this review will facilitate the studies to be carried out to determine the parasitic fauna in cats in Türkiye and also to find the prevalence rates collectively. At the same time, by specifying the methods of parasite detection, it compares different results in different examination methods.

*** Ethics**

Peer-review: Internally peer-reviewed.

*** Authorship Contributions**

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

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A Ruptured Cystic Echinococcosis in the Gallbladder and Intra/Extrahepatic Biliary Tract, Radiological and Surgical Imaging Findings

Safra Kesesi ve İntra/Ekstrahepatik Safra Yollarında Rüptüre Kistik Ekinokokkozisin Radyolojik ve Cerrahi Görüntüleme Bulguları

Saim Türkoğlu[1], Abdurrahman Ekici[2], Leyla Turgut Çoban[3], İlyas Dündar[3], Ozan Okyay[4], Esra Gürbüz[1]

[1]University of Health Sciences Türkiye, Van Training and Research Hospital, Clinic of Radiology, Van, Türkiye
[2]Van Yüzüncü Yıl University Faculty of Medicine, Department of Parasitology, Van, Türkiye
[3]Van Yüzüncü Yıl University Faculty of Medicine, Department of Radiology, Van, Türkiye
[4]Van Yüzüncü Yıl University Faculty of Medicine, Department of General Surgery, Van, Türkiye

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ABSTRACT

Cystic echinococcosis is a common parasitic infestation that can still cause serious complications in endemic areas. Intrabiliary rupture is a well-defined complication, but rupture into the gallbladder is rare. The disease may present with cholecystitis and cholangitis. Clinicians and radiologists working in the emergency room will find the management of the disease much easier if they become familiar with the clinical and radiological findings of the cyst. In this article, a 28-year-old male admitted to the emergency department with acute abdominal pain who was examined for suspected acute cholecystitis and diagnosed with a rupture of the hydatid intra/extrahepatic bile ducts and gallbladder is presented. Our aim is to present the clinical findings and surgical images of the case (ultrasonography, computed tomography, magnetic resonance imaging) and compare them with the literature.
Keywords: Cystic echinococcosis, gallbladder, rupture, ultrasonography, computed tomography, magnetic resonance imaging

ÖZ

Kistik ekinokokkozis, endemik bölgelerde hala ciddi komplikasyonlara neden olabilen yaygın bir parazit enfeksiyonudur. İntrabilier rüptür iyi bilinen bir komplikasyondur, ancak safra kesesine rüptür nadirdir. Hastalık kolesistit ve kolanjit ile kendini gösterebilir. Acil serviste çalışan klinisyenler ve radyologlar, kistin klinik ve radyolojik bulguları iyi bildikleri takdirde hastalığın tedavisini çok daha kolay yapacaklardır. Bu yazıda, akut karın ağrısı şikayeti ile acil servise başvuran, akut kolesistit şüphesiyle muayene edilen ve hidatik intra/ekstrahepatik safra yolları ve safra kesesi rüptürü tanısı alan 28 yaşında erkek hasta sunulmaktadır. Bu çalışmanın amacı olgunun klinik bulgularını ve cerrahi görüntülerini (ultrasonografi, bilgisayarlı tomografi, manyetik rezonans görüntüleme) sunmak ve literatürle karşılaştırmaktır.
Anahtar Kelimeler: Kistik ekinokokkozis, safra kesesi, rüptür, ultrasonografi, bilgisayarlı tomografi, manyetik rezonans görüntüleme

INTRODUCTION

Cystic echinococcosis (CE) is a parasitic infestation most commonly seen in endemic areas and it is caused by *Echinococcus granulosus*. Infection develops when parasitic eggs thrown into the external environment by final host dogs and canines are ingested by humans and natural intermediate hosts, sheep, goats, cattle (1). These cysts are commonly seen in Europe, Mediterranean countries, Asia, the Middle and Far East, South America, and Australia (2).

The clinical signs and symptoms caused by CE depend on the location, size, developmental stage, pressure on

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Address for Correspondence/Yazar Adresi: Abdurrahman Ekici, Van Yüzüncü Yıl University Faculty of Medicine, Department of Parasitology, Van, Türkiye
Phone/Tel: +90 507 704 24 00 **E-mail/E-Posta:** abdurrahman2400@gmail.com **ORCID ID:** orcid.org/0000-0001-6034-513X

Türkiye PARAZİTOLOJİ Dergisi

the surrounding tissues and structures of the cyst and the defense mechanism of the infected person (3).

We present a case of hepatic CE intra-/extrahepatic biliary rupture, which causes obstructive jaundice. It was successfully treated surgically. In this case, our aim is to present the radiological findings and surgical management of parasitic disease, and images used to diagnose it.

CASE REPORT

A 28-year-old male presented to the general surgery clinic two weeks before with abdominal pain. His physical examination revealed pain and hepatomegaly in the epigastric region and palpation in the right upper quadrant of the abdomen. According to the patient's history, he lived in a village and was engaged in animal husbandry. The patient also reported nausea, vomiting, and anorexia. Since this region is endemic in terms of CE, it was suspected in the preliminary diagnosis, and his serum was tested for CE antibodies. Indirect hemagglutination assay (IHA) result was CE positive.

The computed tomography (CT) examination showed a type 3 CE lesion with multiple daughter vesicles and septa in the posterior segment of the right lobe of the liver (Figure 1a). Upon this, we decided to perform elective surgery due to the Coronavirus disease-2019 pandemic and his medical treatment started with albendazole 10-15 mg/kg/day.

Two weeks later, the patient presented to the emergency outpatient clinic with a sudden onset of pain in the right upper quadrant, where physical abdominal pain, tenderness in the abdomen, and cold sweating were observed.

The laboratory findings were as follows: White blood cell count (21.96×10^9) high, serum aspartate transaminase (85 U/L) and alanine aminotransferase (74 U/L) slightly high, direct bilirubin slightly high (0.43 mg/dL), amylase (40 U/L) within normal limits, and gamma-glutamyl transferase high (320 U/L).

Contrast-enhanced CT showed in the right lobe of the liver a connection between the intrahepatic bile ducts in the medial and inferior sections with daughter vesicles (Figure 1b).

Ultrasonographic (US) imaging revealed multiloculated hepatic cystic lesions and dilatation of the intra-/extrahepatic bile ducts. Increased gallbladder size and daughter vesicles were observed in the lumen and common biliary tract (Figure 2a, 2b).

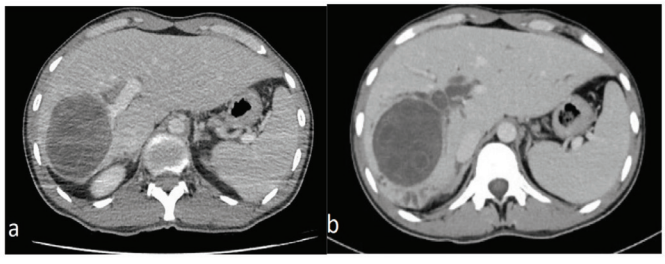

Figure 1a, b. Contrast-enhanced CT image of a 28-year-old male patient admitted to the emergency department with sudden onset abdominal pain two weeks before and at the time of presentation; type 3 cyst hydatid in the liver, before **(a)** and after rupture **(b)** into the intrahepatic biliary tract

CT: Computed tomography

Magnetic resonance imaging (MRI) showed a type 3 CE in the liver and daughter vesicles in the common hepatic duct lumen, as well as daughter vesicles in the gallbladder and common bile duct lumen (Figure 3a-c).

The patient underwent emergency laparotomy in the general surgery unit as well as cystectomy, cyst drainage, cholecystectomy, common bile duct exploration, and T tube drainage. During the operation, we observed CE rupture into the bile ducts and daughter vesicles in the common bile duct. In addition, there were multiple daughter vesicles and cysts in the hydropic gallbladder (Figure 4a, b).

After the operation, all laboratory findings returned to normal values and the patient recovered and was discharged.

DISCUSSION

CE is still an important zoonotic parasitic infection that threatens public health in the world and in our country. It has been reported that there has been a dramatic decrease in the incidence and prevalence of CE throughout the world, especially in recent years. In addition, it is stated that the negative effects of infection on public health and economy continue, especially in developing countries where animal husbandry is common (4,5).

The liver is the most common site of involvement of CE followed by the lungs. All other sites of involvement are considered as unusual, and they include the peritoneal cavity, spleen, kidney, uterus and adnexa, retroperitoneum, pancreas, brain, gallbladder

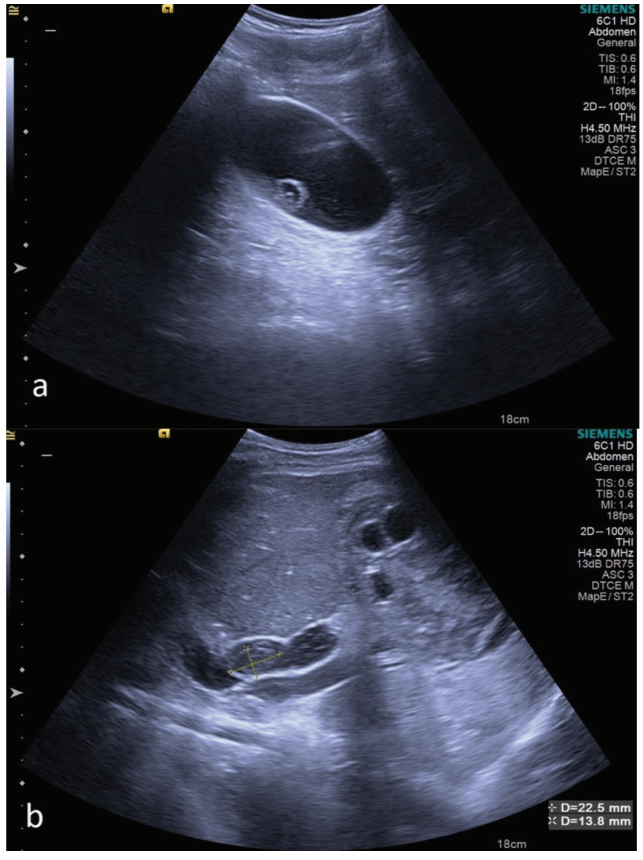

Figure 2a, b. On the ultrasonographic examination, the gallbladder is distended and cholecystitis is observed. Image showing daughter vesicles in the gallbladder lumen (**a**) and common bile duct lumen (**b**)

(<1%) and others (6). CE in the gallbladder is extremely unusual, even in endemic regions. CE may primarily be seen the gallbladder only in rare cases, or secondary invasion may be seen by daughter cysts of a previously infected liver. Depending on whether the cyst develops in the lumen of the gallbladder or on its external surface, the pathogenesis may vary. If the cyst is in the gallbladder lumen, the cysts have spread through the biliary duct, and if the cyst is on the gallbladder's wall, there has been spread through the lymphatic circulation (7). During the operation, we observed CE rupture into the bile ducts and daughter vesicles in the common bile duct. In addition, there were multiple daughter vesicles and cysts in the hydropic gallbladder.

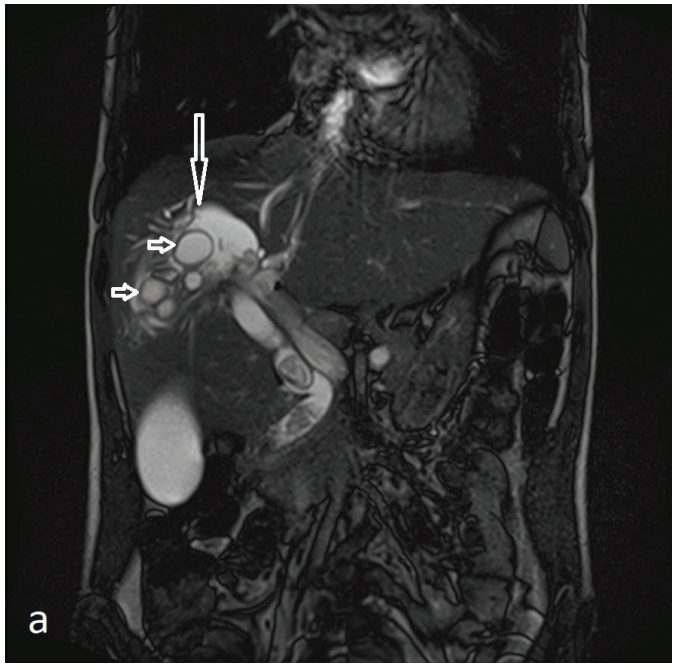

Figure 3a. On MRI coronal examination **(a)**; type 3 cyst hydatid (long arrow) in the liver and daughter vesicles (short arrow) in the gallbladder

MRI: Magnetic resonance imaging

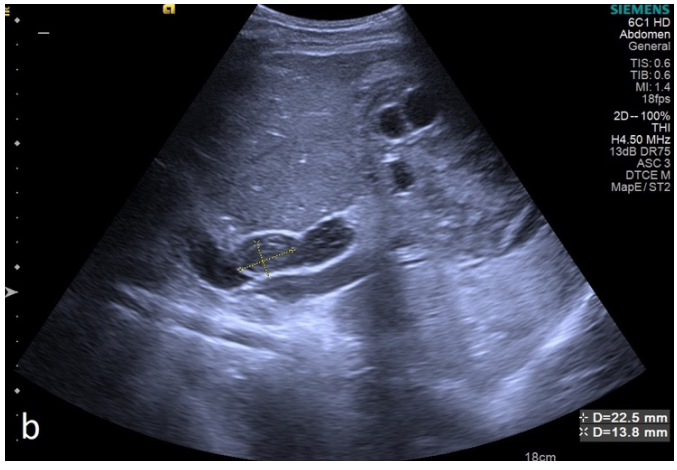

Figure 3b. MRI sagittal T2 WI **(b)** shows daughter vesicles in the gallbladder and liver in images (arrow)

MRI: Magnetic resonance imaging

This parasitic disease can infect all tissues and organs and cause fatal complications. As an acute complication, it causes rupture due to pressure increase in the cyst due to blunt abdominal traumas, stressful situations, or severe cough or defecation causing intraabdominal pressure increase. With biliary rupture of liver hydatid cysts, the most obvious signs and symptoms are right upper quadrant pain, jaundice, and high temperature (8). In our case, sudden onset abdominal pain, nausea, vomiting, and laboratory white blood cell elevation were notable.

The most important clue for recognizing CE disease is the patient's history and clinical suspicion in endemic areas. Various radiological and serological methods can help in the diagnosis (9). Preoperative diagnosis of hepatic CE complications before ultrasonography (USG) or CT was difficult and was based on clinical and laboratory findings. It has been reported that the use of only radiological methods as a diagnostic method may be

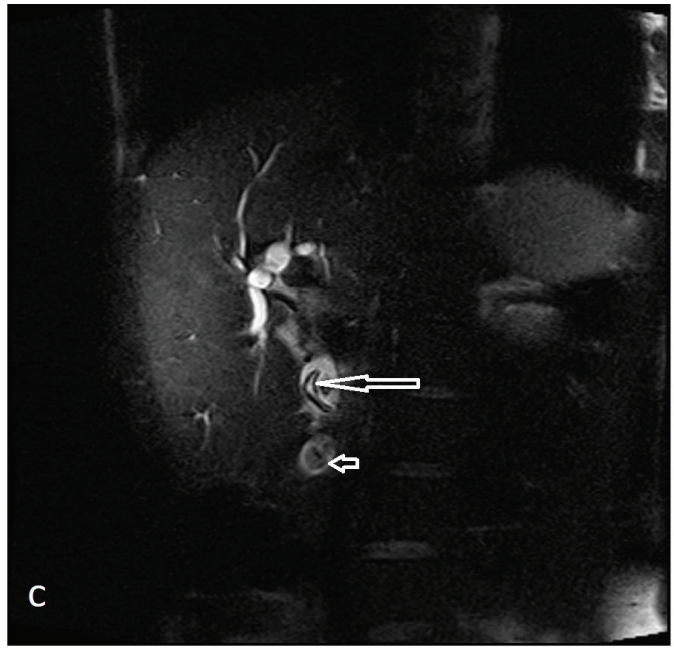

Figure 3c. In the coronal image **(c)** dissociate germinative membrane of the CE (long arrow) and daughter vesicles (short arrow) are observed in common bile duct lumen

CE: Cystic echinococcosis

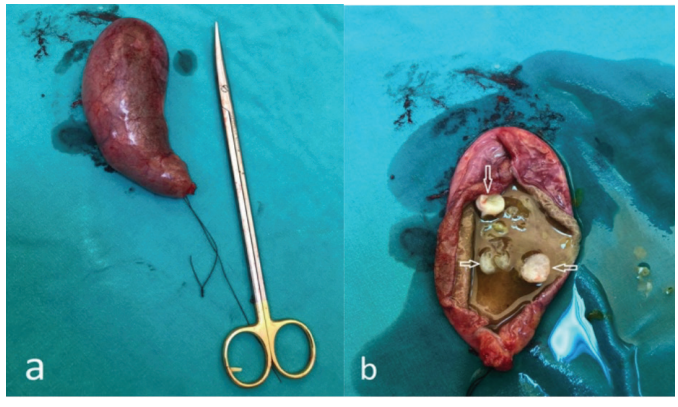

Figure 4a, b. Postoperative hydropic gallbladder specimen (**a**), showing hydatid daughter cysts in the gallbladder (**b**) (white arrow)

insufficient in the differential diagnosis of other lesions, and the use of only serological tests may be insufficient due to reasons such as individual immune response differences and cross-reaction, therefore it is necessary to use both diagnostic methods together (3). In our case, IHA from serological tests, USG, CT and MRI from radiological images were used for the diagnosis of CE.

Nowadays, in the treatment of CE, interventional procedures appear to be less invasive and more comfortable in type 1-3 active cysts compared to Gharbi's US classification (10). If the cysts are very large or subcapsular or if complications have developed, the primary treatment is surgery.

CE is localized in many organs and tissues, especially the liver and lung. Many studies have been conducted on CE, which is located in different organs and tissues in our country. Some of these are as follows; Renal hydatid cyst was found in two patients who were admitted to the hospital with complaints of abdominal pain and swelling in the abdomen. In the USG evaluation of both cases, a cystic mass was observed and the cysts were surgically removed (11). Two pediatric patients admitted to the hospital with different complaints were diagnosed with hepatic echinococcosis by imaging methods and serological methods. Albendazole was followed by PAIR treatment in the treatment of the patients. No side effects and recurrences were observed in the treatment (12). In a study evaluating CE localizing in the pancreas, the patient with acute pancreatitis attack and splenomegaly was found to have pancreatic CE by imaging methods and IHA test. It has been reported that no postoperative complication developed in the patient who underwent albendezole and subsequent surgical intervention (13). In a study investigating whether CE could cause intra-familial infection; CE was detected in the lung and/or liver in a total of four individuals from the same family. A ruptured lung hydatid cyst and multiple cysts in the liver were found in the father from family members, and hydatid cysts in the liver were found in other children. It has been reported that genetic factors may affect the disease and that all family members of individuals living in risky areas should be evaluated for the disease (14). In another study; Although the patient with cough, fever and sputum complaints was initially interpreted as tuberculosis, as a result of the tests performed, a cystic lesion compatible with a hydatid cyst was detected in the left lobe of the liver in USG and CT. It was emphasized that the liver should also be evaluated with ultrasound in cases with suspected pulmonary CE, since the symptoms of ruptured lung CE are similar to tuberculosis (15).

In this study, the patient with abdominal pain, nausea, vomiting and loss of appetite was diagnosed with ruptured CE by CT, USG and MR imaging methods and IHA test. As in the literature reviewed (11,12,14,15), in this study, knowing the differential diagnosis features of CE and The importance of applying appropriate treatment was emphasized.

CONCLUSION

CE is still a common parasitic disease that can lead to serious complications in endemic areas. It can occur in many organs with different complications. Intrabiliary rupture is a well-defined complication, but rupture into the gallbladder is a rare and atypical finding. For clinicians and radiologists working in the emergency room management of the disease may be much easier if they become familiar with the clinical and radiological findings of the cyst.

*** Ethics**

Informed Consent: Informed consent was obtained.

Peer-review: Internally peer-reviewed.

*** Authorship Contributions**

Surgical and Medical Practices: S.T., Concept: İ.D., Design: L.T.Ç., Analysis or Interpretation: O.O., Literature Search: E.G., Writing: A.E.

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

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