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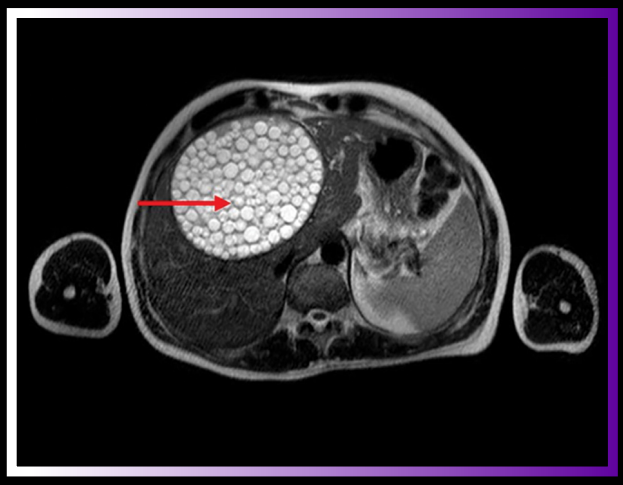

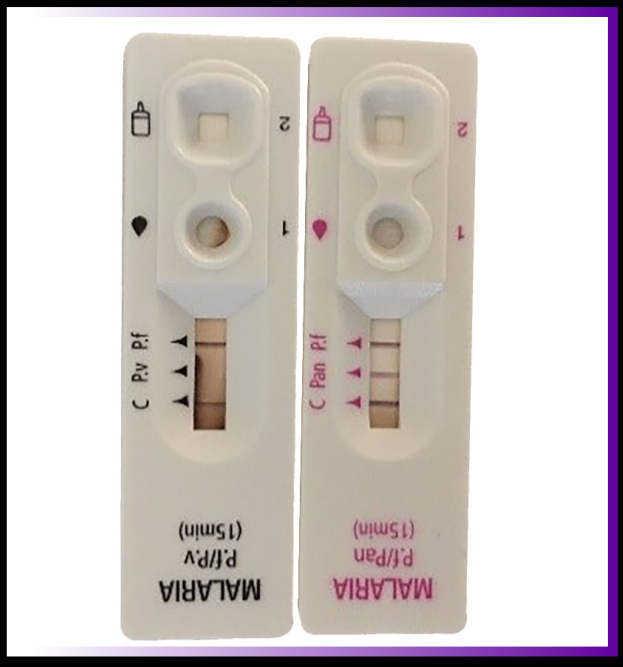

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EDİTÖRDEN

2024 yılının son sayısını 7 özgün araştırma makalesi ve 2 olgu sunumu ile çıkarmaktayız. Özgün araştırmalar arasında; özel kliniklerde çalışan veteriner hekimlerin kanin leishmaniasis sorununa yaklaşımlarını inceleyen bir anket çalışması, bağırsak parazitleri ile ilgili çalışmalar, ülkemizdeki önemli halk sağlığı sorunlarından kist hidatik konusunu irdeleyen çalışmalar ile bir araştırma hastanesindeki *Toxoplasma* test sonuçlarını veren retrospektif bir makale yer almaktadır.

Olgu sunumu olarak; ülkemiz için potansiyel tehdit oluşturabilecek importe *Plasmodium* olgularını ve bir sincapta saptanan ektoparazitleri ele alan birer makaleye yer verilmiştir.

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

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Prof. Dr. Yusuf Özbel

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Management of Canine Leishmaniasis and the Treatment Trends of Veterinarians in Aegean Region of Türkiye: A Questionnaire-based Survey

Türkiye Ege Bölgesi'ndeki Veteriner Hekimlerin Köpek Leishmaniasis Yönetimi ve Tedavi Eğilimleri: Ankete Dayalı Araştırma

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ABSTRACT

Objective: Canine leishmaniasis (CanL) is an important veterinary and public health problem in Mediterranean countries. Although CanL and vector sandflies are widespread in the study area, there are no standardized diagnostic and treatment methods followed by private clinics. This study aimed to survey (i) the treatment, diagnosis, and control measures preferred by veterinarians, (ii) compare the differences in the first-step diagnostic tests applied, and (iii) identify differences in the guidance for CanL.

Methods: This study was conducted between August 2017 and 2019, and an accessible weblink was distributed to veterinary clinics that were registered with the Veterinary Affairs of İzmir. The questionnaire was prepared by following published guidelines and several CanL guidelines and comprised 13 questions. A total of 103 veterinarians voluntarily participated in the study and completed the questionnaire.

Results: Most of the clinicians (n=99; 96.1%) reported that localized alopecia was one of the most common clinical findings in the suspicion of CanL, while only four clinician did not consider this finding in the suspicion of CanL. According to the answers received, 41 clinician (39.8%) frequently observed this finding in CanL-suspected cases, 49 clinician (47.6%) observed it occasionally, and nine clinician (8.7%) rarely observed this finding. The findings of the study indicate that a minority of clinicians employ advanced diagnostic techniques.

Conclusion: The results demonstrate that veterinarians play a unifying role in the fight against CanL. However, the preference for less advanced diagnostic methods over those employed by their European counterparts may result in insufficient detection of particularly asymptomatic patients. It is therefore necessary to validate rapid diagnostic tests, which are the most used method for diagnosing the disease. Furthermore, there is a need to increase awareness of the disease among patient owners and veterinarians in regions where it is endemic, and to recognise it as a notifiable disease in Türkiye.

Keywords: Dog, *Leishmania*, management, Türkiye

ÖZ

Amaç: Köpek leishmaniasis'i (CanL) Akdeniz ülkelerinde önemli bir veteriner ve halk sağlığı sorunudur. Çalışma bölgesinde CanL ve vektör kum sinekleri yaygın olmasına rağmen, özel klinikler tarafından takip edilen standart tanı ve tedavi yöntemleri bulunmamaktadır. Bu çalışmanın amacı (i) veteriner hekimler tarafından tercih edilen tedavi, teşhis ve kontrol önlemlerini araştırmak, (ii) uygulanan ilk adım teşhis testlerindeki farklılıkları karşılaştırmak ve (iii) CanL için rehber farklılıklarını belirlemektir.

Yöntemler: Çalışma, Ağustos 2017 ve 2019 tarihleri arasında gerçekleştirilmiş ve İzmir Veteriner İşleri Müdürlüğü'ne kayıtlı veteriner kliniklerine erişilebilir bir web bağlantısı dağıtılmıştır. Anket, yayınlanmış kılavuzlar ve çeşitli CanL kılavuzları takip edilerek hazırlanmış ve 13 sorudan oluşmaktadır. Toplam 103 veteriner hekim çalışmaya gönüllü olarak katılarak anketi değerlendirmiştir.

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Türkiye PARAZİTOLOJİ Dergisi

Bulgular: Veteriner hekimlerin çoğu (n=99; %96,1) lokalize alopesinin CanL şüphesinde en sık görülen klinik bulgulardan biri olduğunu bildirirken, sadece dört veteriner hekim bu bulguyu CanL şüphesinde dikkate almamıştır. Alınan cevaplara göre, 41 veteriner hekim (%39,8) CanL şüphesi olan olgularda bu bulguyu sıklıkla, 49 veteriner hekim (%47,6) ara sıra ve dokuz veteriner hekim (%8,7) nadiren gözlemlemiştir. Çalışmanın bulguları, klinisyenlerin az bir kısmının ileri tanı teknikleri kullandığını göstermektedir.

Sonuç: Sonuçlar, veteriner hekimlerin CanL ile mücadelede birleştirici bir rol oynadığını göstermektedir. Bununla birlikte, Avrupalı meslektaşları tarafından kullanılanlara kıyasla daha az gelişmiş teşhis yöntemlerinin tercih edilmesi, özellikle asemptomatik hastaların yetersiz şekilde tespit edilmesine neden olabileceği görülmüştür. Bu nedenle, hastalığın teşhisinde özel klinikler tarafından en çok kullanılan yöntem olan hızlı tanı testlerinin doğrulanması gerekmektedir. Ayrıca, hastalığın endemik olduğu bölgelerde hasta sahipleri ve veteriner hekimler arasında hastalık açısından farkındalığın artırılmasına ve Türkiye'de bildirimi zorunlu bir hastalık olarak kabul edilmesine ihtiyaç vardır.

Anahtar Kelimeler: Köpek, *Leishmania*, yönetim, Türkiye

INTRODUCTION

Leishmaniases are a group of diseases caused by several species belonging to the genus *Leishmania*. Since the visceral clinical form mainly has a zoonotic character, natural reservoirs are important in the transmission cycle of the parasite. Both dogs and cats serve as reservoirs of the disease in endemic countries, and several *Leishmania* species [*Leishmania* (L.) *infantum, L. tropica*, and *L. major*] have been detected in both cats and dogs with different clinical manifestations (1-3). All infectious *Leishmania* species are capable of infecting dogs if they share the same geographic region and if the proven or possible vectors are present. A recent study revealed that the isolated *L. infantum* strains from feline leishmaniasis (FeL) cases are identical in terms of growth profile, survival capacity, and genotype to those isolated from human and canine leishmaniasis (CanL) cases (4). Canines, including domestic dogs, are the primary reservoirs of the disease in nature, and *L. infantum* is the most reported causative agent among the others (5). CanL is endemic in over 70 countries and is expanding its borders through the northern European countries due to effects of climate changes and vector spread. The disease is also reported in non-endemic countries like England, Germany, and Austria due to people traveling with their dogs. The distribution of CanL is identified as binomial, and the highest prevalence is reported between 0-3 years old and 8+ years old dogs (6-8).

Türkiye is one of the leishmaniasis endemic countries (9). Both cutaneous and visceral forms of leishmaniasis are present in Türkiye and, to date four causative agents (*L. infantum, L. tropica, L. major*, and *L. donovani*) have been reported (1,2). Visceral leishmaniasis (VL) caused by *L. infantum* is endemic in the Aegean, Marmara, Black Sea, and Mediterranean Regions of Türkiye and more than 20 cases have been reported annually. The cross-sectional surveys carried out in different endemic areas of Türkiye on CanL showed the high veterinary and public health importance of the disease (10). Field studies performed in recent years revealed that the proven vector species (*Phlebotomus tobbi*) is abundant in some endemic regions and the presence of the parasite has been demonstrated via by microscopy and molecular methods (11,12). Besides the other veterinary diseases, CanL is one of the most widespread diseases among both stray and owned dogs in Türkiye (1,13,14). According to recent studies performed in different parts of Türkiye, high molecular (46.66%) and serological (39.13%) positivity rates were reported (1). Each component of the transmission cycle (VL patient, active CanL cases, presence of vector sand fly, and detection of *Leishmania* DNA in vector species) was present in the studied areas, Kuşadası and Karaburun towns located in Ege Region of Türkiye (11,15). Also, several FeL cases were reported in Ege Region stating that there is intense parasite circulations between host and reservoirs occur (2).

Although CanL cases are prevalent in Türkiye, there is currently no mandatory standardized diagnostic or treatment/control approach used by veterinary clinicians in the country. Several diagnostic methods are used in the diagnosis of CanL for suspected cases. The inadequacy of clinical signs in infected dogs makes the use of specific tests necessary (16). Epidemiological and clinical strategies used in the diagnosis of the infection are based on serological and molecular methods (17). Serological tests such as IFA are considered the gold standard in CanL cases and should be performed regardless of clinical findings in endemic areas. As described by Baneth et al. (18), symptomatic cases are only the tip of the iceberg in CanL cases, and routine surveys may help dog owners understand the actual status of a particular region. The diagnosis power of clinician is strongly related to the laboratory infrastructure of the veterinary clinic. Surveillance studies conducted in Türkiye mostly use serological (IFAT) and molecular (ITS1 PCR) tests (1,13,14,19). Among the seven geographic regions, the Mediterranean region had the highest number of diagnosed CanL cases. Serological positivity was always higher in the studied groups compared to molecular tests. The use of molecular tools is on the rise in the diagnosis of CanL, but it is still considered expensive for suspected cases. Furthermore, the lack of trained personnel to perform serological and molecular tests is another reason for undiagnosed/misdiagnosed CanL cases in Türkiye. Since most positive cases do not exhibit any clinical findings, the reported incidence is always lower than the actual incidence (5,20,21). Due to the lack of an effective vaccine and the nature of the disease, integrated approaches such as vector control, the use of insecticide-impregnated collars to prevent sandfly bites, and the use of long-lasting insecticide-impregnated bed nets (LLINs) in endemic areas should be implemented to reduce the spread of the parasite (22).

Some of the measures taken to fight against CanL in different countries include culling (depending on government policy), isolation of infected dogs, and medical treatment for owned dogs. Although dog culling as a strategy to reduce CanL cases is applied in some Asian and South American countries, there is no solid scientific evidence to support its effectiveness in reducing VL incidence (22). Published reports by international study groups suggest that several effective measures such as use of insecticide-impregnated dog collars that might help reduce leishmaniasis transmission from dogs to humans by 48% in endemic areas (23).

Many international foundations and study groups have worked to establish the best practices for the diagnosis and treatment of CanL. To develop diagnostic and treatment guidelines, several symposiums and workshops have been organized, and informative handbooks have been distributed to veterinarians in the study areas. This study aimed to survey (i) the treatment, diagnosis, and control measures preferred by veterinarians, (ii)

compare the differences in the first-step diagnostic tests applied, and (iii) identify differences in the guidance for CanL. Due to the high prevalence of CanL in the Aegean and Mediterranean regions, the questionnaire was limited to registered veterinary clinician located in these areas.

METHODS

Ethically Approval

No clinical material or data were used in this study. The Helsinki Declaration criteria were taken into account in the course of the study.

Background of the Study

Türkiye Leishmaniasis study group has been working in the field for over 20 years with the aim of implementing the one health approach. The group has conducted numerous surveillance studies on vectors, reservoirs, and human leishmaniasis. Given the high risk of CanL in the Mediterranean Region of Türkiye, several meetings and workshops were held with the participation of veterinarians in the study area before the present study. We assume that survey participants have attended one of these symposiums or meetings organized by the study group. Furthermore, guidelines and informative booklets were distributed free of charge to clinician before the study.

Study Design

This study was conducted between August 2017 and 2019 in the Mediterranean Region of Türkiye, and the population of the study included private veterinarians who work on CanL in endemic areas. A total of 109 forms were returned, but six were discarded for various reasons: Three for unknown clinician, one for a duplicate form, one for not receiving dogs, and one for being located outside of the study area. The questionnaire, which comprised 13 questions (eight multiple-choice, three open-ended, and two single select), was prepared by following the published guidelines and several CanL guidelines. To ensure that no different approaches were overlooked, several multiple-choice questions were included in the "other" option. The questions were divided into three major topics: 1) the profile and number of patients, 2) observed symptoms and applied diagnostic tests, and 3) the treatment, follow-up, and protection measures suggested by veterinarians to owners. Veterinary Affairs of İzmir evaluated the questions by following the published guidelines in Türkiye and Mediterranean countries.

To ensure that the participant clinic is in the study area and eliminate the possibility of duplicate forms filled by different veterinarians working at the same clinician, an informative question was asked concerning the name, location, and contact details of the clinic. The last question was open-ended and left for participants to add anything they wanted.

The questionnaire was transferred to the Google Forms system, and an accession link was distributed to all private veterinary clinician registered with Veterinary Affairs of Aegean Region. In 2017, the number of registered clinician was 174, and the accession link was sent to the email addresses of these clinician. Additionally, the accession link was announced on the website of İzmir Veterinary Affairs. All participating clinician were confirmed to be located in Aegean regions.

Statistical Analysis

Descriptive analysis of the data was reported in terms of frequency analyses were performed using the Statistical Package for the Social Sciences (SPSS Inc., Version 21.0; IBM, Armonk, NY, USA).

RESULTS

Access to the questionnaire was activated in August 2017 and deactivated in August 2020 to analyze completed results. Due to the nature of questionnaire studies, the obtained results might not reflect the actual situation. Three clinician without contact details were also discarded. The given answers regarding treatment, diagnosis, and patient profiles were visualized (Figure 1).

1) The profile and the number of the patients

This section investigated general information about clinician and the type of animals admitted, including companion animals, livestock, exotic pets, or zoo animals.

1st question: What is the distribution of animals admitted to your clinic?

Of the evaluated answers, 84 (81.6%; 84/103) of them were receiving only pet animals, such as dogs and cats, and 19 (18.4%; 19/103) were receiving stray animals, such as dogs that were either abandoned or living on the streets without an owner.

2nd question: What is the weekly number of animals admitted to your clinic?

According to the answers received, 10 clinician (9.7%) reported receiving 1-10 patients per week, 23 clinician (22.3%) reported receiving 11-20 patients per week, 31 clinician (30.1%) reported receiving 21-50 patients per week, and 39 clinician (37.9%) reported receiving more than 50 patients per week.

3rd question: How many CanL suspected cases were admitted to your clinic in the last 12 months?

This question in the survey was aimed to gather information on the number of dogs suspected to have CanL at the participating clinician within the last 12 months. It is important to note that the answers provided by the veterinarians only reflect the initial suspicion and do not necessarily indicate a confirmed diagnosis. Out of the 103 clinician, 13 (12.6%) reported not receiving any CanL suspected cases. The remaining 90 clinician reported receiving dogs with suspicion of CanL as follows: 37 clinician (35.9%) received 1-5 dogs, 21 clinician (20.4%) received 6-10 dogs, 19 clinician (18.4%) received 11-20 dogs, 8 clinician (7.8%) received 21-50 dogs, and 5 clinician (4.9%) received more than 50 dogs with suspicion of CanL within the last 12 months.

4th question: Which clinical findings do you base the suspicion on and how often do you encounter those findings?

Based on the answers received for this question, the clinical findings that make clinicians suspect CanL and their frequency of occurrence were recorded. The veterinarians were asked to determine the frequency of several clinical findings, and each finding was classified as occurring frequently, occasionally, or rarely. The overall answers received showed that localized exfoliative dermatitis, ulcerative and/or non-ulcerative lesions, weight loss, exfoliative periocular alopecia and/or blepharitis, and onychogryphosis were the top five clinical findings observed by clinicians in CanL suspected cases.

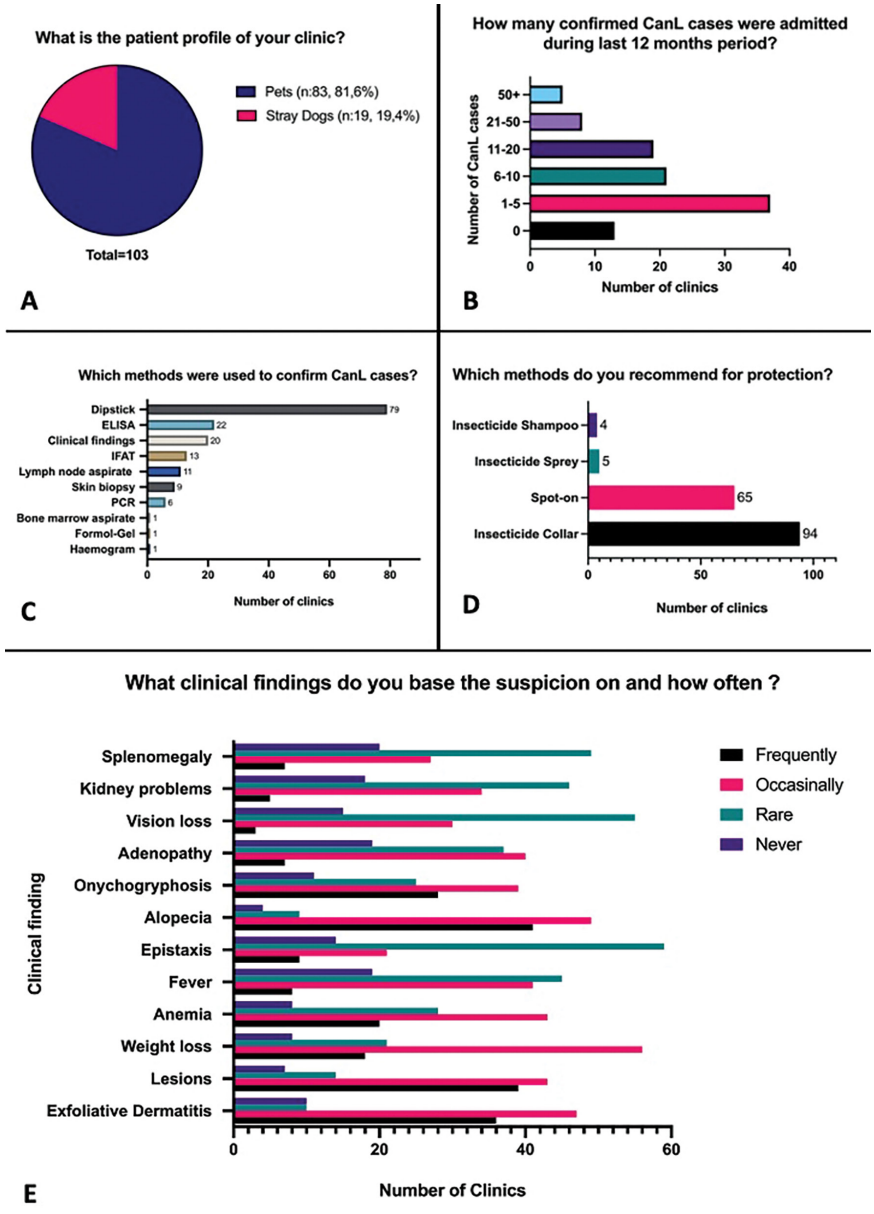

Figure 1. Several answers were visualized as; A) Patient profile of the attended clinician (Question 1), B) The number of confirmed cases during last 12 months period (Question 5), C) Methods used for CanL diagnosis (Question 7), D) Suggested methods by clinician to control CanL (Question 13), and E) Which clinical findings do veterinarians based their diagnosis and how often (Question 4). Graphics were prepared by GraphPad Prism V.9

PCR: Polymerase chain reaciton, ELISA: Enzyme-linked immunosorbent assay, CanL: Canine leishmaniasis

Most clinicians (n=99; 96.1%) reported that localized alopecia was one of the most common clinical findings in the suspicion of CanL, while only four clinician did not consider this finding in the suspicion of CanL. According to the received answers, 41 clinician (39.8%) frequently observed this finding in CanL suspected cases, 49 clinician (47.6%) observed it occasionally, and nine clinician (8.7%) rarely observed it.

Ulcerative and non-ulcerative lesions were other findings stated in the question, and of the 103 participants, 96 clinician (93.2%) reported that the presence of cutaneous lesions is one possible clinical finding in the suspicion of CanL, while seven clinician (6.8%) do not consider this finding in the suspicion of CanL. Of the received answers, 39 clinician (37.9%) frequently noted cutaneous lesions in CanL suspected cases, 43 clinician (41.7%) occasionally noted them, and 14 clinician (13.6%) rarely noted them.

Weight loss was reported frequently by 18 clinician (17.5%), occasionally by 56 clinician (54.4%), and rarely by 21 clinician (20.4%). A total of 95 clinician reported that loss of appetite and weight is one of the findings that make them suspect CanL in suspected cases, while eight clinician (7.8%) do not take this finding into account in the suspicion of CanL.

Exfoliative dermatitis, another clinical finding listed in this question, was notably detected by clinicians (n=93; 90.3%),

while 10 clinician did not report exfoliative dermatitis in CanL suspected cases. According to the received answers, 36 clinician (35%) frequently, 47 clinician (45.6%) occasionally, and ten clinician (9.7%) rarely noted exfoliative dermatitis in CanL suspected cases.

Lastly, 92 clinician (89.3%) reported that they observed onychogryphosis in CanL suspected cases. Eleven clinician did not report onychogryphosis in CanL suspected cases. Twenty-eight clinician (27.2%) frequently, 39 clinician occasionally (37.9%), and 25 (24.3%) clinician rarely noted onychogryphosis in suspected cases.

2) Observed symptoms and applied diagnostic tests

5th question: How many confirmed CanL cases were admitted to your clinic in the last 12 months?

Thirteen clinician (12.6%) reported that they did not have any confirmed cases of CanL during the last 12 months. The remaining clinician (n=90; 87.4%) had at least one confirmed case of CanL. The responses were grouped as follows: 37 clinician (35.9%) had received 1-5 cases, 21 clinician (20.4%) had received 6-10 cases, 19 clinician (18.4%) had received 11-20 cases, eight clinician (7.8%) had received 21-50 cases, and finally five clinician (4.9%) had received more than 50 confirmed cases of CanL during the last 12 months period.

6th question: How many of the confirmed cases were new? (diagnosed over 12 months ago).

Twenty-two of the attended clinician (21.4%) stated that none of the confirmed cases were new. Of the reported new cases, 52 of the clinician (50.5%) stated 1-5, 21 clinician (20.4%) were stated 6-10, four clinician (3.9%) stated 11-20, three clinician (2.9%) stated 21-50, and lastly one clinic (1%) stated more than 50 of the confirmed cases were new.

7th question: Which methods were used to confirm CanL cases?

Several options were provided in the question, and multiple answers were recorded. The three most applied methods were as follows: Rapid diagnosis kit (dipstick) (n=79; 76.7%), ELISA tests (IgG Commercial Kits) (n=22; 21.4%), and clinical findings only (n=20; 19.4%).

8th question: Where did you diagnose the CanL case?

The majority of the attending veterinarians (n=83; 80.6%) stated that they applied diagnostic methods in their clinician. Twenty-two of the veterinarians (21.4%) stated that they sent obtained samples to a private laboratory, and ten clinician (9.7%) sent obtained samples to the nearest veterinary faculty.

9th question: Have these confirmed cases been infected in the area where you work?

According to the received answers, the majority of the clinician (n=72; 69.9%) believed that CanL cases were infected in the area where they worked, while 24 clinician (23.3%) believed that positive cases were infected in another region. Additionally, seven clinician (6.8%) declared that they had no idea about this question.

10th question: Where are those positive dogs kept?

The response to the question was provided in three options. Fifty-two of the clinician (55.3%) stated that diagnosed dogs are kept indoors, 12 clinician (11.7%) stated that dogs are kept outdoors, and 30 clinician (29.1%) stated that diagnosed dogs are kept both indoors and outdoors. Additionally, four clinician declared that they have no idea about this question.

3) The treatment, follow-up, and protection measures suggested by the veterinarian

11th question: What do you use first in treatment?

This question was open-ended, and clinician were able to write any combination of treatments used in their clinician. Of the collected answers, 85 clinician (92.4%) claimed that their first choice of treatment for CanL is Allopurinol. Ten clinician (7.8%) reported using Miltefosine as their first choice of treatment.

12th question: What do you think about the rate of CanL among your patients over the last 10 years?

According to the answers collected from this question, 59 clinician (57.3%) stated that they believe CanL cases have been on the rise over the last 10 years, while 33 clinician (32%) did not report any changes in the number of CanL cases. Only six clinician (5.8%) reported that there has been a decline in CanL cases over the last 10 years period. Five clinician (4.9%) did not answer this question.

13th question: Which methods do you recommend to dog owners for protection?

This question was prepared with multiple choices, and more than one answer was recorded for each clinic. The vast majority of veterinarians (n=94; 91.3%) who answered this question recommended the use of insecticide-impregnated dog collars. Out of 94 clinician recommending the use of insecticide-impregnated dog collars, 61 clinician (59.2%) suggested the use of both a collar and spot-on for protection. Only four clinician did not suggest the use of insecticide-impregnated dog collars for protection.

DISCUSSION

Canine leishmaniasis, caused by *Leishmania infantum*, is endemic in the Aegean, Marmara, Black Sea, and Mediterranean Regions of Türkiye (13). It is of the greatest importance to be aware of the prevalence of the disease in endemic sites in order to gain an understanding of its epidemiology, given the close relationship between dogs and humans and the life cycle of the parasite (24). Various studies conducted in different districts of Türkiye have shown the presence of the parasite in different hosts and vector sand flies (2,10,25,26). In line with these studies, 31.1% of the veterinarians who participated in the survey detected more than 10 CanL cases annually, and 57.3% of them emphasized that the CanL cases had increased in the last 10 years. One possible reason for the increase in CanL cases in the Aegean region might be the changing vector sand fly density, as previously reported by our study group (27,28). According to a recent report by the Aegean Region Municipality, it is estimated that 400,000 stray dogs were living in either indoor or outdoor conditions, and the number of stray dogs annually increased by almost 5,000 (29). Of the 103 veterinarians who participated in the survey, 84 (81.6%) provided consulting services to adopted pet animals, while the remainder worked for stray animals in kennels. In the present study, 57 (55.3%) and 30 (29.15%) of the dogs were staying in outdoor and indoor/outdoor conditions, respectively. Therefore, it can be assumed that 87 (84.4%) of the dogs might stay in suitable outdoor conditions during twilight, evening, and night-active biting times of sand flies. Considering all the factors mentioned above, the rise in CanL cases is not a surprising result.

CanL is a chronic disease that manifests with symptoms such as weakness, reluctance, anemia, generalized lymphadenopathy, dermatitis, alopecia, onychogryphosis, epistaxis, and

asymptomatic proteinuria (17,18). However, the number of dogs showing clinical signs of the disease is believed to be only the tip of the iceberg, especially in endemic areas, as dogs can be infected without showing any symptoms (1,15). In the survey, the veterinarians reported that localized hair loss, weakening, and the presence of persistent wounds in various parts of the body were often associated with CanL in their detailed physical examinations. This finding is consistent with similar studies conducted in other countries (30,31). *Leishmania* invades its host viscero-cutaneously and can cause skin problems such as dermatitis, hair loss, non-healing wounds in various parts of the body, and scaling. However, it should be noted that other vector-borne parasitic diseases, bacteria, neoplasia, metabolic or autoimmune diseases can also cause similar symptoms. Epistaxis was reported as a rare symptom found in only 4% of the cases in the literature (32), but according to the survey results in Slovenia (33), 54% of the veterinarians reported that they frequently observed epistaxis. Veterinarians often suspect CanL with abnormal nail growth and anemia, but fever and poor renal prognosis are rarely attributed to CanL by the participants. Immunocomplex deposition in the renal glomeruli is a symptom with high prognostic value together with ocular disorders, providing information about the poor progression of the disease. Suspicious clinical findings are widely reported in the literature, and even a single concordant sign in endemic regions could be a strong indicator for CanL (31,32,34-37).

The clinical findings of the infection are mostly insufficient in the diagnosis of the disease, and advanced diagnostic methods are needed for the correct diagnosis (16). In the management of CanL, it is important to perform a differential diagnosis to exclude other vector-borne or non-vector-borne diseases that may cause skin lesions (32). Although direct microscopy of the parasite is a definitive diagnostic method, it is not commonly used in clinician due to several reasons, such as the invasive nature of skin and lymph puncture sampling procedures required to visualize the causative agent, patient owners' preference, low sensitivity (38), and dependence on the skill of the user (39). Therefore, veterinarians participating in the study are unlikely to choose this method for diagnosis in their clinician. The flexibility of dog owners in allowing diagnostic tests to be performed in veterinary clinician can significantly affect the frequency and number of detected cases (30). In the diagnosis of CanL, it is known that the interpretation of serological or molecular techniques such as indirect fluorescent antibody test (IFAT) and polymerase chain reaction (PCR), which have been frequently used in recent years, along with clinical findings and blood biochemistry, will increase the accuracy of diagnosis. However, ELISA-based rapid diagnostic kits, which take less time than other methods in diagnosis and allow for fast therapeutic measures, are insufficient for diagnosis alone, and are frequently used by veterinarians as a preliminary diagnostic method in clinician. Serological testing is used in up to 95% of symptomatic dogs which often have high antibody levels, and the tests are easy and inexpensive to perform. However, most assays fail to detect low antibody titers and to diagnose asymptomatic cases (17,40). The fact that serological testing is the preferred first diagnostic method by veterinarians may provide inaccurate information about the actual infection rate (20). Therefore, the disease should be followed up with a quantitative serological or molecular method (31,41). When evaluating the diagnostic methods used by the veterinarians in the study, it was found that 86.4% and 93.8% did not prefer the IFA test and PCR methods, respectively. Furthermore, it was determined that 99% of the attending physicians did not use blood biochemistry values, which are considered valuable for clinical staging in CanL and recommended by LeishVet guidelines (https://www.leishvet.org/) as an aid in diagnosis. Although IFAT is described as the gold standard method for diagnosing clinical cases and ELISA is a highly sensitive and specific test for clinically expressed CanL, they are not sufficient for detecting asymptomatic cases. In Spain, Slovenia, Italy, and France, studies have shown that IFA and ELISA tests are used more frequently to detect CanL than in Türkiye. Therefore, it is important to consider the limitations of these methods and use quantitative serological or molecular tests to monitor the disease progression accurately (20,31,33,42,43). While most veterinarians diagnose the disease in their clinician, it is noticed that private laboratories and laboratories of veterinary faculties, where more equipped and advanced diagnostic tests can be performed, are much less preferred. Hence, it should not be overlooked that the number of animals found to be sick or infected with *Leishmania* spp. might be underreported.

Despite the high sensitivity and specificity of ELISA, IFA, and PCR methods, 76.7% of the veterinarians who participated in the study reported that they frequently use rapid diagnostic tests due to their ease of use in field conditions. While high specificity and sensitivity are essential for veterinarians to make an accurate diagnosis of the disease, numerous rapid diagnostic kits have been developed for this purpose. One of the rapid kits developed for this aim is the rk39 immunochromatographic test strip, which is produced based on the rk39 antigen. The rk39 antigen is a 39 amino acid repeat B-cell epitope in a protein conserved between *L. infantum* and *L. donovani* (44). Among the rapid diagnostic kits, the rk39 test is widely used. Courtenay et al. (45) determined the sensitivity of the rk39 test to be 78% in their study and evaluated this value as low for use in effective prevention programs. The low overall sensitivity of rk39 to detect infection suggests that it is not an effective tool for estimating the prevalence of the disease or for identifying infected dogs in control programs (46). Dual-Path Platform (DPP®), another rapid diagnostic kit used in the field, detects antibodies against *L. infantum* rk26/rk39 fusion protein by colloidal gold-based immunochromatography. Grimaldi et al. (47) stated that the DPP kit is effective in detecting dogs with severe disease and may be a diagnostic marker for active disease, but it has a very low sensitivity in detecting asymptomatic dogs. SNAP® Canine Leishmania antibody test, which is prepared by purifying antigens of *L. infantum* promastigotes, is another rapid diagnosis kit frequently preferred by veterinarians. It was reported by Souza et al. (48) to have higher sensitivity and specificity, and it was stated in their study that it would be a good alternative to DPP®. Despite the low sensitivity to detect infected dogs, the rapid kit's high specificity and the brief time between sampling and results make it a preferred choice for veterinarians. Encouraging the use of further diagnostic tests in addition to the use of rapid kits will contribute to more effective detection of the disease and thus control programs. Moreover, further research is needed to develop more sensitive rapid kits using different antigen combinations and to increase the validation of currently used kits. Increasing the diagnostic accuracy will contribute to the fight against the CanL by ensuring that the agents to be selected in the treatment of the disease are used appropriately and effectively.

The most used antileishmanial agent by the surveyed veterinarians was allopurinol (either alone or in combination with other drugs) (79.6%). Although a synergistic effect was reported for the combination of drugs (49), only 4.9% of veterinarians in our study used a combination of antimony and allopurinol in treatment, possibly due to the route of administration and high cost. When examining the results of surveys conducted in other countries, it is observed that the majority of veterinarians in Portugal and Slovenia, as well as Türkiye, prefer to use allopurinol alone in treatment. However, the combined use of allopurinol and antimony is very widespread in France, Italy, and Spain (31). In the present study, only 3% of veterinarians used allopurinol and domperidone, which were reported to be more effective when used together as a CanL treatment protocol (50). A recently published study conducted in Spain (51) shows that Spanish veterinarians use this treatment protocol more often than their Turkish counterparts. Fortunately, amphotericin B, which is the first choice in human leishmaniasis cases but poorly tolerated by dogs and not suitable for veterinary use (31), has not been included in any practitioner's treatment protocol in this study. Miltefosine, which is not licensed in Türkiye yet, is included in the treatment protocols of 7.71% of our survey participants, who use it together with allopurinol. Obtaining a license for Miltefosine in Türkiye in the future will ensure that the combination of Miltefosine and Allopurinol, which is recommended by LeishVet and ESCCAP, will be used more and may increase the success of the treatment (5,52). While some similarities in practices exist, the different treatment protocols used in various countries underscore the need to standardize CanL treatment, particularly considering the parasite's drug resistance, to effectively control both human and canine leishmaniasis.

In all guidelines concerning public and veterinary health, the importance of preventive measures against CanL is emphasized (30). However, the results of this study indicate that a significant proportion of veterinarians (50.5%) who responded to the questionnaire did not have access to any presentations, brochures, or guidelines regarding the management of the disease published by the World Organization for Animal Health or other organizations such as LeishVet. This highlights the need for improved dissemination and accessibility of information and guidelines related to the prevention and management of CanL among veterinary professionals (53). The majority of veterinarians who participated in the study preferred the use of insecticide-impregnated collars as a preventative measure, as they can reduce the likelihood of vector flies feeding on the animal and increase their mortality rate (54,55). The use of insecticide-impregnated collars as a prophylactic measure is crucial in interrupting the biological cycle of *Leishmania* species by preventing vector-host communication. The majority of veterinarians participating in the study preferred this control method, as it reduces the blood-sucking of vector flies and their chances of survival, thereby contributing to the prevention of both canine and human leishmaniasis cases. Recommending or using insecticide-containing collars to prevent dogs from encountering vector flies, regardless of their health status, is an important step in preventing a disease that poses a threat to public health (32,56). In addition, a majority of veterinarians (66.3%) in the study favored using topical spot-on insecticides as a preventive measure to protect dogs from the disease. A recent study has reported that the combined use of topical insecticides and insecticide collars gave more effective results in the fight against sand flies. However, some veterinarians (5.1%) recommended alternative, non-specific control measures such as shampoos, sprays, and other insect repellents (57). Besides this, 2.91% of the participants expressed apathy towards the risk of CanL and cited the cost of treatment as a factor. In contrast, studies conducted in European countries have shown that most veterinarians are concerned about the disease and prefer using topical insecticides or repellents as a preventive measure (30,58,59).

The risk of leishmaniasis is closely tied to public and animal health, so further research should focus on increasing awareness of guidelines and promoting standardization in practice through greater cooperation between the two professions, utilizing the one health approach (42). Additionally, guidelines on the disease should be translated into native languages to make them more accessible, and more frequent updates incorporating current ecological and epidemiological data can lead to more effective disease control (20). The questionnaire responses suggest that quantitative serological and molecular methods, which are critical in CanL diagnosis, are underutilized. Therefore, veterinarians should be encouraged to perform these tests more frequently, whether in endemic regions or not. This can help identify unnoticeable cases, provide more accurate information on seroprevalence and epidemiology, and indirectly improve public health. Veterinarians should also inform pet owners about the health effects of CanL, which is closely related to public health.

In addition to chemical or biological agents, traditional methods such as keeping doors-indoors at night when flies are active and protecting pets with mosquito nets should be recommended in the fight against CanL. Advising pet owners on these measures can increase awareness and aid in disease prevention.

CONCLUSION

This article provides an overview of the current clinical management of CanL in endemic regions of Türkiye based on data reported by private veterinarians. While the study reveals that many veterinarians follow similar practices in preventing and managing CanL, the lower preference for advanced diagnostic methods compared to their European counterparts may lead to a lack of detection of asymptomatic patients. Therefore, it is crucial to emphasize the following points: (i) CanL should be a notifiable disease in the veterinary field, (ii) private veterinarians' knowledge about the disease should be increased, and (iii) commercial rapid diagnostic tests should be validated in different endemic regions of Türkiye.

*Ethics

Ethics Committee Approval: No clinical material or data were used in this study. The Helsinki Declaration criteria were taken into account in the course of the study.

Informed Consent: N/A.

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Footnotes

*Authorship Contributions

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

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2017-2022 Yılları Arasında (6 Yıllık Dönemde), Çocuklarda Saptanan Parazitlerin Sıklığı ve Dağılımı

Frequency and Distribution of Parasites Detected in Children Between 2017-2022 (6-year Period)

Yelda Sorguç

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ÖZ

Amaç: Çalışmada, 2017-2022 yılları arasında Sağlık Bilimleri Üniversitesi Dr. Behçet Uz Çocuk Hastalıkları ve Cerrahisi Eğitim Araştırma Hastanesi, Mikrobiyoloji Laboratuvarı'na ishal, kabızlık, karın ağrısı, bulantı-kusma gibi gastrointestinal şikayetler, kilo kaybı, gece işemesi, anüs çevresinde kaşıntı, malnütrisyon, malabsorbsiyon, anemi, zeka ve gelişme geriliği gibi çeşitli klinik semptomlarla başvuran hastalarda intestinal parazitlerin sıklığının ve dağılımının belirlenmesi amaçlanmıştır.
Yöntemler: Çalışmada Ocak 2017-Eylül 2022 tarihleri arasında mikrobiyoloji laboratuvarına gönderilen 33.249 dışkı ve 3499 selofan bant örneğinin sonuçları değerlendirilmiştir. Dışkı örnekleri salin-Lugol, modifiye Kinyoun asit-fast ve trikrom boyama yöntemleriyle incelenmiştir.
Bulgular: Toplam 36.748 hastanın %1,8'inin dışkı örneğinde parazit saptanmıştır. En yüksek oranda saptanan bağırsak paraziti *Entamoeba histolytica*'dır (%67). Bunu sıklık sırasına göre; *Enterobius vermicularis* (%19), *Giardia intestinalis* (%12), *Ascaris lumbricoides* (%0,6), *Hymenolepis nana* (%0,2) *Blastocystis* spp. (%0,2) *Hymenolepis diminuta* (%0,2) izlemektedir.
Sonuç: Başvuran hastalarda parazit görülme oranı 2017 yılında %19,9, 2018 yılında %24,6, 2019 yılında %28,6 saptanırken, 2020 yılında bu oran %4,3'e, 2021 yılında %16'ya ve 2022 yılında %6,6'ya düşmüştür. Parazitlerin yıllara göre dağılım yüzlerine bakıldığında 2020 yılında Koronavirüs hastalığı-2019 pandemisinin başlamasıyla parazit görülme oranında bir düşüş olduğu görülmektedir. Şiddetli akut solunum yolu sendromu-koronavirüs-2'nin yayılmasını azaltmak için yapılan enfeksiyon kontrol önlemleri ve karantina kurallarının sıkı şekilde işletilmesi, pandemi döneminde parazit pozitiflik oranını dikkate değer oranda düşürmüş olabileceği düşüncesindeyiz. Türkiye'de intestinal parazitlerle ilgili veriler bölgesel farklılıklar göstermekte ve özellikle çocuklarda ulusal düzeyde yapılacak sürveyans çalışmalarına gereksinim duyulmaktadır.
Anahtar Kelimeler: İntestinal parazit, çocuk, dağılım

ABSTRACT

Objective: In the study, various complaints such as gastrointestinal complaints such as diarrhea, constipation, abdominal pain, nausea and vomiting, weight loss, night urination, itching around the anus, malnutrition, malabsorption, anemia, intelligence and developmental delay were reported to University of Health Sciences Türkiye, Dr. Behçet Uz Pediatric Diseases and Surgery Training and Researhc Hospital, Microbiology Laboratory between 2017 and 2022. It was aimed to determine the frequency and distribution of intestinal parasites in patients presenting with clinical symptoms.
Methods: In the study, the results of 33,249 stool and 3499 cellophane tape samples sent to the microbiology laboratory between January 2017 and September 2022 were evaluated. The stool samples were examined with saline-Lugol, modified Kinyoun acid-fast and trichrome staining methods.
Results: Parasites were detected in the stool samples of 1.8% of a total of 36,748 patients. The intestinal parasite detected at the highest rate was *Entamoeba histolytica* (67%). In order of frequency: *Enterobius vermicularis* (19%), *Giardia intestinalis* (12%), *Ascaris lumbricoides* (0.6%), *Hymenolepis nana* (0.2%) *Blastocystis* spp. (0.2%) is followed by *Hymenolepis diminuta* (0.2%).
Conclusion: While the rate of parasite occurrence in patients applying was found to be 19.9% in 2017, 24.6% in 2018, and 28.6% in 2019, this rate decreased to 4.3% in 2020, 16% in 2021 and 6.6% in 2022. Looking at the distribution of parasites by years, it is seen that there was a decrease in the rate of parasite occurrence with the start of the Coronavirus disease-2019 pandemic in 2020. We believe that infection control measures and strict enforcement of quarantine rules to reduce the spread of severe acute respiratory syndrome-coronavirus-2 may have significantly reduced the parasite positivity rate during the pandemic period. Data on intestinal parasites in Türkiye show regional differences, and national surveillance studies are needed, especially in children.
Keywords: Intestinal parasite, child, distribution

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Türkiye PARAZİTOLOJİ Dergisi

GİRİŞ

Fakir ve sınırlı kaynaklara sahip ülkelerde bağırsak parazitleri en yaygın sağlık sorunu olup, bebek ve çocuk ölümlerinin çoğunun bulaşıcı hastalıklar ve özellikle paraziter hastalıklardan kaynaklandığı bildirilmektedir (1).

Parazit görülme sıklığına etki eden olası faktörlerden biri de yaştır. Yaklaşık 450 milyon çocuk bağırsak parazitleri tarafından enfekte olmaktadır (2).

Cinsiyetin bağırsak parazitleri açısından kadın ve erkek arasında anlamlı fark yaratmadığını bildiren çalışmalara rastlansa da, bunun tersini destekleyen, erkeklerin %46,87, kadınların %38,82'sinde parazite rastlandığını bildiren, ancak istatistiksel olarak yorum yapılmayan çalışmalar da mevcuttur (3).

Bağırsak parazitleri en çok çocukları etkilemekte ve malabsorbsiyon, malnütrisyon, anemi, büyüme geriliği, bilişsel bozukluklar ve öğrenme güçlüğü gibi süreğen sorunlara, uzun süreli parazit enfeksiyonlarının sonucu olarak da, özellikle çocuklarda bedensel ve zihinsel gelişme bozukluklarına neden olabilmektedir (4,5).

Çocuk sağlığı açısından dünyada en yaygın parazitozlardan biri olan Giardiyoz, gelişmekte olan ülkelerde ishal ve beslenme eksikliği oluşturmasının yanı sıra, gastrointestinal geçirgenliğin artmasıyla, gıda alerjenlerinin emilimini artırarak gıda alerjisine neden olabilir (6).

Bu çalışmanın amacı 6 yıllık bir süreçte Sağlık Bilimleri Üniversitesi, Dr. Behçet Uz Çocuk Hastalıkları ve Cerrahisi Eğitim Araştırma Hastanesi, Mikrobiyoloji Laboratuvarı'na polikliniklerden veya kliniklerde yatırılarak izlenen hastalardan gelen, ishal, kabızlık, karın ağrısı, bulantı-kusma gibi gastrointestinal şikayetler, kilo kaybı, gece işemesi, anüs çevresinde kaşıntı, malnütrisyon, malabsorbsiyon, anemi, zeka ve gelişme geriliği gibi çok geniş spektrumda klinik tabloyla başvuran hastalarda görülen intestinal parazitlerin sıklığını ve dağılımını belirlemektir. Sonuçlar geriye dönük olarak değerlendirilerek parazit dağılımlarının belirlenmesi amaçlanmıştır.

YÖNTEMLER

Ocak 2017-Eylül 2022 tarihleri arasında hastanemize başvuran hastaların dışkı inceleme sonuçları laboratuvar bilgi sisteminden elde edilerek retrospektif olarak değerlendirilmiştir. Hastanemiz polikliniklerine başvuran ve serviste takip edilen 1 ay-18 yaş aralığında bulunan hastalardan, parazit incelemesi yapılması için laboratuvarımıza gönderilen 33.249 dışkı ve 3499 selofan bant örneği incelenmiştir.

Dışkı örnekleri öncelikle; koku, kıvam, mukus-kan içeriği ve parazitlerin erişkin formlarına ait yapılar yönünden makroskobik olarak incelenmiştir. Hastalardan alınan dışkı örnekleri makroskobik bakının ardından, nativ-Lugol yöntemi ile mikroskobik incelemeye alınmıştır. Nativ-Lugol yönteminde dışkı örneğinden bir kürdan ucuyla bir pirinç tanesi kadar dışkı alınıp aynı lam üzerinde serum fizyolojik ile, ayrıca Lugol solüsyonu içerisinde ezilerek homojen hale getirilip ikinci bir yayma hazırlanmıştır. Hazırlanan preparatlar önce X10'luk büyütme ile helmint yumurtaları yönünden incelenmiştir. Sonra nativ-Lugol yöntemi ile hazırlanan yaymalar X40'lık büyütme ile bağırsak protozoonları ve helmintlerine ait erişkin, yumurta, trofozoid ve kistlerin varlığı yönünden incelenmiştir. Trikrom ile *Entamoeba histolytica/E. dispar* tespit edilen örneklerde *E. histolytica*'nın kesin tanısı için adezin antijen testi (Entamoeba celisa path, Cellabs, Avustralya) uygulandı.

Sabah ilk dışkılamadan önce selofan bant yöntemi ile örnek alınarak değerlendirildi. Selofan bant yöntemi ile alınan örnekler ışık mikroskobunda X10 büyütme ile incelenmiştir.

Laboratuvara uygun örnek kabı ile getirilmeyen, örnek alındıktan sonra belirlenen süreler içerisinde laboratuvara ulaştırılmayan dışkı ve selofan bant örnekleri kalite doküman yönetim sisteminde belirtilen ret kriterlerine göre reddedilmiştir.

Etik Onay

Bu çalışma Sağlık Bilimleri Üniversitesi, Dr. Behçet Uz Çocuk Hastalıkları ve Cerrahisi Eğitim Araştırma Hastanesi Etik Kurulu tarafından onaylandı (karar no: 2022/20-10, tarih: 24.11.2022).

İstatistiksel Analiz

Çalışmada elde edilen parazit sıklık verileri sayı ve yüzde olarak ifade edilmiştir. Saptanan her bir parazit türü için 2017-2022 yıllarını kapsayan veriler için tablolar oluşturulmuştur.Verilerin değerlendirilmesinde kategorik değişkenler arasındaki dağılım ilişkisi ki-kare testi ile incelenmiştir. İstatistiksel anlamlılık için $p<0,05$ değeri kabul edildi ve veriler IBM SPSS 22 programında değerlendirildi. Her bir satır için ki-kare değeri bulundu.

Ascaris lumbricoides, Hymenolepis nana/diminuta ve *B. hominis* sayıları çok düşük olduğundan istatistiksel olarak değerlendirilemedi.

Yine yıllara göre parazit dağılımına bakıldığında istatistiksel olarak korelasyon bulunamamıştır. Bunun için en büyük ki-kare değerine sahip olan *E. vermicularis* ve *G. intestinalis* analiz dışı bırakılarak, en fazla saptadığımız *E. histolytica* ve yıllara göre dağılımı yeniden hesaplanmıştır. Ki-kare değeri =0,91 olarak hesaplanmış ve yıllara göre değişen sıklıkta görülen *E. histolytica* sayısı anlamlı bulunmuştur.

BULGULAR

Hastanemiz mikrobiyoloji laboratuvarına 2017-2022 yılları arasında (6 yıllık dönemde) toplam 33.249 dışkı ve 3499 selofan bant örneği kabul edilmiş ve bu örneklerin 684'ünde parazit tespit edildiği görülmüştür.

2017-2022 yılları arasında saptanan parazit türleri, görülme oranlarının yıllara göre dağılımı Tablo 1'de gösterilmiştir

2017 yılında 7616 dışkı örneğinin 136'sında, 2018 yılında 7335 dışkı örneğinin 168'inde, 2019 yılında 7495 dışkı örneğinin 196'sında, 2020 yılında 3874 örneğin 30'unda, 2021 yılında 4867 örneğin 110'unda, 2022 yılında 5561 örneğin 44'ünde parazit tespit edildiği belirlenmiştir.

Yıllar, Hasta Sayısı, Pozitif Olgu Sayısı (%)

Mikrobiyoloji laboratuvarına gönderilen 36.748 gaita örneğinin %20,7'si (n=7616) 2017, %19,9'u (n=7335) 2018, %20,3'ü (n=7495) 2019, %10,5'i (n=3874) 2020, %13,2'si (n=4867) 2021, %15,1'i (n=5561) 2022 yılında gönderilen örneklerdir.

Örneklerin %55'i (n=20211) erkek, %45'i (n=16537) kadın hastalardan alınmıştır.

Altı yıl boyunca en sık saptanan parazit türleri sırasıyla, *E. histolytica, E. vermicularis, G. intestinalis* olarak bulunmuştur. Bunu takip eden parazitler, *A. lumbricoides, H. nana, H. dimunata, B. hominis* olarak kaydedilmiştir (Tablo 1).

Tablo 1. Saptanan parazitlerin sayısı ve yıllara göre dağılımı

Parazit	2017	2018	2019	2020	2021	2022	Toplam
E. histolytica	91 (%66,9)	118 (%70,3)	131 (%66,8)	21 (%70)	77 (%70)	25 (%56,8)	463 (%67,7)
E. vermicuaris	28 (%20,6)	30 (%17,8)	36 (%18,6)	6 (%20)	23 (%20,9)	11 (%25)	134 (%19,7)
G. intestinalis	16 (%11,8)	20 (%11,9)	23 (%11,6)	3 (%10)	10 (%9)	5 (%11,4)	77 (%11,4)
A. lumbricoides			4 (%2)			1 (%2,3)	5 (%0,7)
H. nana			2 (%1)				2 (%0,2)
H. diminuta	1 (%0,7)						1 (%0,1)
B. hominis						2 (%4,5)	2 (%0,2)
Toplam	136	168	196	30	110	44	684

En fazla saptadığımız *E. histolytica* ve yıllara göre dağılımı hesaplandığında ki-kare değeri =0,91 olarak hesaplanmış ve yıllara göre değişen sıklıkta görülen *E. histolytica* sayısı anlamlı bulunmuştur.

Bu çalışmada; dördü protozoon ve üçü helmint olmak üzere toplam yedi farklı parazit türüne rastlandı. Bulunan parazit türlerinin %99'unu protozoonlar, sadece %1'ini helmintler oluşturuyordu. Şekil 1'de *E. vermicularis* yumurtası X40'lık büyütme mikroskop görüntüsü yer almaktadır.

Tüm klinik bölümler içinde en çok parazit saptanan bölümlerin dağılımı Tablo 2'de belirtilmiştir. %53,9 oranı ile en çok genel pediatri bölümünden örnek gönderilmiştir.

TARTIŞMA

Sağlık Bilimleri Üniversitesi, Dr. Behçet Uz Çocuk Hastalıkları ve Cerrahisi Eğitim Araştırma Hastanesi, yıllık yaklaşık 600.000 ayaktan tedavi ve 24.000 yatış kapasitesi ile 360 yataklı üçüncü basamak bir hastanedir ve Ege Bölgesi'nde bulaşıcı hastalıklar için bir referans merkezidir. Sonuçlar intestinal paraziter enfeksiyonları hakkında fikir vermekte olup tıp literatürüne katkı sağlayacağı düşünülmektedir.

Şekil 1. *E. vermicularis* yumurtası (Selofan bant incelemesi)

Başta gelişmekte olan ülkeler olmak üzere halen dünyada önemli bir sağlık sorunu olmaya devam eden parazit enfeksiyonları, alt yapı yetersizliği, hijyenik kurallara yeterince uyulmaması ve çevre şartlarının parazitlerin yaşaması ve yayılması için uygun olması nedeniyle, güncelliğini korumaktadır. Ekolojik faktörler açısından incelediğimizde ülkemizin de içinde bulunduğu subtropikal iklim kuşağı paraziter hastalık etkenlerinin gelişip çoğalmasına olanak vermektedir (7).

Yaptığımız çalışmada 2002 yılına göre son on yılda, bağırsak parazitleri sıklığının önemli ölçüde azaldığını (%1,8) görüyoruz. Nitekim, 2002 yılında yapılan çalışmada Sağlık Bilimleri Üniversitesi, Dr. Behçet Uz Çocuk Hastalıkları ve Cerrahisi Eğitim Araştırma Hastanesi bağırsak paraziti oranı %19,3 olarak bulunmuştur (8). Aynı bölgedeki verilerin periyodik olarak bu denli değişkenliği, intestinal parazitlerle mücadelede bize yol gösterecektir.

Gülbudak ve ark. (9) tarafından yapılan bir çalışmada çocuklarda akut gastroenterit etkeni olarak saptanan parazitlerden Giardia lamblia %3,6 oranında bulunmuşken, başka bir çalışmada *G. intestinalis*, %62 gibi yüksek bir oranla en sık saptanan parazit olarak değerlendirilmiştir (10).

Çakar ve ark.'nın (11) yaptığı çalışmada %3,6 oranında parazit saptanmış, bu parazitlerin çoğunun (%69,5) *G. intestinalis* olduğu görülmüştür. Bu oran %1,8 olarak bulduğumuz çalışma sonuçlarına yakın bulunmuştur.

Ülkemizde yapılan araştırmalarda parazit insidansı bölgesel farklılıklar gösterdiğini, farklılıkların ve sosyo-ekonomik durumun parazit yüzdesini belirgin şekilde değiştirebildiğini bilsek de, İzmir'de %1,8 olarak bulduğumuz parazit pozitiflik

Tablo 2. Parazit saptanan hasta örneklerinin gönderilen kliniklere göre dağılımı

Parazit bulunan birimler	Sayı	%
1. Genel pediatri	**369**	**53,9**
2. Ped. acil servis ve acil poliklinik	**167**	**24,5**
3. Ped. gasroenteroloji servisi ve poliklinik	**73**	**10,8**
4. Ped. alerji-immünoloji servisi ve poliklinik	**27**	**3,9**
5. Ped. enfeksiyon hastalıkları servisi ve poliklinik	**18**	**2,8**
6. Ped. cerrahi servisi ve poliklinik	**16**	**2,6**
7. Yanık ve diğer servisler	**9**	**1,5**
Toplam	**684**	**100**

oranı kendimizi sorgulamamıza neden olmuştur. Nüfusun önemli bir kısmının kentsel alanda yaşıyor olması ve sosyo-ekonomik koşulların çok kötü olmaması intestinal helmint sıklığını azaltmış olabilir diye düşündük.

Ocak 2008 ve Aralık 2017 tarihlerini kapsayan dönemde Ege Üniversitesi Tıbbi Parazitoloji Direkt Tanı Laboratuvarı'ndan saptanan parazitlere baktığımızda bu oran, %18,3 başka bir çalışma da ise 6-10 yaş çocuklarda parazit görülme oranı %23,9 bulunmuştur (12,13).

Değişen literatür bulguları ışığında, bağırsak parazitlerinin görülme sıklığının toplumların sosyo-ekonomik durumu,yaş, kişisel hijyen alışkanlıkları gibi faktörler kadar bölgesel farklılıklara bağlı olarak da değişik dağılımlar gösterdiğini söylemek mümkündür.

Bizim çalışmamızda 684 pozitif olgunun 369'u erkek (%53,9), 315'i (%46,1) kız olarak bulunmuştur.

Aynı coğrafyada farklı sonuçlar elde etmiş olsak da, %1,8 olarak bulduğumuz parazit pozitiflik oranını hastalarımızın çocukluk yaş döneminde olmasına, aynı coğrafik konum içerisinde intestinal parazit sıklık ve dağılımının kişisel hijyen, nüfus özellikleri, sosyo-ekonomik düzey, beslenme alışkanlıkları gibi birtakım özelliklere göre farklılıklar gösterebilmesine (14) ve çalışmamızın pandemi dönemini kapsıyor oluşuna bağladık.

Benzer şikayetler ile hastanemize başvuran çocuklarda viral gastroenteritlerin en sık nedeni Rotavirüs olarak bildirildiğinden (15) etiyolojinin viral de olabileceğinin üzerinde durduk.

Öncelikle iyi bir parazitolojik değerlendirme için aynı hastadan en az üç örneğe ihtiyaç duyulmaktadır. Yapılan çalışmalarda, bu şekilde *G. intestinalis* için %11,3, *E. histolytica* için %22,7'lik bir tanı düzeyine erişilebileceği bildirilmiştir (16).

Çalışmanın Kısıtlılıkları

Çalışmamızın en büyük kısıtlılığı, dışkının uygun sayıda ve uygun koşullarda toplanmamış olmasıdır. Çalışmamızda bunu sorgulamasak da pek çok hastanın antibiyotik kullanıyor olma ihtimali, özellikle *E. histolytica* trofozoitlerini yakalama açısından dışkının bekletilmemiş olması gibi faktörler, çocuk hastaların uyumunu güçleştirmiş ve bu durum dezavantaj olarak karşımıza çıkmış, dolayısıyla mikroskopik inceleme sonuçlarımız olumsuz yönde etkilenmiş olabilir diye düşünüyoruz.

Literatüre baktığımızda, çevre koşullarına son derece dayanıklı kistleriyle su ve gıda kaynaklı salgınlara yol açabilen *G. intestinalis*, kist formunun aralıklı olarak dışkıyla atılması veya dışkıdaki kist sayısının az olması halinde tek bir örnekteki dışkı bakısının duyarlılığı oldukça düşüktür. Duodenal villuslara emici diskleriyle yapışmış olan *Giardia trofozoitleri*, epitel hücrelerinin 72 saatte bir dökülmesi ile dışkıdan atıldıklarından parazitin her zaman dışkıda gösterilmesi mümkün olamamaktadır (17) ifadesi de bu görüşümüzü desteklemektedir.

İntestinal protozoonların yayılmasında kişisel hijyen koşullarının ve sağlıklı içme suyuna erişimin önemli olduğu (18) göz önüne alındığında, şehrin alt yapısı ve hijyenik koşullarında iyileştirme yapılması, temiz su kaynaklarının düzenli olarak kontrol edilmesi, binaların sıhhi tesisatlarının daha kaliteli ve temiz suya erişimin daha kolay olması gibi nedenler toplum sağlığı açısından umut verici olup, kayda değer bir insidans farkı yaratmış olabilir.

Bunun yanı sıra, yaptığımız çalışmada, *E. nana, C. mesnili, I.* büstchlii, *E. coli* vb. gibi protozoon parazitler ve X40 büyütmede her mikroskop sahasında beşin altında *B. hominis*, apatojen parazit kisti olarak kabul edildiğinden parazit saptanan olgu sayımızın düşük çıkmış olabileceğini düşünüyoruz.

Çalışmamızda *B. hominis* oranını %0,2 olarak bulduk. Bu oran Harran Üniversitesi Tıp Fakültesi, Çocuk Hastalıkları tarafından yapılan bir çalışmada %1,8 bulunurken (19) Vidal ve Catapani (16) tarafından yapılan çalışmada %39,8 olarak bulunmuştur.

B. hominis için önemli bir patojenite kriteri olan X40 büyütmede her mikroskop sahasında beş ve üstünde parazitin görülmesi halinde örnek pozitif olarak kabul edilmiştir. Patojenite açısından değerlendirilme kriterlerinin değişkenliği yüzünden, başta *B. hominis* olmak üzere tüm intestinal parazitlerde istatistiksel olarak anlamlı farklar olabileceğini fark ettik.

SONUÇ

Çalışmamızda elde edilen verilere göre laboratuvarımızın hizmet verdiği çocuk hastalarda en yüksek oranda saptanan bağırsak paraziti *E. histolytica*'dır. Kişisel hijyen ve gıda konusundaki olumlu alışkanlıklar toplum sağlığı açısından umut verici olup, bölgemizdeki intestinal parazit enfeksiyonlarının sıklığını azaltmış olabileceği sonucuna varılmıştır.

Yukarıda da belirttiğimiz gibi parazitlerin yıllar içindeki dağılım oranına bakıldığında 2020 yılında Koronavirüs hastalığı-2019 pandemisinin başlamasıyla pozitiflik oranında bir düşüş olduğu göze çarpmaktadır. Şiddetli akut solunum yolu sendromu-koronavirüs-2'nin yayılmasını azaltmak için yapılan enfeksiyon kontrol önlemleri neticesinde, pandemi döneminde parazit pozitiflik oranını dikkate değer oranda düşürmüş olabileceği düşüncesindeyiz.

Türkiye'de intestinal parazitlerle ilgili veriler bölgesel farklılıklar göstermekte ve özellikle çocuklarda ulusal düzeyde yapılacak sürveyans çalışmalarına gereksinim duyulmaktadır.

* Etik

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Evaluation of Hydatid Cyst Cases: A Single-center Retrospective Study

Kist Hidatik Olgularının Değerlendirilmesi: Tek Merkezli Retrospektif Bir Çalışma

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ABSTRACT

Objective: Cystic echinococcosis (CE) is a zoonotic condition that can be encountered, particularly in developing countries, and leads to significant economic losses. This study was planned to observe the treatment options, complications, in the patients we followed.

Methods: Patients aged 18 and over who were diagnosed with hydatid cyst and followed in our hospital between January 2018 and December 2023 were included in the study. Data were obtained from the hospital's record system. The patients with CE were retrospectively evaluated in terms of age, gender, cyst location, treatment method applied, presence of relapse, and complications.

Results: A total of 30 patients, with a mean age of 42.8 years (range: 19-68), were included in the study; 13 (43.3%) were male and 17 (56.7%) were female. The most common presenting complaint was abdominal pain (n=14, 46.7%), and 6 patients (20.0%) were asymptomatic. Sixteen patients had multiple cysts in the same region, and 6 patients had cysts in different regions. The most common site of involvement was the liver (n=21, 70.0%), followed by the lungs in 4 patients (13.3%). Single cases of brain, spinal cord, spleen, kidney, and bone involvement were observed. Diagnosis was made by ultrasonography in 16 patients (53.3%), magnetic resonance imaging in 8 patients (26.7%), and computed tomography in 6 patients (20.0%). Surgical intervention was performed in 20 patients (66.7%), and percutaneous drainage in 3 patients (10.0%). All patients received albendazole treatment. Complications included intra-abdominal abscess in three patients (10.0%) and rupture in one patient. One patient with intracranial involvement died.

Conclusion: Although observed worldwide, CE maintain their importance in terms of morbidity and mortality, particularly in developing countries.

Keywords: Hydatid cyst, cystic echinococcosis, zoonotic disease, *Echinococcus granulosus*

ÖZ

Amaç: Kistik ekinokokoz (KE), dünya üzerinde özellikle gelişmekte olan ülkelerde karşılaşılabilen, ciddi ekonomik kayıplara yol açan zoonotik karakterli bir hastalıktır. Takip ettiğimiz hastalardaki tedavi seçenekleri, relaps ve komplikasyonları görebilmek için bu çalışmanın yapılması planlandı.

Yöntemler: Ocak 2018-Aralık 2023 yılları arasında hastanemizde takip edilen ve kist hidatik tanısı alan 18 yaş ve üstü hastalar çalışmaya dahil edildi. Veriler hastane kayıt sisteminden elde edildi. Kist hidatik tanısı alan hastalar yaş, cinsiyet, kistin yerleşim yeri, uygulanan tedavi yöntemi, komplikasyonlar açısından retrospektif olarak değerlendirildi.

Bulgular: Yaş ortalaması 42,8 (19-68), 13'ü (%43,3) ve erkek ve 17'si (%56,7) kadın toplam 30 hasta çalışmaya dahil edildi. En sık başvuru şikayeti (n=14, %46,7) karın ağrısıydı, 6 (%20,0) hastada herhangi bir şikayet yoktu. On altı hastada aynı bölgede birden fazla ve 6 hastada da farklı bir bölgede de kist mevcuttu. En sık tutulum bölgesi (n=21, %70,0) karaciğerdi, 4 (%13,3) hastada akciğer tutulumu görüldü. Bunu birer hasta ile beyin, omurilik, dalak, böbrek ve kemik tutulumu izledi. On altı (%53,3) hastada tanı ultrasonografi, 8 (%26,7) hastada manyetik rezonans görüntüleme ve 6 (%20,0) hastada bilgisayarlı tomografi ile kondu. Yirmi hastaya cerrahi (%66,7) ve 3 (%10,0) hastaya perkütan drenaj uygulandı. Tüm hastalara albendazol tedavisi verildi. Üç (%10,0) hastada komplikasyon olarak batın içi apse ve bir hastada rüptür gelişti. İntrakraniyal tutulumu olan bir hasta öldü.

Sonuç: Dünyanın her yerinde görülmekle birlikte özellikle gelişmekte olan ülkelerde kist hidatik morbidite ve mortalite açısından önemini korumaktadır.

Anahtar Kelimeler: Kist hidatik, kistik ekinokokoz, zoonotik hastalık, *Echinococcus granulosus*

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Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Cystic echinococcosis (CE) is a zoonotic disease that, while more concentrated in certain regions globally, can be found in every country. It causes significant public health issues and serious economic losses (1). The common causative agents of the disease are *Echinococcus granulosus* and *E. multilocularis*. In Türkiye, it is quite prevalent as a zoonosis, with most cases caused by *E. granulosus* (2,3). Although there are case series in our country, a study has found that the number of publications on CE has decreased in recent years (4). Liver involvement is the most common manifestation, it can affect various parts of the body including the heart, eyes, brain, kidneys, and bones (5,6).

Transmission to humans occurs through ingestion of eggs from contaminated hands, water, and food, typically via the feces of infected animals. The eggs reach various organs either through direct inoculation or secondary dissemination. While liver involvement is the most common, the disease can manifest anywhere in the body (7,8). Symptoms are not observed in the initial stages of infection, and it is almost always asymptomatic. Clinical findings vary depending on the affected organ and the size of the cyst. If the cyst is small and the cyst wall is calcified, it may not cause symptoms (8). In particular, liver hydatid cysts may not show symptoms until they reach a size of 10 cm (7-9). If a vital organ is affected, complications can arise, including mass effect, edema due to blood or lymphatic flow obstruction, systemic symptoms in case of rupture, and sometimes death or secondary bacterial infection (7,8).

At the time of diagnosis, most patients are asymptomatic (8). Diagnosis is established through serological tests such as indirect hemagglutination (IHA) and enzyme-linked immunosorbent assay (ELISA), along with radiological methods such as ultrasound (USG), magnetic resonance imaging (MRI), and computed tomography (CT) (9). Routine laboratory tests are generally normal unless a complication is present. When a cyst is associated with bile ducts, elevated liver function tests and cholestasis enzymes may occur; leukocytosis can be seen if there is an infected cyst. In 25% of patients, eosinophilia may develop following cyst rupture or leakage of cyst fluid. Serological tests have a sensitivity of 90%. However, serological tests can produce false positives, and a negative result does not definitively exclude the diagnosis of CE (2,9).

The classification of CE based on their morphology has been established according to the World Health Organization and Gharbi criteria (10,11). According to this classification, Type I cysts are unilocular and consist solely of fluid; Type II cysts have a multiloculated wall; Type III cysts contain daughter cysts; Type IV cysts have a heterogeneous echo; and Type V cysts have a calcified inactive wall (10,11). Alveolar echinococcosis (AE) is caused by the larval stage of the fox tapeworm (*Echinococcus multilocularis*) and is often diagnosed as a space-occupying lesion in the liver. The PNM classification system is used for AE because the growth pattern resembles that of a malignant tumor (P = parasitic mass in the liver, N = involvement of adjacent organs, and M = metastasis) (12). The management of CE includes medical therapy, percutaneous therapy, and surgical intervention. Surgery is the treatment of choice for pulmonary hydatid cysts. A total splenectomy is the treatment of choice for splenic CE. Differential diagnoses should include simple liver cysts, hemangiomas, hepatocellular carcinoma, liver and lung abscesses, Caroli disease, hemangioendotheliomas, and mesenchymal hamartomas (13).

METHODS

The study was planned as a retrospective, cross-sectional, descriptive study in a hospital located in northwestern Türkiye. Patients over the age of 18 who were followed up in our hospital between January 2018 and December 2023 and diagnosed with CE were included in the study. The patients were reached using the ICD-10 diagnosis codes B67.8 (hepatic echinococcosis, unspecified) and B 67.9 (echinococcosis, other and unspecified). The information was obtained from the hospital registry system. The study was conducted in accordance with the principles of the Declaration of Helsinki. The study was approved by the Clinical Research Ethics Committee of University of Health Sciences Türkiye, Kartal Dr. Lütfi Kırdar City Hospital with the decision numbered 2024/010.99/2/47 on 27.03.2024.

Patients were retrospectively evaluated in terms of age, gender, cyst location, imaging methods used for diagnosis, laboratory tests used for CE diagnosis, treatment applied, presence of relapse, and complications. Ultrasound, CT or MRI, depending on the location of the cyst, were used as imaging methods, and *Echinococcus* IHA was used as laboratory test.

Statistical Analysis

The statistical analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS) for Windows version 25.0. Numerical variables were presented as mean ± standard deviation and minimum-maximum values. Continuous variables were expressed as medians, while categorical variables were expressed as frequencies and percentages.

RESULTS

The study included a total of 30 patients, aged between 19 and 68 years with a mean age of 42.8 years. Among these patients, 13 (43.3%) were male and 17 (56.7%) were female. Twenty-one (70.0%) of the patients had no underlying disease. In 6 (20.0%) patients who had no complaints at the time of admission, cysts were detected in the liver during imaging performed for other complaints. The most common presenting complaint among the other patients was abdominal pain (n=14, 46.7%). Other presenting complaints included cough, itching, seizures, and back pain (Table 1). Four (13.3%) of these patients had a prior history of CE.

Of the patients, 16 (53.3%) had multiple cysts in the same organ. In six patients (20.0%), CE were simultaneously detected in another organ outside the primary region. The liver (n=21, 70.0%) was the most commonly affected organ, and lung involvement was observed in 4 (13.3%) patients. This was followed by involvement of the brain, spinal cord, spleen, kidney, and bone in one patient each (Table 2). The smallest cyst, measuring 20x20 mm, was located in the liver, while the largest cyst, measuring 170x110 mm, was located in the kidney. Of the liver hydatid cysts, 15 (50.0%) were in segment 8, and 7 (23.3%) were in segment 7, predominantly in the right hepatic regions. In 11 (36.7%) patients, cysts were present in more than one segment. All lung cysts were located in the left lung.

The most commonly used imaging method for diagnosis was USG, employed in 16 patients (53.3%). Depending on the

Table 1. General characteristics of the patients

Demographic characteristics		**Application assessment**	
Gender	**n (%)**	**Complaint**	**n (%)**
Female/male	17 (56.7)/13 (43.3)	Abdominal pain	14 (46.7)
Age distribution		Cough	4 (13.3)
18-20	2 (6.7)	Side pain	2 (6.7)
21-30	6 (20.0)	Hip pain	1 (3.3)
31-40	5 (16.7)	Seizures	1 (3.3)
41-50	6 (20.0)	Itching	1 (3.3)
51-60	5 (16.7)	Swelling in the back	1 (3.3)
>60	6 (20.0)	No complaints	6 (20.0)
-	-	-	30 (100.0)

Table 2. Cyst characteristics

Location		**Dimensions**		**Classification**	
Site	**n (%)**	**Millimeter (mm)**	**n (%)**	**Stage* (WHO-IWGE)**	**n (%)**
Liver	21 (70.0)	0-10	-	1	3 (10.0)
Lung	4 (13.3)	11-30	6 (20.0)	2	4 (13.3)
CNS**	2 (6.7)	31-50	6 (20.0)	3	1 (3.3)
Spleen	1 (3.3)	51-100	11 (36.7)	4	4 (13.3)
Kidney	1 (3.3)	>100	7 (23.3)	5	2 (6.7)
Bone	1 (3.3)	-	-	-	-
	30 (100.0)	-	30 (100.0)	-	14 (46.7)

*: The World Health Organization Informal Working Group on Echinococcosis, **: Central nervous system

location, MR was used in 8 patients (26.7%), and CT was used in 6 patients (20.0%). *Echinococcus* indirect hemagglutination assay (IHA) was used as a biochemical test for cyst diagnosis. Among the 21 patients tested, 11 (36.7%) had an *Echinococcus* IHA titer >2560. Cysts of two patients with positive *Echinococcus* IHA are shown as examples in Figure 1. *Echinococcus* IHA test results were negative in 4 (13.3%) patients. No significant difference was found between *Echinococcus* IHA titers in hepatic and extrahepatic CE (p=1.00). There was also no significant difference in terms of CE localization site in patients with negative *Echinococcus* IHA (p=1.00). Cysts were demonstrated in these patients by imaging methods (Figure 2). No examination was requested for 5 (16.7%) patients whose CE diagnosis was confirmed by MRI in 2 patients and by CT imaging in 3 patients. Initial laboratory examinations revealed eosinophilia (eosinophil percentage >4%) in 8 patients (26.7%), elevated gamma-glutamyl transferase (GGT) levels in 9 patients (30.0%), elevated alkaline phosphatase levels in 5 patients (16.7%), and elevated aspartate aminotransferase and alanine aminotransferase levels in 3 patients (Table 3).

Surgery was used in the treatment of 20 patients (66.7%), while cyst fluid drainage was applied in 3 patients (10.0%). Relapse occurred in 5 (16.7%) patients, 4 (20.0%) of the patients who underwent surgery and 1 patient who underwent drainage. In addition to surgical and drainage treatments, all patients were given albendazole therapy for a minimum of 3 months. Three patients (10.0%) developed intra-abdominal abscesses, and one patient experienced a rupture. One patient with intracranial involvement died.

DISCUSSION

CE remains a significant and preventable zoonotic condition worldwide, particularly in developing countries (14). Many cases can progress asymptomatically without proper medical diagnosis and treatment, but it can also present with various symptoms and findings depending on the organ in which the cyst is located (9,15). In our study, alongside asymptomatic cases, the majority of symptoms included abdominal pain, and less frequently, symptoms such as cough, itching, seizures, and back pain were observed.

The disease is observed in 50-70% of cases in the liver and 20-30% in the lungs, with other organs being affected less frequently. In adults, two-thirds of patients have liver involvement, whereas pulmonary involvement is higher in pediatric patients. Although typically only one organ is affected, concurrent cysts can be seen in other organs (9,16,17). Similar to the literature, in our cases, 70.0% presented with liver involvement (particularly the right liver), and 13.3% presented with lung involvement. Among the patients with CE in the liver, cysts were also detected in the lungs in 2 patients (6.7%) and in the pancreas in one patient (3.3%), albeit with smaller diameters. Additionally, there were cases of cyst involvement in the brain, spinal cord, spleen, kidneys, and bones.

Although radiological examinations are mostly sufficient for diagnosis, it is recommended that serological methods be used concurrently. USG is the first choice for diagnosis, differential diagnosis, staging, guiding interventional treatment, and follow-up. In cases where USG is insufficient, such as for lung or brain hydatid cysts, or in obese patients, CT can be used.

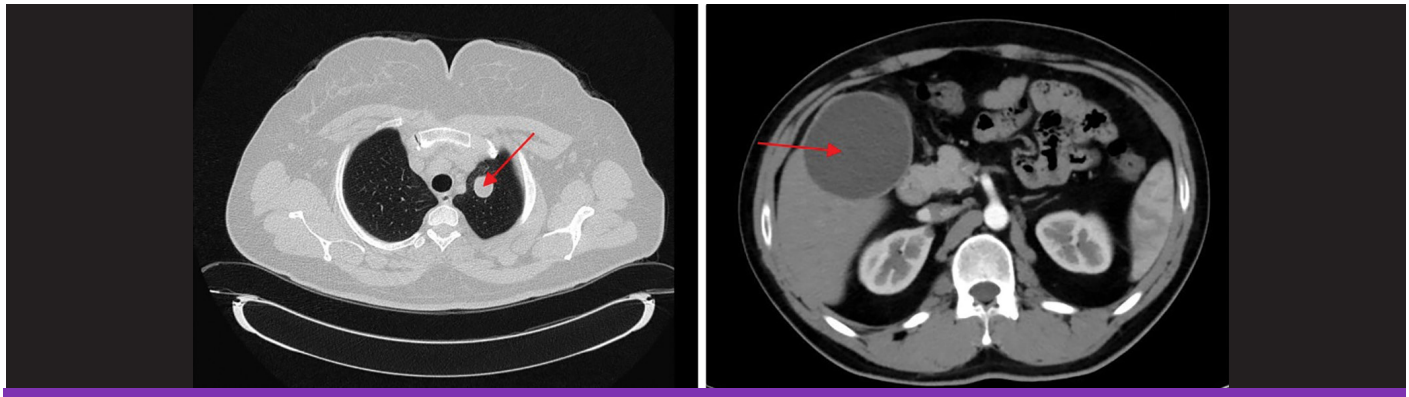

Figure 1. Cyst appearances on lung CT and abdominal CT in 2 different patients with positive *Echinococcus* IHA
CT: Computed tomography, IHA: Indirect hemagglutination

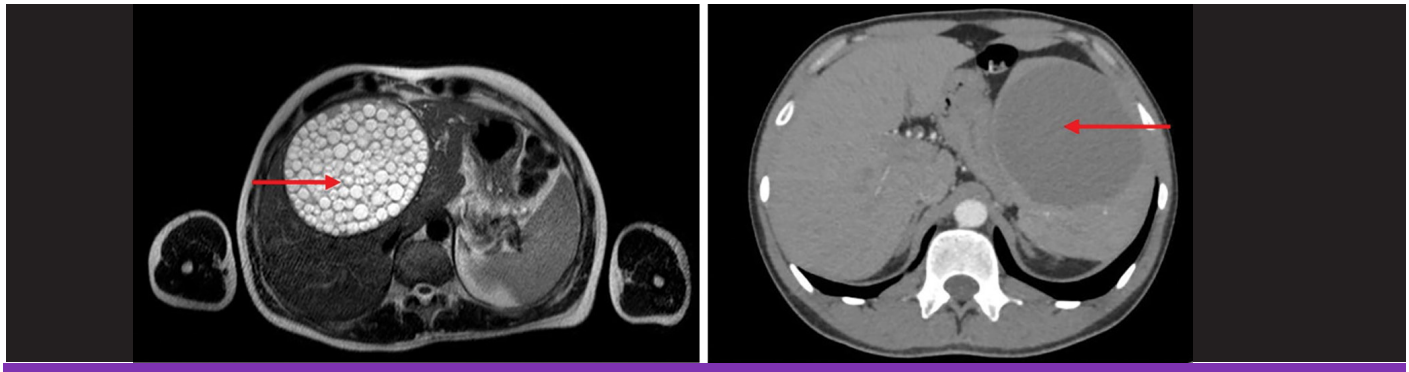

Figure 2. Cyst appearances on abdominal MRI and abdominal CT in 2 different patients with negative *Echinococcus* IHA
MRI: Magnetic resonance imaging, CT: Computed tomography, IHA: Indirect hemagglutination

Table 3. Diagnostic methods and laboratory results

Imaging method		**Indirect hemagglutination**		**Laboratory findings**		
Method	**n (%)**	**Result**	**n (%)**	**Parameter**	**n (%)**	**Location**
USG	16 (53.3)	Negative	4 (13.3)	Eosinophilia (>4%)	8 (26.7)	Liver, lung, kidney
MRI	8 (26.7)	No test requested	5 (16.7)	ALT, AST elevation (>35 U/L)	3 (10.0)	Liver, CNS
CT	6 (20.0)	1/320-1/1280	10 (33.3)	ALP elevation (>98 U/L)	5 (16.7)	Liver, CNS
-	-	>2560	11 (36.7)	GGT elevation (>38 U/L)	9 (30.0)	Liver, lung, CNS

USG: Ultrasound, MRI: Magnetic resonance imaging, CT: Computed tomography, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alcaline phosphatase, GGT: Gama-glutamyl transferase, CNS: Central nervous system

MRI may also be necessary to show cyst wall defects and neural involvement (9,13). In our patients, the most frequently used imaging method, especially for liver cysts, was USG (n=16, 53.3%). CT (n=6, 20.0%) was used for lung CE and in obese patients, while MRI was particularly used for bone, brain, and spinal cord involvement. The sensitivity of serological tests varies depending on the characteristics of the case; for liver cysts, sensitivity ranges from 80% to 100% and specificity from 88% to 96%, whereas it is lower for lung infections (50-56%) or other organ involvements (9). A negative serological test does not rule out CE. In 4 (13.3%) of our patients, *Echinococcus* IHA was found negative. Among 21 patients with a titer >160, in 11 (36.7%) cases the *Echinococcus* IHA titer was >2560. IHA positivity in liver CE was detected at 81.0% (n=17). Serological testing was not requested for 5 (16.7%) patients whose CE diagnosis was confirmed by imaging methods.

Routine laboratory tests for cases are usually normal; however, leukocytosis and eosinophilia can be observed in 25% of cases with infected CE (18). The incidence of eosinophilia in our cases (n=8, 26.7%) was similar to the literature. Leukocytosis was present in two patients who developed intra-abdominal abscesses and in one patient with cyst rupture. Elevated GGT levels were detected in nine patients (30.0%) and elevated ALP levels in five patients (16.7%), while three patients exhibited elevated liver function tests.

The type of lesion is important in treatment planning. Types I, II, and III, as well as Type IV cysts containing fluid, are considered active and require treatment (10). Surgery, percutaneous drainage, and concurrent administration of albendazole or mebendazole may be employed (9,10). Among patients who undergo surgery, the average mortality rate is 2.2%, with a recurrence rate of approximately 6.5% (14). Surgical treatment was applied to

66.7% (n=14) of patients with liver cysts. Percutaneous drainage was performed on a total of three patients: two with liver cysts and one with a spleen cyst. All patients with lung or other organ involvement underwent surgical treatment. Additionally, all patients received albendazole treatment for at least three months. Although percutaneous drainage is often preferred due to shorter procedure times and reduced hospitalization, it does not show a significant difference in relapse rates compared to surgical treatment. In fact, some studies report higher relapse rates in patients who underwent drainage (19,20). In our study, relapse was observed in one patient with intracranial involvement and three patients with liver involvement. All patients who experienced relapse had previously undergone surgical treatment. Patients with small cysts, who did not receive surgical treatment or drainage, were treated with albendazole for a minimum of six months.

The incidence of complications in patients is approximately 10%. Depending on the cyst's location in relation to the bile ducts, it may open into the bile ducts or a bronchus. The cyst contents can cause luminal obstruction or post-obstructive bacterial infection. One significant complication is the secondary spread of daughter cysts to other parts of the body, leading to the development of new foci in different organs (9). In our study, intra-abdominal abscesses developed in three patients (10.0%) as complications, and one patient experienced a rupture. One patient with intracranial involvement was died.

Türkiye's rural areas are particularly endemic for CE. Globally, it is estimated that over one million people are infected (21). Preventing unsanitary slaughtering practices, ensuring proper collection and disposal of waste, maintaining adequate environmental hygiene, and informing the public about transmission routes will reduce the emergence of new cases (22). Since dogs play a crucial role in transmission, vaccination programs for infected animals will help reduce the spread of the disease (23).

Study Limitations

Due to the retrospective nature of the study, accessing patient information through medical records and the small number of cases were limitations of the research.

CONCLUSION

In conclusion, CE is a preventable public health issue for our country, and the expenses related to its diagnosis and treatment hold significant importance for the national economy. Individuals presenting at hospitals with various complaints and symptoms in endemic and rural areas should be evaluated for CE. Prevention and control programs are crucial to reducing the incidence of CE. Although there are case series in our country, the number of publications on CE has been decreasing in recent years. More comprehensive, prospective, multicenter studies are needed on this disease, which is endemic in our country.

*Ethics

Ethics Committee Approval: The study was conducted in accordance with the principles of the Declaration of Helsinki. The study was approved by the Clinical Research Ethics Committee of University of Health Sciences Türkiye, Kartal Dr. Lütfi Kırdar City Hospital with the decision numbered 2024/010.99/2/47 on 27.03.2024.

Informed Consent: Retrospective study.

Footnotes

*Authorship Contributions

Concept: S.Ş., B.K., Design: S.Ş., B.K., Data Collection or Processing: S.Ş., B.K., Analysis or Interpretation: S.Ş., B.K., Literature Search: S.Ş., B.K., Writing: S.Ş., B.K.

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Investigation of Cystic Echinococcosis Knowledge Levels of Animal Breeders and Butchers in Ardahan Province in Türkiye

Ardahan İlindeki Hayvan Yetiştiricilerinin ve Kasapların Kistik Echinococcosis Hakkındaki Bilgi Düzeyinin Araştırılması

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ABSTRACT

Objective: The objective of this study was to ascertain the knowledge level of animal breeders and butchers regarding cystic echinococcosis (CE) in the Ardahan province and its districts situated in the Eastern Anatolia Region of Türkiye. Additionally, the study aimed to identify the potential risk factors that may contribute to the occurrence of the disease.

Methods: The research was conducted between November 2021 and May 2022. A total of 402 animal breeders and 23 butchers participated in the survey. In order to ascertain the extent of awareness regarding CE, a series of multiple-choice questions were administered in a face-to-face setting.

Results: Upon evaluation of the data obtained from the research in a socio-demographic context, it shows that people's awareness of CE increases with age, and women have the most knowledge on this subject (45.85%). A total of 71.76% (305/425) of participants reported owning a dog. Of these, 48.52% (148/305) typically feed their dog food waste. Additionally, 58.03% (177/305) of dog owners do not regularly take their dogs to the vet. The animals were slaughtered by the participants (53.65%; 228/425), and the infected organs found during slaughter were destroyed by deep burial (72.81%; 166/228). The majority of participants (93.41%; 397/425) expressed a desire to gain further insight into the subject.

Conclusion: It was thus established that the districts and province of Ardahan are deficient in the availability of information regarding CE among animal breeders and butchers. It was therefore concluded that the inhabitants of the aforementioned region should be made aware of CE and other zoonotic diseases prevalent in the Ardahan province.

Keywords: Animal breeders, Ardahan, butcher, cystic echinococcosis, level of knowledge

ÖZ

Amaç: Bu çalışmada, Türkiye'nin Doğu Anadolu Bölgesi'nde yer alan Ardahan ili ve ilçelerinde hayvan yetiştiricileri ve kasapların kistik ekinokokkozis (KE) hakkındaki bilgi düzeylerinin belirlenmesi ve hastalığın oluşmasına neden olabilecek risk faktörlerinin belirlenmesi amaçlandı.

Yöntemler: Araştırma Kasım 2021 ile Mayıs 2022 tarihleri arasında gerçekleştirildi. Anket çalışması 402 hayvan yetiştiricisi ve 23 kasap ile gerçekleştirildi. KE ile ilgili bilgi düzeyini belirlemek amacıyla çoktan seçmeli sorulardan oluşan anket soruları gönüllülük esasına göre yüz yüze uygulandı.

Bulgular: Sosyo-demografik açıdan incelendiğinde; KE'nin çoğunlukla kadınlar (%45,85) tarafından bilindiği ve yaş arttıkça kişilerin hastalık hakkında daha fazla bilgi sahibi olduğu belirlendi. Katılımcıların %71,76'sının (305/425) köpeğinin olduğu; köpeklerini genelde yemek atıklarıyla beslediklerini (%48,52; 148/305); köpeklerinin düzenli veteriner hekim muayenesinden geçmediklerini (%58,03; 177/305) ifade ettiler. Hayvanların katılımcılar tarafından kesildiği (%53,65; 228/425) ve kesim sırasında karşılaştıkları enfekte organları derine gömerek (%72,81; 166/228) imha ettiklerini belirttiler. Katılımcıların büyük çoğunluğunun (%93,41; 397/425) konu hakkında bilgi almak istediğini ifade ettiler.

Sonuç: Sonuç olarak Ardahan ili ve ilçelerinde hayvan yetiştiricileri ve kasaplar arasında KE hakkında yeterli bilginin olmadığı, Ardahan ilinde bölgede yaşayan halkın KE ve diğer zoonotik hastalıklar konusunda bilgilendirilmesi gerektiği tespit edilmiştir.

Anahtar Kelimeler: Hayvan yetiştiricileri, Ardahan, kasap, kistik echinococcosis, bilgi düzeyi

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Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Cystic echinococcosis (CE) represents a significant public health concern in developing countries, including Türkiye, where intensive animal husbandry practices are prevalent (1,2). This disease, classified as an important zoonosis by the World Health Organization, is widely distributed throughout Türkiye (3,4). It is known that *Echinococcus granulosus sensu stricto* is the predominant species causing CE with a wide distribution worldwide (5,6). The definitive hosts of *Echinococcus* species are canids, particularly dogs, while their intermediate hosts are herbivorous animals. Infection of final hosts occurs by eating infected organs and subsequent expulsion of eggs in faeces. The eggs are then dispersed in the environment, depending on prevailing environmental conditions. The transmission occurs when intermediate hosts ingest the eggs through digestion and respiration. The emergence of adult parasites occurs in the final host, which ingests the cyst-containing internal organs of the intermediate host (7). In intermediate hosts, the cysts typically localise to the liver (50-70%) and, in some cases, the lungs (20-30%). Cysts may also be observed in ocular, osseous, muscular, renal, splenic, and peritoneal tissues. In less common instances, the condition may also manifest in various internal organs, including the brain, heart, cerebellum, medulla spinalis, salivary glands, pancreas, uterus, ovary, and diaphragm (8-12). The prevalence of CE is higher in areas where sheep are raised due to the higher rate of fertile cysts among intermediate hosts (13-15). The transmission routes of the disease are as follows: Contamination of food and water with *E. granulosus* eggs, ingestion of soil containing the eggs, inhalation of contaminated dust, and contact with infected final hosts. Moreover, it has been postulated that intrauterine transmission may be a potential avenue for transmission. A number of studies have documented the presence of hydatid cysts in human fetuses (7,16-18).

The breeding of sheep (13-15), the presence of intermediate or final hosts in habitats, uncontrolled animal transfers, the careless disposal of infected organs, the spread of infected organs into the environment in slaughterhouses, and their easy access to final hosts represent a multitude of risk factors for CE. To avoid this outcome, it is essential to undertake the destruction of the infected organ in a manner that is both appropriate and controlled. A further risk factor is constituted by the lack of knowledge about CE and the lack of concern for the problem among the general public (17,19). Failure to guarantee socio-economic and socio-cultural growth, as well as neglect of owned or stray dogs, constitutes a risk factor (20).

To date, the Ardahan province has not undergone a comprehensive investigation into the prevalence of CE or the extent of public awareness of the illness. The objective of the study was to ascertain the level of knowledge that animal breeders and butchers in the Ardahan province and its districts had regarding CE. Furthermore, the objective is to ascertain the risk factors that are associated with the disease. This was achieved through the administration of a survey.

METHODS

Ethics Committee Approval

The necessary permissions for this study were obtained from the Kafkas University Non-Interventional Research Ethics Committee (letter dated: 02.11.2021, numbered: 81829502.903/248).

The study was conducted between November 2021 and May 2022 among animal breeders and butchers in the Ardahan province and district. A total of 425 participants were interviewed in person, comprising 402 animal breeders and 23 butchers. A voluntary survey form comprising 32 questions and an informed consent form verifying the volunteers' consent were also utilized. Please refer to Appendix 1 for the survey form used in the research project. The survey included questions on demographics, dog ownership, livestock ownership, CE, and wishes and expectations.

Statistical Analysis

Data were analysed using Pearson chi-square test and the IBM statistical analysis package program (SPSS 26.0).

RESULTS

The data set was compiled using the information provided in responses to survey questions regarding the occurrence of CE in the Ardahan province and district. In order to facilitate comparison between animal breeders and butchers, this study employed a multiple-choice format to examine a range of socio-demographic characteristics, dog ownership, farm animal ownership, and the extent of knowledge regarding CE.

1. The Results on the Socio-demographic Distribution of Butchers and Animal Breeders

Table 1 presents an analysis of the relationship between gender and educational level among animal breeders and butchers. A low percentage of the participants in the survey were women, representing only 11.29% (48/425) of the total sample.

Table 1. A socio-demographic analysis of animal breeders and butchers

Question	Answer	Butcher (%)	Animal breeder (%)	Total (%)	p
Gender	**Female**	0 (0)	48 (11.94)	48 (11.29)	0.093
	Male	23 (100)	354 (88.05)	377 (88.70)	
Level of education	**Literate**	0 (0)	2 (0.50)	2 (0.47)	0.930
	Primary school	3 (13.04)	65 (16.17)	68 (16)	
	Secondary school	9 (39.13)	178 (44.28)	187 (44)	
	High school and its equivalent	8 (34.78)	119 (29.60)	127 (29.88)	
	University	3 (13.04)	38 (9.45)	41 (9.65)	
Total		23 (5.41)	402 (94.59)	425 (100)	

A distribution between the groups revealed that 11.94% (48/402) of the farmers were female. It was determined that gender was not a significant factor, as all butchers were male. A gender-based evaluation of the participants' knowledge about the disease revealed that women were the most informed group, with 45.85% (22/48) having heard about CE. The results of our research indicate that the majority of individuals engaged in animal care are women. Nevertheless, the majority of business owners are male. With regard to gender, the proportions of individuals in both groups who had heard of the disease were similar, although female participants demonstrated a greater awareness of the disease than their male counterparts (Table 2). The results of the survey indicated that women demonstrated a greater awareness of the disease than men when evaluated according to gender. The responses provided to the questions pertaining to the socio-demographic characteristics in the survey were subjected to a comprehensive evaluation. Upon examination of the variables of gender and education level, it was determined that the observed difference was not statistically significant ($p>0.05$).

In order to evaluate the level of knowledge about CE, the participants were divided into two age groups: Those between 20 and 39 years of age and those aged 40 years and older. As indicated in Table 3, an analysis was conducted to determine the relationship between age and the participants' awareness of the disease. The results demonstrated that the likelihood of awareness increased with age.

Three groups were constituted on the basis of the participants' level of education, with the objective of evaluating their level of knowledge regarding CE (Table 4). The study cohort comprised the majority of primary, secondary, and high school graduates, as well as their equivalents.

Of the 425 individuals who participated in the survey, 190 (44.70%) indicated that they possessed knowledge about the disease. Of those who have heard of the disease, 0.53% (1/190) are literate, 89.45% (170/190) have graduated from primary, secondary, or high school, and 10% (19/190) have obtained a university degree. The evaluation of the participants' awareness of the disease, according to their educational background, revealed a negative correlation between the level of education and the probability of being aware of the disease. As the level of education increased, the likelihood of being aware of the disease decreased.

Table 5 presents an examination of the relationship between the participants' educational attainment and the manner in which they feed the dogs under their care. No correlation was identified between educational status and nutritional habits.

Table 2. Animal breeders and butchers' awareness of CE by gender

Group	Female			Male			Total
	n (%)	Yes (%)	No (%)	n (%)	Yes (%)	No (%)	
Animal breeder	48 (11.95)	22 (45.85)	26 (54.15)	354 (88.05)	164 (46.33)	190 (53.67)	402 (94.59)
Butcher	0	0	0	23 (100)	4 (17.39)	19 (82.61)	23 (5.41)
Total	48 (11.30)	22 (45.85)	26 (54.15)	377 (88.70)	168 (44.56)	209 (55.44)	425 (100)

n: The number of respondents is shown

Table 3. Animal breeders and butchers' awareness of CE by age

Group	20 and 39 years of age			40 years and older			Total
	n (%)	Yes (%)	No (%)	n (%)	Yes (%)	No (%)	
Animal breeder	117 (29.10)	52 (44.44)	65 (55.56)	285 (70.90)	134 (47.02)	151 (52.98)	402 (94.59)
Butcher	5 (21.74)	1 (20)	4 (80)	18 (78.26)	3 (16.67)	15 (83.33)	23 (5.41)
Total	122 (28.71)	53 (43.44)	69 (56.56)	303 (71.29)	137 (45.21)	166 (54.79)	425 (100)

n: The number of respondents is shown

Table 4. Animal breeders and butchers' awareness of CE by education level

Group	Literate			Primary/secondary/high school and its equivalent			University			Total
	n (%)	Yes (%)	No (%)	n (%)	Yes (%)	No (%)	n (%)	Yes (%)	No (%)	
Animal breeder	2 (0.50)	1 (0.50)	1 (0.50)	362 (90.05)	168 (46.41)	194 (53.59)	38 (9.45)	17 (44.74)	21 (55.26)	402 (94.60)
Butcher	0	0	0	20 (86.95)	2 (10)	18 (90)	3 (13.05)	2 (66.67)	1 (33.37)	23 (5.40)
Total	2 (0.47)	1 (0.50)	1 (0.50)	382 (89.88)	170 (44.50)	212 (55.50)	41 (9.65)	19 (46.34)	22 (53.66)	425 (100)

n: The number of respondents is shown

Table 6 presents the distribution of responses to the question "Do you administer anti-parasitic medication to your dog?" posed in the survey. The objective of the analysis was to ascertain whether there was a correlation between the level of education and the administration of anti-parasitic medication to dogs. It has been established that as the level of education increases, the average number of individuals who administer parasitic drugs to their dogs decreases. A comparable situation was identified with regard to animal breeders when the data were analyzed according to occupational groups. It was established that the status of butchers remained unchanged.

The responses of the animal breeders and butchers who participated in the survey to the question "How do you dispose of infected organs following the slaughter of the animal?" were evaluated in terms of their level of education (Table 7). It was determined that the likelihood of destroying the infected organ decreased as the level of education increased, with the exception of those who were literate. Upon evaluation of the participants according to their respective groups, it was observed that the proportion of individuals who selected the option of deep burial and other alternatives decreased, while the rate of those who opted to surrender the animals to the relevant authorities increased. Although the practice of deep burial has become more prevalent among butchers, the proportion of those who elect to transfer animals to the appropriate authorities or select alternative options has declined.

2. The Results of the Study on Animal Breeders' and Butchers' Dog Ownership

The responses of animal breeders and butchers in Ardahan province and its districts regarding dog ownership are presented in Table 8. While 88.45% of the 425 participants indicated that they have a street dog in their vicinity, 71.76% stated that they are responsible for a dog. A total of 48.52% of participants reported feeding their dogs with food waste, while 58.03% revealed that they do not take their dogs for regular veterinary check-ups. A total of 51.48% of respondents indicated that they protect their dogs from disease by administering anti-parasitic medication. Furthermore, 96.39% of respondents stated that they always wash their hands after contact with dogs.

3. The Results of the Study on Animal Breeders' and Butchers' Animal Ownership

Table 9 illustrates the distribution of responses to questions on animal ownership, with the objective of determining the level of knowledge of animal breeders and butchers in Ardahan province about CE. Upon evaluation of the responses to the survey on the characteristics of animal ownership among animal breeders and butchers, it becomes evident that the vast majority of participants (98.82%; 420/425) own animals, with the majority of them engaged in cattle rearing (72.62%; 305/420).

4. The Results Regarding the Slaughtering Procedures Applied by Animal Breeders and Butchers

The questions posed to animal breeders and butchers regarding their slaughtering practices were designed to assess their level of knowledge about CE. The responses received are presented in Table 10. The data indicates that 197 participants (46.35%) do not engage in the practice of slaughter, while 228 participants (53.65%) do. It was established that 220 participants (96.50%) proceeded to dispose of the infected organ subsequent to the slaughter, whereas 8 participants (2%) did not dispose of the infected organ.

Table 5. Dog feeding habits and the educational level distribution of butchers and animal breeders

Group	Literate				Primary/secondary/high school and its equivalent				University				Total
	n (%)	Food residue (%)	Dog food (%)	Animal offal (%)	n (%)	Food residue (%)	Dog food (%)	Animal offal (%)	n (%)	Food residue (%)	Dog food (%)	Animal offal (%)	
Animal breeder	2 (0.65)	0	2 (100)	0	267 (89.3)	129 (48.30)	112 (41.95)	26 (9.75)	30 (10.05)	19 (63.35)	8 (26.65)	3 (10.00)	299 (98.05)
Butcher	0	0	0	0	4 (66.65)	0	0	4 (100)	2 (33.35)	0	0	2 (100)	6 (1.95)
Total	2 (0.65)	0	2 (100)	0	271 (88.85)	129 (47.60)	112 (41.30)	30 (11.10)	32 (10.50)	19 (59.40)	8 (25)	5 (15.60)	305 (100)

n: The number of respondents is shown

Table 6. Distribution of butchers and animal breeders by educational level and whether or not they treat their dogs with parasitic drugs

Group	Literate			Primary/secondary/high school and its equivalent			University			Total
	n (%)	Yes (%)	No (%)	n (%)	Yes (%)	No (%)	n (%)	Yes (%)	No (%)	
Animal breeder	2 (0.65)	2 (100)	0	267 (89.30)	136 (50.95)	131 (49.05)	30 (10.05)	13 (43.35)	17 (56.65)	299 (98.05)
Butcher	0	0	0	4 (66.65)	4 (100)	0	2 (33.35)	2 (100)	0	6 (1.95)
Total	2 (0.65)	2 (100)	0	271 (88.85)	140 (51.66)	131 (48.34)	32 (10.50)	15 (46.88)	17 (53.12)	305 (100)

n: The number of respondents is shown

Table 7. Distribution of animal breeders and butchers according to level of education and the way diseased organs are disposed of

Level of education	Answer	Animal breeder (%)	Butcher (%)	Total
Literate	n	2 (0.96)	0	2 (0.90)
	Deep burial	2 (100)	0	2 (100)
	By discarding	0	0	0
	Giving it to the authorities	0	0	0
	By burning	0	0	0
	Others	0	0	0
Primary/secondary/high school and its equivalent	n	183 (88.41)	18 (85.71)	201 (88.16)
	Deep burial	145 (79.24)	2 (11.11)	147 (73.13)
	By discarding	0	0	0
	Giving it to the authorities	14 (7.65)	14 (77.78)	28 (13.93)
	By burning	0	0	0
	Others	24 (13.11)	2 (11.11)	26 (12.94)
University	n	22 (10.65)	3 (14.30)	25 (10.95)
	Deep burial	16 (72.72)	1 (33.33)	17 (68)
	By discarding	0	0	0
	Giving it to the authorities	4 (18.18)	2 (66.75)	6 (24)
	By burning	0	0	0
	Others	2 (9.09)	0	2 (8)
Total		207 (90.79)	21 (9.21)	228 (100)

n: The number of respondents is shown

Table 8. Distribution of animal breeders and butchers according to their dog ownership characteristics

Question	Answer	Animal breeder (%)	Butcher (%)	p-value
Are there any stray dogs around you?	Yes	358 (89.05)	18 (78.25)	0.167
	No	44 (10.95)	5 (21.75)	
Do you have a dog?	Yes[a]	299 (74.35)	6 (26.10)	<0.001*
	No[b]	103 (25.65)	17 (7.90)	
Is your dog in your living area?	No answer[a]	103 (25.65)	17 (73.90)	<0.001*
	Yes[b]	299 (74.35)	6 (26.10)	
	No	0 (0)	0 (0)	
What type of food do you feed your dog?	No answer[a]	103 (25.65)	17 (73.90)	<0.001*
	Food residues[b]	148 (36.80)	0 (0)	
	Dog food[b]	122 (30.35)	0 (0)	
	Animal offal[a]	29 (7.20)	6 (26.10)	
Are routine veterinary examinations performed for your dog?	No answer[a]	103 (25.65)	17 (73.90)	<0.001*
	Yes[b]	124 (30.85)	4 (17.40)	
	No[b]	175 (43.50)	2 (8.70)	
Do you wash your hands after contact with your dog?	No answer[a]	103 (25.65)	17 (73.90)	<0.001*
	Always[b]	289 (71.90)	5 (21.75)	
	Sometimes/occasionally[a,b]	10 (2.45)	1 (4.35)	
Do you administer anti-parasitic medication to your dog?	No answer[a]	103 (25.65)	17 (73.90)	<0.001*
	Yes[b]	151 (37.55)	6 (26.10)	
	No[c]	148 (36.80)	0 (0)	

*: A p-value of less than 0.05 is statistically significant, [a, b, c]: The letters in the answers to the questions in the same column indicate statistical differences

It was indicated that 166 individuals (72.81%) disposed of the infected organs by interring them at a depth sufficient to ensure their destruction, 34 individuals (14.90%) by delivering them to the relevant authorities, and 28 individuals (12.30%) by employing alternative procedures.

5. The Results on the Distribution of CE Knowledge Levels of Animal Breeders and Butchers

Table 11 presents the distribution of the survey findings, which were designed to assess the level of knowledge among butchers and animal breeders regarding CE. Of the 425 participants, 235 lacked awareness of the disease (55.30%) and 239 did not know which tissues and organs were affected (56.25%). In response to the question, "What specific tissues and organs are affected by CE?", 66 participants (35.48%) indicated that the disease affects the liver, while 120 participants (64.52%) stated that the disease affects the lungs. Most respondents (56.25%, 239/425) did not answer the question "What are the clinical symptoms of CE?" 68.09% (128/188), of participants reported nausea and vomiting, 29.26% (58/188) pain and 1.06% (2/188) other symptoms. Most participants said the disease affected sheep (25.65%; 109/425) and dogs (1.90%; 8/425). 88.44% (176/199) knew how it was transmitted. Most participants (72.24%) were unaware that the disease could be fatal. Among those who had an opinion (22.35%), all stated that it could be. However, 43.05% believed it could be treated. The majority of respondents (95.30%; 405/425) indicated that they had not received any education about the disease from any source.

Twenty percent of participants knew they could safeguard their health by eating fruit and veg after washing. Butchers didn't provide a response. 74.82% of the sample took disease-related precautions. Furthermore, 397 individuals (93.41%) had expectations and desires regarding the survey. One hundred and fifty nine individuals (37.41%) answered "all" to the question comprising eight answer options ($p<0.05$).

It was determined that very few people in the environment of the participants had the disease (7.53%; 32/425) and those who had it were not relatives (2.82%; 12/425). It was stated that only one of these people was a relative of the butcher ($p<0.05$).

Table 9. Distribution of animal breeders and butchers according to their animal ownership characteristics

Question	Answer	Animal breeder (%)	Butcher (%)	p-value
Do you breed animals such as ovine, caprine, and bovine species?	**Yes[a]**	402 (100)	18 (78.25)	<0.001*
	No[b]	0 (0)	5 (21.75)	
If yes, specify animal species.	**No answer[a]**	0 (0)	5 (21.75)	<0.001*
	Large animal[b]	290 (72.15)	15 (65.20)	
	Small animal[b]	21 (5.20)	2 (8.70)	
	Mix[b]	91 (22.65)	1 (4.35)	
Total		402 (100)	23 (100)	

*: A p-value of less than 0.05 is statistically significant, [a, b, c]: The letters in the answers to the questions in the same column indicate statistical differences

Table 10. Distribution according to slaughtering practices of animal breeders and butchers

Question	Answer	Animal breeder (%)	Butcher (%)	p-value
Do you slaughter?	**Yes[a]**	207 (51.50)	21 (91.30)	<0.001*
	No[b]	195 (48.50)	2 (8.70)	
Where is the slaughter conducted?	**No answer[a]**	195 (48.50)	2 (8.70)	<0.001*
	In the garden of the house[a]	165 (41.05)	0 (0)	
	Abattoir [b]	42 (10.45)	21 (91.30)	
After slaughter, do you destroy diseased organs?	**No answer[a]**	195 (48.50)	2 (8.70)	<0.001*
	Yes[b]	199 (49.50)	21 (91.30)	
	No[a,b]	8 (2)	0 (0)	
After slaughter, how are diseased organs disposed of?	**No answer[a]**	195 (48.50)	2 (8.70)	<0.001*
	Deep burial[a]	163 (40.55)	3 (13.05)	
	By discarding	0 (0)	0 (0)	
	Giving it to the authorities[b]	18 (4.50)	16 (69.55)	
	By burning	0 (0)	0 (0)	
	Others[a]	26 (6.45)	2 (8.70)	
Total		402 (100)	23 (100)	

*: A p-value of less than 0.05 is statistically significant, [a, b, c]: The letters in the answers to the questions in the same column indicate statistical differences

Table 11. Distribution according to CE knowledge levels of animal breeders and butchers

Question	Answer	Animal breeder (%)	Butcher (%)	p-value
Have you heard of cystic echinococcosis disease?	**Yes[a]**	186 (46.25)	4 (17.40)	0.008
	No[b]	216 (53.75)	19 (82.60)	
What specific tissues and organs are affected by cystic echinococcosis?	**No answer**	220 (54.75)	19 (82.60)	0.030
	Liver	64 (15.90)	2 (8.70)	
	Lung	118 (29.35)	2 (8.70)	
	Kidney	0 (0)	0 (0)	
	Brain	0 (0)	0 (0)	
	Spleen	0 (0)	0 (0)	
	Heart	0 (0)	0 (0)	
	Others (muscle-bone)	0 (0)	0 (0)	
What are the clinical symptoms of cystic echinococcosis?	**No anwser[a]**	218 (54.20)	19 (82.60)	<0.001*
	Nauseation, vomiting[b]	128 (31.85)	0 (0)	
	Pain around the cysted organ[a,b]	56 (13.95)	2 (8.70)	
	Others (cough, fever)[c]	0 (0)	2 (8.70)	
Which species of animals are affected by cystic echinococcosis?	**Sheep[a]**	108 (26.85)	1 (4.35)	0.004
	Dog[b]	6 (1.50)	2 (8.70)	
	Cat	0 (0)	0 (0)	
	Horse	0 (0)	0 (0)	
	Mouse	0 (0)	0 (0)	
	No idea[a,b]	288 (71.65)	20 (86.95)	
How does humans become infected with cystic echinococcosis?	**No answer[a]**	207 (51.50)	19 (82.60)	0.027
	Water and food[a,b]	26 (6.45)	0 (0)	
	Air[b]	148 (36.80)	2 (8.70)	
	Blood[a,b]	1 (0.25)	0 (0)	
	Contact[a,b]	20 (5)	2 (8.70)	
Is cystic echinococcosis a fatal disease?	**Yes**	94 (23.40)	1 (4.35)	0.093
	No	21 (5.20)	2 (8.70)	
	No idea	287 (71.40)	20 (86.95)	
Is cystic echinococcosis a treatable disease?	**Yes[a]**	182 (45.25)	1 (4.35)	<0.001*
	No[b]	0 (0)	1 (4.35)	
	No idea[c]	220 (54.75)	21 (91.30)	
Have you received training or information anywhere about cystic echinococcosis?	**Yes**	17 (4.20)	3 (13.05)	0.086
	No	385 (95.80)	20 (86.95)	
Do you know ways to protect against cystic echinococcosis?	**Yes**	83 (20.65)	3 (13.05)	0.593
	No	319 (79.35)	20 (86.95)	
Which of the ways to protect against cystic echinococcosis do you know?	**Vaccine**	17 (4.25)	0 (0)	0.165
	Hand washing	33 (8.20)	3 (13.05)	
	Antihelmintic drug treatment	0 (0)	0 (0)	
	Destruction of infected organs	0 (0)	0 (0)	
	Washing fruits and vegetables	67 (16.65)	0 (0)	
	Informing the public	40 (9.95)	2 (8.70)	
	No idea	245 (60.95)	18 (78.25)	
Are you taking any preventative measures against cystic echinococcosis?	**Yes[a]**	312 (77.60)	6 (26.10)	<0.001*
	No[b]	90 (22.40)	17 (73.90)	

Table 11. Continued

Question	Answer	Animal breeder (%)	Butcher (%)	p-value
Do you have expectations and wishes to prevent the spread of cystic echinococcosis?	**Yes**	377 (93.80)	20 (86.95)	0.187
	No	25 (6.20)	3 (13.05)	
If you have expectations and wishes, what are they?	**Giving importance to personal hygiene[a]**	118 (29.35)	0 (0)	<0.001*
	Information should be provided through mass media[a,b]	48 (11.95)	2 (8.70)	
	There should be health education in schools[a,b]	8 (2)	0 (0)	
	Seminars should be given	0 (0)	0 (0)	
	Fighting should be done without polluting the environment	0 (0)	0 (0)	
	Collaboration should be made with an expert on the subject[a,b]	84 (20.90)	4 (17.40)	
	All[b]	144 (35.80)	15 (65.20)	
	Others[c]	0 (0)	2 (8.70)	
Is there anyone around you who has CE disease?	**Yes**	11 (2.75)	21 (91.30)	0.152
	No	391 (97.25)	2 (8.70)	
If so, what is the degree of closeness?	**No answer[a]**	391 (97.25)	21 (91.30)	<0.001*
	Relative[b]	0 (0)	1 (4.35)	
	Others[a]	11 (2.75)	1 (4.35)	

*: A p-value of less than 0.05 is statistically significant, [a, b, c]: The letters in the answers to the questions in the same column indicate statistical differences

DISCUSSION

CE, a disease caused by helminths, is a prevalent illness affecting both humans and animals. It has been identified in all regions of Türkiye (4). Apart from the two studies on this disease (21,22), no further epidemiological data related to this region has been identified. The objective of this study was to ascertain the level of awareness among animal breeders and butchers in the province of Ardahan regarding the disease and to collate the most recent information on CE in order to facilitate awareness and implement preventative measures.

This study examines the relationship between socio-demographic factors, including age, gender, and education, and CE. Previous research has identified a correlation between infection and age and gender (21,23-26). However, only a few studies have addressed this topic (27,28). Our findings demonstrate that as age increases, both the probability of encountering the disease and knowledge about it rise, in accordance with the findings of Aydın et al. (29). The proportion of female participants is minimal, comprising only 11.29% (48/425) of the total sample. The evaluation of knowledge about the disease according to gender revealed that among the participants, women were the most likely to have heard of or know about CE (45.85%; 22/48). This result is consistent with other studies (27,30).

The literature shows that dog ownership raises the risk of CE (29,31,32). 71.76% of participants (305/425) were dog owners. Dogs not adequately cared for pose a CE risk to their owners (29,31,32). The study data support these findings. Occupations with intense contact with dogs, including shepherds, hunters, farmers, and butchers, are at high risk of CE (12,33). 26.10% (6/23) of butchers and 74.35% (299/402) of animal breeders kept dogs. Previous studies have shown that both occupational groups are at risk of CE (12,33). The participants also lacked sufficient information on the potential for dogs to transmit certain diseases, particularly CE.

The disease is on the rise, particularly in light of the rising number of stray dogs (34). The prevalence of CE in dogs ranges from 1% to 65% (35-37). 71.76% of participants owned at least one dog. In the intergroup evaluation, 74.35% of animal breeders stated that they own dogs and do not have regular veterinary checks conducted on them. 26.10% (6/23) of butchers surveyed reported owning dogs, with the majority (17.40%; 4/6) taking them for regular check-ups. Most butchers who own dogs protect their pets from disease by giving them parasitic drugs. The dog is at risk of CE due to a high population, lack of records, and lack of effective medication. A factor has been identified as a risk for the disease in the region. Further studies are needed to detect and control the disease.

It has been established that there is a direct correlation between education level and the feeding of dogs (offal, fetus) (29), the administration of antiparasitic drugs to dogs (38), and the destruction of organs by producers (25). People who own dogs stated that they applied parasitic drugs to their dogs (51.48%; 157/305). However, they did not provide information about how frequent the application was or which parasitic drug they used. Additionally, when this situation is associated with the level of education, it has been observed that as the education level increases, the average number of individuals applying parasitic drugs to their dogs decreases. This situation is thought to be due to the lack of equal distribution between the groups according to the education levels of the participants.

It has been demonstrated that contact with dog feces and exposure to the *Echinococcus granulosus* parasite, which causes CE, are associated with an increased risk of seropositivity in humans (39). A statistically significant relationship has been identified between CE cases and offal consumption by dogs (40). In a study conducted by Varcasia et al. (41), it was found that 17% of farmers utilize offal as a source of nutrition for their canine companions. In light of the aforementioned evidence, it can be reasonably deduced that the dietary habits of the fetus and offal, which have been identified as a contributing factor to the disease, play a pivotal role in the disease's emergence and dissemination. The data obtained from this research indicated that as the level of education increases, the status of feeding animal offal also increases. These findings differ from those of other studies (29,39-41). This discrepancy is attributable to the geographical context of the other studies. It is established that the region where the study was conducted has a higher incidence of animal slaughter than other regions, coupled with a harsh winter period of 5-6 months. It is hypothesized that this is caused by feeding dogs offal that is rich in protein and fat, which is likely to increase in prevalence as the level of education increases.

In a study conducted in Aydın province, it was stated that the organs that were considered unsafe for consumption after slaughter (88.66%) were mostly buried and 6.40% were thrown away (27). In a study conducted in Karaman province, it was reported that butchers disposed of internal organs incorrectly at a rate of 59.2% (28). In the study conducted by Aydın et al. (29), in parallel with the previous study, it was stated that animal breeders' practices regarding the destruction of infected internal organs were inappropriate at a rate of 57.9%. Of those who took the survey, 96.50% (220/228) admitted to destroying organs. Upon evaluation of the results of the study conducted in Ardahan, it was determined that despite the participants' lack of awareness regarding the disease, they opted to destroy the infected organs, which play a crucial role in disease transmission, rather than feeding them to the final host dogs (either by burying them or handing them over to the authorities). Furthermore, they employed an appropriate disposal method to prevent disease spread.

It is hypothesized that animal husbandry in Türkiye is predominantly undertaken by individuals with limited educational levels. It is of paramount importance for those engaged in animal breeding to be able to identify cysts associated with CE, given the prevalence of the illness in Türkiye. A number of studies have demonstrated a positive correlation between educational level and knowledge of CE. This is evidenced by the findings of Akalin et al. (42), Demir et al. (25) and Aydın et al. (29). The findings of this study indicate that the proportion of individuals with awareness of the disease was higher among secondary school graduates (44%; 187/425). Furthermore, there was no statistically significant correlation between education level and awareness of the disease (p=0.930). It is hypothesized that this discrepancy is attributable to an imbalance in the distribution of educational attainment between the groups of participants.

Raw meat consumption, education/knowledge status, contact with dogs, not giving antiparasitic drugs to dogs, and inadequate hygiene conditions are reported to be important risk factors for CE (29,43). Control programs have been implemented with the objective of protecting dogs from echinococcosis (44). "Do you administer anti-parasitic medication to your dog?" 51.48% of participants responded positively to the inquiry. In the study conducted by Aydın et al. (29), it was determined that 77.5% of dog owners did not administer antiparasitic drugs to their dogs. In our study, 48.52% (148/305) of participants reported administering antiparasitic drugs to their dogs, which is a lower prevalence than that reported by Aydın et al. (29). It is hypothesized that the aforementioned situation was caused by the fact that the participants lacked the requisite knowledge to identify the specific parasitic disease they were utilizing the pharmaceutical agents against, coupled with a dearth of information pertaining to CE.

Upon inquiry as to which animal in the study CE affected, the majority of respondents (25.65%; 109/425) indicated that it affected sheep, while only 1.90 percent (8/425) stated that it affected dogs. The data indicated that animal breeders reported a prevalence of 26.85% (108/402) in sheep and 1.50% (6/402) in dogs. However, the prevalence reported by butchers was 4.35% (1/23) in sheep and 8.70% (2/23) in dogs. A review of the literature reveals that CE affects both ruminants, which serve as intermediate hosts (25,45), and dogs, which act as final hosts (37,46). The findings of this study corroborate those of previous research in this field. Despite their lack of awareness regarding the illness, the participants demonstrated an understanding of the animals affected, suggesting that they may not have provided a conscious response to the relevant inquiry.

CE has been demonstrated to affect a number of internal organs, with the liver and lungs being the most commonly affected in intermediate hosts (47). This is supported by a number of studies, including those conducted by Gundogdu et al. (21), Hakverdi et al. (23), Aksu et al. (24), Dashti et al. (48), Türkoğlu et al. (49) and Aydın and Adıguzel (50). In response to the question, "What specific tissues and organs are affected by CE?", 66 participants (35.48%) indicated that the disease affects the liver, while 120 participants (64.52%) stated that the disease affects the lungs. Although fewer individuals are aware of CE than those who are not, it can be inferred that the participants answered the pertinent question without a comprehensive understanding of the subject matter.

The failure to dispose of infected organs in an appropriate manner, or the lack of attention to this issue, represents a significant contributing factor to the transmission of CE. In our study, 88.44% (176/199) of participants indicated awareness of the disease's mode of transmission. In other studies, the route of transmission of the disease has been identified as a factor in 61.5% of cases (40), 67.4% of cases (51) and 81.3% of cases (29). The findings of our study indicated a higher prevalence of this phenomenon. Although the number of individuals who are aware of the disease is less than those who are not, the fact that they indicate that they are aware of the manner in which the disease is transmitted suggests that the participants responded to the pertinent question without focusing on it.

In the study conducted by Aydın et al. (29), it was determined that those who heard/know the disease attended courses about CE. Upon evaluation of the level of education and the source of any received education or information, it was determined that the likelihood of having received such education or information increased in conjunction with the level of education, with the exception of those who were literate. Group evaluations yielded consistent results. These findings corroborate those of previous studies (29,51), indicating that the desire to receive education or

information about CE is positively correlated with rising levels of education.

The persistence of CE in many countries, including Türkiye, is attributed to the ineffectiveness of eradication programs, the inadequate use of antiparasitic drugs in stray dogs, uncontrolled animal slaughter, and misapplications resulting from a lack of knowledge in humans (18,50,52). The results of our survey indicate that there is a lack of awareness among animal owners and butchers regarding the infection and its transmission route. In particular, 44.70% (190/425) of respondents reported a lack of knowledge about the infection, while 20.24% (86/425) were similarly unaware of the available protection methods. Conversely, the majority of respondents (88.44%; 176/199) demonstrated a good understanding of the transmission route. Additionally, the majority of infected organs are destroyed after slaughter (51.76%; 220/425), and the majority of animal offal is not fed to dogs (11.48%; 35/305). Upon evaluation of the responses provided in the survey, it can be reasonably inferred that the disease is prevalent in Ardahan province. It is evident that disease screening on both intermediate and final hosts in the region is essential to gain a comprehensive understanding of the situation. Nevertheless, it is imperative that the local population is made aware of this illness and provided with education about it.

CONCLUSION

The research was conducted in the Ardahan Province and its districts. Butchers and animal breeders lacked knowledge of CE. To avoid health risks, we must inform animal breeders, butchers, and the public about how the disease is spread and how to stop it. Control programs are needed to protect humans and animals from this disease. These programs must address the treatment and protection of stray dogs, controlled slaughtering, the disposal of infected organs, and public awareness. The region is at risk of disease, so further studies are needed to detect and control it.

*Ethics

Ethics Committee Approval: The necessary permissions for this study were obtained from the Kafkas University Non-Interventional Research Ethics Committee (letter dated: 02.11.2021, numbered: 81829502.903/248).

Informed Consent: A voluntary survey form comprising 32 questions and an informed consent form verifying the volunteers' consent were also utilized.

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Footnotes

*Authorship Contributions

Concept: N.A., E.G.A., Design: N.A., E.G.A., Data Collection or Processing: E.G.A., Analysis or Interpretation: N.A., E.G.A., Literature Search: E.G.A., Writing: N.A., E.G.A.

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Appendix 1.
1- Gender a-Female b-Male
2- Level of education a- Literate b- Primary school c- Secondary school d- High school and its equivalent d- University
3- Your job a- Animal breeder b- Butcher
4- Are there any stray dogs around you? a-Yes b-No
5- Do you have a dog? a-Yes b-No
6- Is your dog in your living area (home)? a-Yes b-No
7- What type of food do you feed your dog? a- Food residues b- Dog food c- Animal offal
8- Are routine veterinary examinations performed for your dog? a-Yes b-No
9- Do you wash your hands after contact with your dog? a- Always b- Sometimes/occasionally
10- Do you administer anti-parasitic medication to your dog? a-Yes b-No
11- Do you breed animals such as ovine, caprine, and bovine species? a-Yes b-No
12- If yes, specify animal species. a-Large animal b-Small animal c-Mix
13- Do you slaughter? a-Yes b-No
14- Where is the slaughter conducted? a- In the garden of the house b- Abattoir
15- After slaughter, do you destroy diseased organs? a-Yes b-No
16- After slaughter, how are diseased organs disposed of? a- Deep burial b- By discarding c- Giving it to the authorities d- By burning e-Others
17- Have you heard of cystic echinococcosis disease? a-Yes b-No
18- What specific tissues and organs are affected by cystic echinococcosis? a-Liver b-Lung c-Kidney d-Brain e-Spleen f-Heart g-Others (Muscle-Bone)
19- What are the clinical symptoms of cystic echinococcosis? a- Nauseation, vomiting b- Pain around the cysted organ c- Others (cough, fever)
20- Which species of animals are affected by cystic echinococcosis? a-Sheep b-Dog c-Cat d-Horse e-Mouse f-No idea
21- How does humans become infected with cystic echinococcosis? a-Water and food b- Air c-Blood d-Contact
22- Is cystic echinococcosis a fatal disease? a-Yes b-No c-No idea
23- Is cystic echinococcosis a treatable disease? a-Yes b-No c-No idea
24- Have you received training or information anywhere about cystic echinococcosis? a-Yes b-No
25- Do you know ways to protect against cystic echinococcosis? a-Yes b-No
26- Which of the ways to protect against cystic echinococcosis do you know? a- Vaccine b- Hand washing c- Antihelmintic drug treatment d- Destruction of infected organs e- Washing fruits and vegetables f- Informing the public g-No idea
27- Are you taking any preventative measures against cystic echinococcosis? a-Yes b-No
28- Do you have expectations and wishes to prevent the spread of cystic echinococcosis? a-Yes b-No
29- If you have expectations and wishes, what are they? a- Giving importance to personal hygiene b- Information should be provided through mass media c- There should be health education in schools d- Seminars should be given e- Fighting should be done without polluting the environment f- Collaboration should be made with an expert on the subject g- All h- Others
30- Is there anyone around you who has CE disease? a-Yes b-No
31- If so, what is the degree of closeness? a-Relative b-Others

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The Association Between the Presence of Human Facial Mites *Demodex* (Acari, Demodicidae) and Dermatological Symptoms in Rosacea Patients in Central Anatolia, Türkiye

Türkiye'nin Orta Anadolu Bölgesi'ndeki Rosacea Hastalarında İnsan Yüz Akarları Demodex (Acari, Demodicidae) Varlığı ile Dermatolojik Semptomlar Arasındaki İlişki

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ABSTRACT

Objective: This study aimed to investigate the potential relationship between the presence of *Demodex* mites and dermatological symptoms in patients with rosacea in the Central Anatolian Region of Türkiye.

Methods: A total of 63 patients diagnosed with rosacea were enrolled in the study. Skin samples were obtained using a standardized skin surface biopsy technique, and a questionnaire was administered to assess skin symptoms. Statistical analysis was performed to evaluate the association between *Demodex* infestation and specific skin symptoms.

Results: Among the study participants, 65.1% exhibited *Demodex* infestation. Statistical analysis revealed significant associations between the presence of *Demodex* and skin burning (p=0.018), skin pain (p=0.012), and skin stinging (p=0.001). However, no statistically significant associations were observed between *Demodex* presence and gender, skin dryness, itching, irritation, skin rash or the presence of red/pink bumps on the skin.

Conclusion: This study provides evidence suggesting a potential role for *Demodex* mites in the pathogenesis of rosacea, specifically in relation to skin symptoms such as burning, pain, and stinging. The findings underscore the complexity of the relationship between *Demodex* infestation and rosacea and highlight the need for further research, including longitudinal and mechanistic studies, to better understand this association and its clinical implications. Ultimately, understanding the role of *Demodex* mites in rosacea may lead to innovative therapeutic approaches, offering hope for improved management of this challenging dermatological condition.

Keywords: *Demodex*, rosacea, dermatological symptoms

ÖZ

Amaç: Bu çalışmada, Türkiye'nin İç Anadolu Bölgesi'ndeki rosacea hastalarında *Demodex* akarlarının varlığı ile dermatolojik semptomlar arasındaki potansiyel ilişkinin araştırılması amaçlanmıştır.

Yöntemler: Çalışmaya rosacea tanısı alan toplam 63 hasta dahil edilmiştir. Deri örnekleri standart bir deri yüzeyi biyopsisi tekniği kullanılarak elde edilmekte ve deri semptomlarını değerlendirmek için bir anket uygulanmıştır. *Demodex* istilası ile spesifik deri semptomları arasındaki ilişkiyi değerlendirmek için istatistiksel analiz yapılmıştır.

Bulgular: Çalışmaya katılanların %65,1'inde *Demodex* istilası görüldü. İstatistiksel analiz, *Demodex* varlığı ile deri yanması (p=0,018), deri ağrısı (p=0,012) ve deri batması (p=0,001) arasında anlamlı ilişkiler olduğunu ortaya konulmuştur. Ancak *Demodex* varlığı ile cinsiyet, deri kuruluğu, kaşıntı, tahriş, deri döküntüsü veya deride kırmızı/pembe şişliklerin varlığı arasında istatistiksel olarak anlamlı bir ilişki gözlenmemiştir.

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Türkiye PARAZİTOLOJİ Dergisi

Sonuç: Bu çalışma, özellikle yanma, ağrı ve batma gibi deri semptomlarıyla ilişkili olarak, rosacea patogenezinde *Demodex* akarlarının potansiyel bir rol oynadığını öne süren kanıtlar sunmaktadır. Bulgular, *Demodex* istilası ile rosacea arasındaki ilişkinin karmaşıklığının altını çizmekte ve bu ilişkiyi ve klinik sonuçlarını daha iyi anlamak için boylamsal ve mekanik çalışmalar da dahil olmak üzere daha fazla araştırmaya ihtiyaç duyulmaktadır. Sonuçta, *Demodex* akarlarının rosaceadaki rolünün anlaşılması, yenilikçi terapötik yaklaşımlara yol açarak bu zorlu dermatolojik durumun daha iyi açıklanmasını umut etmektedir.

Anahtar Kelimeler: *Demodex*, rosacea, dermatolojik semptomlar

INTRODUCTION

Rosacea is a chronic and common dermatological condition that predominantly affects the central regions of the face, including the cheeks, nose, chin and forehead. This skin condition is characterised by a range of symptoms including facial erythema, persistent flushing, papules, pustules and telangiectasias. Despite its prevalence, the aetiology of rosacea remains incompletely understood, posing significant challenges to its management and treatment (1-3). The pathogenesis of rosacea is thought to be multifactorial, involving complex interactions between genetic predisposition, environmental triggers, neurovascular dysregulation and immune dysfunction. In addition, the impact of rosacea goes beyond physical symptoms and significantly affects patients' quality of life and self-esteem. Given the complexity of the disease, elucidating the underlying mechanisms and identifying effective therapeutic strategies is of paramount importance (2,4).

Demodex is a genus of microscopic parasitic mites that commonly inhabit the skin and hair follicles of mammals, including humans. The two species that primarily affect humans are *Demodex folliculorum* and *D. brevis* (5,6). These mites are considered commensals as they generally coexist harmlessly with their host. However, under certain conditions, such as a weakened immune system or an overpopulation of mites, *Demodex* can contribute to skin problems. They are particularly associated with skin conditions such as rosacea and demodicosis, the latter being a more severe infestation that can cause skin inflammation and irritation (7,8). *Demodex* mites feed on sebum and cellular debris and are primarily found in the pilosebaceous units where hair follicles and sebaceous glands are located. Although *Demodex* mites are relatively common, more research is needed to better understand their role in skin health and the factors that contribute to their proliferation (6).

The presence of *Demodex* mites in rosacea patients has been associated with various symptoms and could exacerbate the severity of the condition. Studies have shown a positive correlation between *Demodex* mite density and the severity of rosacea symptoms, including facial redness, papules, and pustules. Individuals with higher *Demodex* counts were more likely to experience dryness, itchiness, and skin irritation. Furthermore, *Demodex* mite infestation was significantly associated with rough, scaly skin in rosacea patients. Treatments aimed at reducing *Demodex* mite counts have shown improvements in skin symptoms such as stinging and pain sensations. Though the exact mechanisms are still being investigated, the presence of *Demodex* mites appears to influence rosacea symptoms, suggesting potential avenues for targeted therapeutic interventions (6,9-11).

The primary aim of this study is to investigate the possible relationship between the presence of *Demodex* and the dermatological symptoms of rosacea patients in the Central Anatolian Region of Türkiye. By conducting an analysis of *Demodex* infestation and associated symptoms, we aim to contribute to the understanding of the aetiological factors underlying rosacea.

METHODS

Study Population and Ethical Considerations

The study included 63 patients diagnosed with rosacea who registered at Elazığ Universal Eye Hospital Dermatology Clinic and Fırat University Dermatology Outpatient Clinic between November 2022 and February 2023. Individuals with concomitant skin or systemic diseases, a history of dermatological surgery, or receiving systemic/topical treatment were excluded to minimise potential confounding factors.

The study received ethical approval from the Clinical Research Ethics Committee of Sivas Cumhuriyet University, with the approval number 2022-10/02.

Sample Collection

Skin samples were taken from the cheek, nasolabial and chin regions of each participant using a standardised skin surface biopsy (SSSB) technique. The sampling sites were first cleaned with alcohol and dried. An area of one square centimetre (cm^2) was then marked on a clean glass slide. A drop of cyanoacrylate adhesive was applied to the opposite side of the slide, in the centre of the marked area. The marked area of skin was gently pressed onto the adhesive and then carefully lifted after approximately one minute.

Survey Administration

The questionnaire designed to assess the relationship between the presence of *Demodex* and skin symptoms was administered face-to-face to the rosacea patients participating in the study.

Statistical Analysis

The data collected were entered into SPSS software (Statistical Package for the Social Sciences, version 22.0 for Windows). Descriptive statistics such as percentiles and means were used to evaluate the data. Chi-square tests were also used, with a significance level of 0.05.

RESULTS

A total of 63 patients diagnosed with rosacea were included in this study to investigate the prevalence of *Demodex* spp. infestation and its association with various skin symptoms. Among the study participants, *Demodex* spp. was detected in 65.1% of rosacea patients (Table 1).

Analysis of the relationship between the presence of *Demodex* spp. and individual skin symptoms revealed statistically significant associations with specific symptoms. *Demodex* spp. infestation was significantly associated with skin burning (p=0.018),

skin pain (p=0.012) and skin stinging (p=0.001). However, no statistically significant associations were observed between the presence of *Demodex* spp. and gender (p=0.388), skin dryness (p=0.340), skin itching (p=0.432), skin irritation (p=0.087), skin rash (p=0.053) or the presence of red/pink bumps on the skin (p=0.237). Among the different age groups of rosacea patients, the highest incidence of *Demodex* infestation was observed in individuals aged 51-69 years. However, our statistical analysis showed no significant difference in *Demodex* prevalence between age groups (Table 1).

DISCUSSION

The association between *Demodex* mites and rosacea has been the subject of considerable interest in the field of dermatology (12,13). Several studies have suggested a possible link between the presence of *Demodex* mites, particularly *D. folliculorum* and *D. brevis*, and the development or exacerbation of rosacea symptoms (5,14,15). A meta-analysis of 1.513 rosacea patients showed that the prevalence (70.4% vs. 31.8%) of *Demodex* mites were significantly higher in rosacea patients compared to controls (7,16). Our findings revealed that *Demodex* mites were detected in a substantial proportion of our rosacea patient population, with a prevalence of 65.1%. This observation aligns with previous studies suggesting a high prevalence of *Demodex* infestation in individuals with rosacea.

Rosacea, which usually appears in men after the age of 50 depending on gender and age factors, has been shown to start at an earlier age in women and is therefore three times more common in women than in men (9,17,18). Females accounted for 85.7% of rosacea patients in the study group. However, the presence of *Demodex* in rosacea patients was not statistically significantly different between the sexes.

One of the key findings of our study was the significant association between *Demodex* infestation and specific skin symptoms. We observed statistically significant relationships between the presence of *Demodex* and symptoms such as burning, pain and stinging of the skin. These findings raise the possibility that *Demodex* mites may actively contribute to the development or aggravation of these specific symptoms in rosacea patients. These microscopic ectoparasites are commonly found in human hair follicles and sebaceous glands, and their overpopulation is thought to trigger inflammatory responses that contribute to the and other micro-organisms in the follicles. However, this process can cause damage to the host's follicular epithelium. As the number

Table 1. Age, gender and skin symptoms associated with *Demodex* spp. presence

		n (%)	*Demodex* spp. (+) - n (%)	p-value
Rosacea patients		63	41 (65.1)	-
Age, (mean ± SD) -				
19-30		13 (20.6)	11 (17.4)	0.434
31-40		7 (11.1)	4 (6.3)	
41-50		16 (25.4)	8 (12.7)	
51-68		27 (42.8)	18 (28.6)	
Sex				
Female		54 (85.7)	34 (54.0)	0.388
Male		9 (14.3%)	7 (11.1)	
Skin symptoms				
Dryness of the skin	+	50 (79.4)	34 (54.0)	0.340
	-	13 (20.6)	7 (11.1)	
Itching of the skin	+	44 (69.8)	30 (47.6)	0.432
	-	19 (30.2)	11 (17.5)	
Irritation of the skin	+	48 (76.2)	34 (54.0)	0.087
	-	15 (23.8)	7 (11.1)	
Burning on the skin	+	54 (85.7)	32 (50.8)	0.018
	-	9 (100.0)	9 (22.0)	
Rashes on the skin	+	44 (69.8)	32 (50.8)	0.053
	-	19 (30.2)	9 (14.3)	
Stinging on the skin	+	35 (55.6)	15 (23.8)	0.001
	-	28 (44.4)	26 (41.3)	
Pain on the skin	+	18 (28.6)	16 (25.4)	0.012
	-	45 (71.4)	25 (39.7)	
Red/pink bumps on the skin	+	60 (95.2)	40 (63.5)	0.237
	-	3 (4.8)	1 (1.6)	

SD: Standard deviation

of mites increases, they mechanically occlude the hair follicles and sebaceous glands, causing tissue damage and disruption of the cutaneous barrier. The dying *Demodex* releases the chitin layer that forms the exoskeleton and the mite's own pathogenesis of rosacea. While the exact mechanisms underlying this association are still under investigation, it is clear that *Demodex* mites may play a role in the inflammatory cascade characteristic of rosacea (19-21). In a favourable microenvironment (skin pH, humidity, sebum lipid profiles, etc.) *Demodex* lives in balance with the host immune system. The human immune system has an inhibitory effect on the proliferation of mites. Their numbers are under control without any inflammatory reaction or symptoms. However, if this balance is disturbed, clinical symptoms occur. *Demodex* uses lipase enzymes to digest the sebum from the lipids. In this way it digests bacteria contents, including bacterial antigens, are shed into the environment. The onset of the inflammatory process and the clinical symptoms and severity of the resulting demodicosis are increased in certain inflammatory diseases such as rosacea (22,23).

Interestingly, we did not find statistically significant differences between the presence of *Demodex* and other common skin symptoms associated with rosacea, including dryness, itching, irritation, rash, and the presence of red/pink bumps on the skin. This suggests that the relationship between *Demodex* infestation and rosacea may be specific to certain symptoms, highlighting the complexity of the condition.

In a study by Forton and Seys (24) the presence of *Demodex* was associated with itching symptoms. In different studies, a statistically significant difference was found between itching, redness and rash symptoms in rosacea patients with *Demodex* (10,25-29). According to our research results, contrary to the findings in the literature, no relationship was found between itching and the presence of *Demodex*.

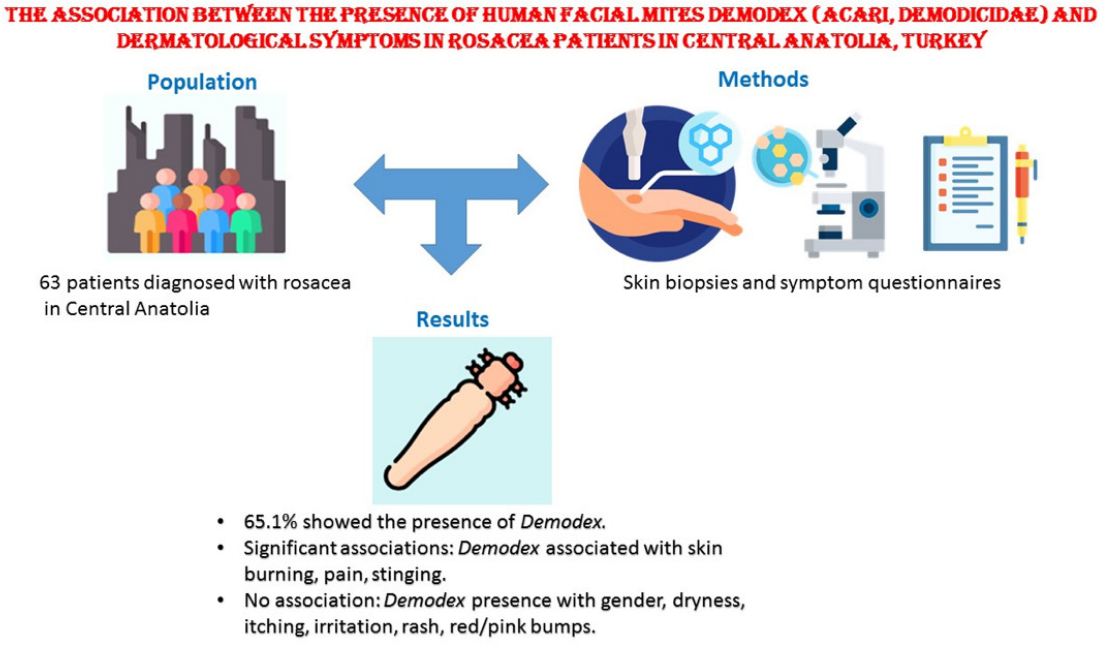
Our study found a statistically significant association between the presence of *Demodex* mites and the occurrence of symptoms such as burning, pain and stinging in rosacea patients (Graphic 1). This finding adds to the growing body of evidence suggesting that *Demodex* mites may play an important role in the aetiology of rosacea-related symptoms. While the exact mechanisms by which *Demodex* mites exacerbate these symptoms require further investigation, our findings highlight the need for continued research in this area. Understanding the interactions between *Demodex* mites and the host's skin microenvironment could potentially lead to novel therapeutic strategies to manage and alleviate the discomfort associated with rosacea, offering hope for an improved quality of life for those affected. It's important to acknowledge several limitations of our study. First, our sample size was relatively small, which may limit the generalizability of our findings to the broader population of rosacea patients. Additionally, our study design was cross-sectional, and therefore, we cannot establish causality or the temporal relationship between *Demodex* infestation and the observed skin symptoms.

Study Limitations

Small sample size is a limitation of the study. The lack of an objective scoring system in the diagnosis of *Demodex* infestation is another limitation.

CONCLUSION

In conclusion, our study adds to the growing body of evidence suggesting a potential role for *Demodex* mites in the pathogenesis of rosacea. The significant associations between *Demodex* infestation and specific skin symptoms such as burning, pain and stinging provide important insights into the multifaceted nature of rosacea. Understanding the role of *Demodex* mites in rosacea may open new avenues for therapeutic intervention and improve the management of this challenging dermatological condition. Although this study provides evidence suggesting a potential role for *Demodex* mites in the pathogenesis of rosacea,



Graphic 1. Study of 63 rosacea patients in Central Anatolia explored *Demodex* mite ties to symptoms. Significant link found: *Demodex* presence correlated with skin burning, pain and stinging. No significant associations seen with other symptoms or demographic factors

it is observational in nature and does not evaluate symptom improvement after treatment. Further longitudinal and interventional studies are needed to clarify the causal relationship and therapeutic implications.

*Ethics

Ethics Committee Approval: The study received ethical approval from the Clinical Research Ethics Committee of Sivas Cumhuriyet University, with the approval number 2022-10/02.

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

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Footnotes

*Authorship Contributions

Concept: M.K., Z.A.P., Design: M.K., Z.A.P., Data Collection or Processing: M.K., Z.A.P., M.E., F.Ç.G., Analysis or Interpretation: M.K., Z.A.P., Literature Search: M.K., Z.A.P., Writing: M.K., Z.A.P.

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Retrospective Evaluation of *Toxoplasma gondii* Test Results in Patients Applying to A Research Hospital

Bir Araştırma Hastanesine Başvuran Hastalarda Toxoplasma gondii Test Sonuçlarının Retrospektif Olarak Değerlendirilmesi

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ABSTRACT

Objective: This study retrospectively evaluates our laboratory *Toxoplasma gondii* immunoglobulin (Ig)M, IgG antibody and avidity test results to determine the distribution of the pathogen according to sex, age, clinics and years.
Methods: The serum samples sent to Pamukkale University Healthcare Research and Practice Hospital's Medical Microbiology laboratory between January 2016 and December 2023 were evaluated for *T. gondii* seropositivity. *Toxoplasma* antibodies and avidity testing were studied using chemiluminescent microparticle immunological testing method (Abbott Architect i2000SR, Weisbaden, Germany).
Results: The study evaluated 42,817 tests ordered from patients with suspected toxoplasmosis. Five hundred-three (2.1%) were found to be anti-*T. gondii*-IgM positive and 4.406 (24.7%) to be anti-*T. gondii*-IgG positive. Anti-*T. gondii*-IgM and anti-*T. gondii*-IgG positivity were significantly higher in women than in men (p=0.0001; p=0.0001, respectively). Anti-*T. gondii*-IgM positivity was found to be high between the ages of 19 and 49 (p=0.0001). The anti-*T. gondii*-IgG positivity rate increased with age (p=0.0001). Anti-*T. gondii*-IgM and anti-*T. gondii*-IgG positivity rates have decreased significantly over the last four years (2020-2023) compared to the first four years (2016-2019) (p=0.0001; p=0.0001, respectively). When the results of 582 patients with *T. gondii*-avidity requests were evaluated, 38.1% of the anti-*T. gondii*-IgM positive patients and 7.8% of the anti-*T. gondii*-IgM negative patients had low avidity.
Conclusion: *T. gondii* seroprevalence was found to be similar to the global average, with female sex, age and nationality among the risk factors. It has also been observed that seroprevalence has significantly decreased in recent years.
Keywords: *Toxoplasma gondii*, seroprevalence, age, change over years

ÖZ

Amaç: Çalışmamızda laboratuvarımız *Toxoplasma gondii* immünoglobulin (Ig)M, IgG antikor ve IgG avidite test sonuçlarını retrospektif olarak değerlendirerek patojenin cinsiyete, yaşa, kliniklere ve yıllara göre dağılımının belirlenmesi amaçlanmıştır.
Yöntemler: Pamukkale Üniversitesi Sağlık Araştırma Uygulama Hastanesi Tıbbi Mikrobiyoloji Laboratuvarı'na Ocak 2016-Aralık 2023 tarihleri arasında gönderilen serum örneklerinde *T. gondii* seropozitifliği değerlendirildi. Toxoplazma antikorları ve avidite testi kemolüminasan mikropartikül immünolojik test yöntemi (Abbott Architect i2000SR, Weisbaden, Germany) ile çalışıldı.
Bulgular: Çalışmada, toksoplazmozis şüphesi olan hastalardan istenen 42.817 test sonucu değerlendirilmiştir. Beş yüz üçünde (%2,1) anti-*T. gondii*-IgM ve 4406'sında (%24,7) anti-*T. gondii* -IgG pozitif olarak saptanmıştır. Anti-*T. gondii*-IgM ve anti-*T. gondii*-IgG pozitifliği kadınlarda erkeklere göre anlamlı şekilde yüksek bulunmuştur (sırasıyla p=0,0001; p=0,0001). Anti-*T. gondii*-IgM pozitifliğinin 19-49 yaş aralığında yüksek olduğu görülmüştür (p=0,0001). Anti-*T. gondii*-IgG pozitiflik oranı ise yaş ilerledikçe artmıştır (p=0,0001). Anti-*T. gondii*-IgM ve anti-*T. gondii*-IgG pozitiflik oranı son dört yılda (2020-2023) ilk dört yıla (2016-2019) göre anlamlı şekilde azalmıştır (sırasıyla p=0,0001; p=0,0001). IgG avidite istemi olan 582 hastanın sonuçları değerlendirildiğinde; anti-Toxo IgM pozitif hastaların %38,1'inin ve anti-Toxo IgM negatif hastaların %7,8'inin düşük aviditeye sahip olduğu görüldü.
Sonuç: *T. gondii* seroprevalansının küresel ortalama ile benzerlik gösterdiği, kadın cinsiyet, yaşın ve uyruğun risk faktörleri arasında yer aldığı bulunmuştur. Aynı zamanda son yıllarda seroprevalansın anlamlı şekilde azaldığı görülmüştür.
Anahtar Kelimeler: *Toxoplasma gondii*, seroprevalans, yaş, yıllar arası değişim

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Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Toxoplasmosis caused by *Toxoplasma gondii* (*T. gondii*) is a common infection worldwide (1). *T. gondii* is an obligate intracellular protozoan parasite in the Apicomplexa family. Cats are the definitive hosts and they can spread a large number of oocysts containing two sporocysts with four sporozoites each. Alongside humans, intermediate hosts consist of many other warm-blooded animals, including birds, rodents, pigs, chickens, goats, sheep (2). Transmission to intermediate hosts can occur by consuming fruit, vegetables and water contaminated with sporulated oocysts, eating raw or uncooked meats containing bradyzoite forms of the parasite, congenitally from mother to baby via the placenta, blood transfusion and transplantation of organs containing cysts or tachyzoites (3).

Most cases of toxoplasmosis are asymptomatic or have mild symptoms. Infection can result in congenital toxoplasmosis and sometimes death of the fetus. The most common clinical manifestations in congenital toxoplasmosis are hydrocephalus, cerebral calcification and chorioretinitis. In some cases, it can be a cause of chorioretinitis in patients with sufficient immunity. In patients with suppressed immune systems, encephalitis may occur because of activation of latent infection (3-5).

There are differences in *T. gondii* seroprevalence rates between countries. This difference is caused by the complex interaction of human and environmental factors (6). This study retrospectively evaluates our laboratory *T. gondii* immunoglobulin (Ig)M, IgG antibody and avidity test results to determine the distribution of the pathogen according to sex, age, nationality, clinics and years.

METHODS

Ethically Approval

This study was approved by the Ethics Board for Non-Invasive Clinical Research at Pamukkale University (date: 06/03/2024, number: E.501178).

Study Design

The results of the samples delivered to Pamukkale University Healthcare Research and Practice Hospital's Medical Microbiology Laboratory between January 01, 2016 and December 31, 2023 with *T. gondii* IgM, IgG antibody and avidity requests were evaluated retrospectively. In case patients had recurrent results, only the first results were included in the study. Toxoplasma antibodies and avidity testing were studied with chemiluminescent microparticle immunological testing method (Abbott Architect i2000SR, Weisbaden, Germany). Index values of toxoplasmosis IgM antibodies were reported to be <0.50 non-reactive, ≤0.50-<0.60 gray zone, ≥0.60 reactive; and Toxo IgG antibodies were reported to be <1.6 IU/mL non-reactive, 1.6-<3 IU/mL gray zone, and ≥3 IU/mL reactive. The studied avidity results were evaluated according to clinical request. Those with an avidity index value of 50% were considered low avidity (infection within the last four months) 50% to <60% gray zone, and ≥60% were considered high avidity (infection acquired at least four months ago). The results of the patients were grouped according to the age groups 0-18, 19-49, >49 years, the clinics where they were referred, and years. In addition, the first four-year and last four-year periods were compared.

Statistical Analysis

All statistical analyses were performed using SPSS 25.0 (IBM SPSS Statistics 25 software (Armonk, NY: IBM Corp.). Categorical variables were defined by number and percent. Chi-square test was used for categorical variables comparisons. Bonferroni adjustment method was used for the examinations of multiple comparisons (clinics). Statistical significance was determined as $p<0.05$.

RESULTS

In the study, there were 42,817 test requests from patients with suspected toxoplasmosis. 24,395 were evaluated for anti-*T. gondii*-IgM, 17,840 for anti-*T. gondii*-IgG and 582 for *T. gondii*-avidity. The number of samples evaluated was 18,656 (43.6%) between 2016 and 2019 and 24,161 (56.4%) between 2020 and 2023. Of the samples, 26,717 (62.4%) belonged to female patients, 16,100 (37.6%) to male patients and 42,270 (98.7%) to Turkish nationals and 547 (1.3%) to foreign patients. There were 15,748 (36.8%) samples aged 0-18, 18,518 (43.2%) samples aged 19-49 and 8,551 (20.0%) samples aged 50 and older. Anti-*T. gondii*-IgM and anti-*T. gondii*-IgG were detected positive in 503 (2.1%) and 4406 (24.7%) samples, respectively.

Anti-*T. gondii*-IgM and anti-*T. gondii*-IgG positivity were higher in women (2.6%; 26.3%, respectively) than in men (1.1%; 22.4%, respectively) (p=0.0001; p=0.0001, respectively). The anti-*T. gondii*-IgM positivity rate was higher in the 19-49 age (3.3%) range than in other age groups (1.1%, 1.1%) (p=0.0001). The anti-*T. gondii*-IgG positivity rate increased with age (p=0.0001). Anti-*T. gondii*-IgM and anti-*T. gondii*-IgG positivity rates were found to have decreased significantly over the last four years (2020-2023) (1.8%; 23.1%, respectively) compared to the first four years (2016-2019) (2.4%; 26.9%, respectively) (p=0.0001; p=0.0001, respectively) (Table 1).

Table 1. Anti-T. *gondii*-Ig M and anti-*T. gondii*-IgG

		IgM			IgG		
		Positive	**Negative**	**p**	**Positive**	**Negative**	**p**
Sex	Female	403 (2.6%)	15,218 (97.4%)	0.0001* (cs=57.702)	2,797 (26.3%)	7,858 (73.7%)	0.0001* (cs=34.321)
	Male	100 (1.1%)	8,674 (98.9%)		1,609 (22.4%)	5,576 (77.6%)	
Age	0-18	85 (1%)	8,562 (99%)	0.0001* (cs=155.969)	538 (7.6%)	6,524 (92.4%)	0.0001* (cs=2513.024)
	19-49	364 (3.3%)	10,609 (96.7%)		2,018 (28.2%)	5,141 (71.8%)	
	50 and above	54 (1.1%)	4721 (98.9%)		1,850 (51.1%)	1,769 (48.9%)	
Years	2016-2019	266 (2.4%)	10,592 (97.6%)	0.0001* (cs=14.58)	2,026 (26.9%)	5,493 (73.1%)	0.0001* (cs=35.308)
	2020-2023	237 (1.8%)	13,300 (98.2%)		2,380 (23.1%)	7,941 (76.9%)	

*: p<0.05 statistically significant, cs: Chi-square test, Ig: Immunoglobulin

Anti-*T. gondii*-IgM and anti-*T. gondii*-IgG positivity did not differ by sex in patients aged 0-18 (female 1.1%; 8.1%, male 0.9; 7.2; respectively) (p=0.464; p=0.142, respectively). Although statistically significant differences were not observed, women's positivity (3.5%) was higher than men's in clinical terms. Anti-*T. gondii*-IgM positivity was found to be higher in women (3.5%) than in men (2.2%) in the 19-49 age group, whereas anti-*T. gondii*-IgG positivity did not differ by gender (female and male; 28.2%) (p=0.003; p=0.992, respectively). In patients aged 50 years and older, anti-*T. gondii*-IgM and anti-*T. gondii*-IgG positivity rates were higher in women (1.5%; 53.9, respectively) (male; 0.8%, 48.3% respectively) (p=0.689; p=0.067, respectively) (Table 2).

No significant differences were found in anti-*T. gondii*-IgM and anti-*T. gondii*-IgG positivity rates for age groups between the first four years (2016-2019) and the last four years (2020-2023) (p>0.05). However, despite the absence of significant difference, the anti-*T. gondii*-IgM (1.1%; 0.9%, respectively) and anti-*T. gondii*-IgG (8.4%; 7.2%, respectively) positivity rates in the first four years between the ages of 0-18 were higher than the positivity rate in the last four years. Similarly, despite the absence of significant difference, the anti-*T. gondii*-IgM (3.6%; 3%, respectively) and anti-*T. gondii*-IgG (29.2%; 27.2%, respectively) positivity rates in the first four years between the ages of 19-49 were higher than the positivity rate in the last four years. The anti-*T. gondii*-IgM positivity rate (1.2%) in the first four years of the age group 50 and older was found to be high relative to the positivity rate (1.1%) in the last four years, and the anti-*T. gondii*-IgG positivity rate was low (50.7%, 51.1%, respectively) (Table 2).

When the anti-*T. gondii*-IgM and anti-*T. gondii*-IgG requests of clinics were evaluated, the highest number of requests according to the frequency were found in pediatric health and diseases with 32.9% (13,885/42,235), followed by internal diseases with 28.4% (11,993/42,235), obstetrics and gynecology with 19.5% (8,241/42,235), infectious diseases with 5.4% (2,266/42,235), organ transplant clinic with 3.9% (1,638/42,235) and eye diseases with 2.5% (1,052/42,235), respectively. An evaluation of the anti-*T. gondii*-IgM results showed that the clinics with the highest rate of positivity were infectious diseases (18.6%) and psychiatry (3.6%) clinics, respectively. Anti-*T. gondii*-IgG positivity was highest in cardiology (54.1%) chest disease (52.6%) and adult intensive care (45.4%) samples, respectively (Figure 1). In samples sent from internal medicine clinic and adult intensive care, no statistically significant difference was found between anti-*T. gondii*-IgM and anti-*T. gondii*-IgG positivity rates (p=0.028; p=0.352, respectively). When compared anti-*T. gondii*-IgM positivity rates assessed in the pediatric health and diseases clinic and in the child intensive care departments, there was no statistically significant difference (p>0.05). Anti-*T. gondii*-IgG positivity rates were higher in samples sent from the pediatric health and diseases clinic than in samples sent from the child intensive care department (p=0.082; p=0.0001, respectively).

Anti-*T. gondii*-IgM positivity rates were evaluated according to years, a statistically significant difference was found. There is a significant difference between the years; 2016-2017 (p<0.0001) 2016-2018 (p<0.0001), 2016-2019 (p<0.0001), 2016-2020 (p=0.0005), 2016-2021 (p<0.0001), 2016-2022 (p<0.0001), 2016-2023 (p<0.0001), 2017-2022 (p<0.0001), 2018-2020 (p<0.0001), 2019-2020 (p<0.0001), 2020-2022 (p<0.0001), 2020-2023 (p<0.0001). Anti-*T. gondii*-IgG positivity rates were evaluated according to years, a statistically significant difference was found. There is a significant difference between the years; 2016-2017 (p<0.0001), 2016-2018 (p<0.0001), 2016-2020 (p=0.0001), 2017-2021 (p<0.0001), 2017-2022 (p<0.0001), 2017-2023 (p<0.0001), 2018-2021 (p<0.0001), 2018-2022

Table 2. Evaluation of anti-*T. gondii*-IgM and anti-*T. gondii*-IgG results by age group

			IgM			IgG		
			Negative	**Positive**	**p**	**Negative**	**Positive**	**p**
0-18	Sex	Female	4091 (98.9%)	44 (1.1%)	0,464 (cs=0.535)	3,084 (91.9%)	272 (8.1%)	0,142 (cs=2.152)
		Male	4,471 (99.1%)	41 (0.9%)		3,440 (92.8%)	266 (7.2%)	
	Total		8,562 (99%)	85 (1%)		6,524 (92.4%)	538 (7.6%)	
19-49	Sex	Female	8,807 (96.5%)	324 (3.5%)	0,003* (cs=9.059)	3938 (71.8%)	1546 (28.2%)	0,992 (cs=0)
		Male	1,802 (97.8%)	40 (2.2%)		1203 (71.8%)	472 (28.2%)	
	Total		10,609 (96.7%)	364 (3.3%)		5,141 (71.8%)	2,018 (28.2%)	
50 and above	Sex	Female	2,320 (98.5%)	35 (1.5%)	0,022* (cs=5.247)	836 (46.1%)	979 (53.9%)	0,001* (cs=11.59)
		Male	2,401 (99.2%)	19 (0.8%)		933 (51.7%)	871 (48.3%)	
	Total		4721 (98.9%)	54 (1.1%)		1,769 (48.9%)	1,850 (51.1%)	
0-18	Years	2016-2019	3048 (98.9%)	33 (1.1%)	0,537 (cs=0.382)	2183 (91.6%)	200 (8.4%)	0.08 (cs=3.066)
		2020-2023	5514 (99.1%)	52 (0.9%)		4341 (92.8%)	338 (7.2%)	
	Total		8,562 (99%)	85 (1%)		6,524 (92.4%)	538 (7.6%)	
19-49	Years	2016-2019	5,626 (96.4%)	210 (3.6%)	0.08 (cs=3.072)	2,558 (70.8%)	1,053 (29.2%)	0,065 (cs=3.405)
		2020-2023	4,983 (97%)	154 (3%)		2,583 (72.8%)	965 (27.2%)	
	Total		10,609 (96.7%)	364 (3.3%)		5,141 (71.8%)	2,018 (28.2%)	
50 and above	Years	2016-2019	1,918 (98.8%)	23 (1.2%)	0.77 (cs=0.086)	752 (49.3%)	773 (50.7%)	0,658 (cs=0.196)
		2020-2023	2803 (98.9%)	31 (1.1%)		1,017 (48.6%)	1,077 (51.4%)	
	Total		4721 (98.9%)	54 (1.1%)		1,769 (48.9%)	1,850 (51.1%)	

*: p<0.05 statistically significant, cs: Chi-square test, Ig: Immunoglobulin

(p<0.0001), 2018-2023 (p<0.0001), 2019-2021 (p<0.0001), 2019-2022 (p<0.0001), 2019-2023 (p<0.0001), 2020-2021 (p<0.0001), 2020-2022 (p<0.0001), 2020-2023 (p<0.0001) anti-*T. gondii*-IgM and anti-*T. gondii*-IgG positivity rates vary over the years (Figure 2).

When the results of 582 patients with *T. gondii*-avidity requests were evaluated, 38.1% of the anti-*T. gondii*-IgM positive patients and 7.8% of the anti-*T. gondii*-IgM negative patients had low avidity. Of the 124 patients with low avidity, 105 (84.7%) were female patients, of whom 90 (85.7%) were in the fertility age group. It was found that 44.4% of anti-*T. gondii*-IgM positive patients and 87.3% of anti-Toxo IgM-negative patients had high avidity (Table 3).

DISCUSSION

T. gondii causes toxoplasmosis and is found all over the world. It can remain in the body for life in humans and animals. Most people with a healthy immune system have no symptoms. Toxoplasmosis is usually diagnosed by the detection of Toxoplasma-specific IgG, IgM, IgA or IgE antibodies. These antibodies are detected by dye test, Indirect fluorescent antibody test, enzyme immunoassays (immunoblots), Avidity test and Agglutination test (1).

This study examined *T. gondi* prevalence and the changes in prevalence by sex, age, clinics and over years. Anti-*T. gondii*-IgG and anti-*T. gondii*-IgM positivity rates were 24.7% and 2.1%, respectively. The global *T. gondii* seroprevalence ranges widely from 0.5% to 87.7%, and the average seroprevalence is 25.7% (6). Seroprevalence is influenced by geographical location and there are differences in the prevalence rates between continents. Africa (61.4%) and Oceania (38.5%) have high prevalence, while Asia (16.4%) presents low prevalence (6). The overall seroprevalence of the European continent is estimated at 32.1% (7). The seroprevalence of *T. gondii* in neighboring countries of Türkiye with similar geography has been reported as 27% in Bulgaria, 2.3% for anti-*T. gondii*-IgM and 31.1% for anti-*T. gondii*-IgG in Iran, and 37.6% in the city of Basra in Iraq (8-10). The prevalence in Türkiye ranges from 17.5% to 69.5% (4). Türkiye is located in Europe and Asia, and our study was conducted in a province of the Aegean region in the western part of Türkiye. The prevalence of *T. gondii* in our study is similar to the global average.

In our study, *T. gondii* IgM and IgG positivity was higher in female sex (female, 2.6%; 26.3%; male, 1.1%; 22.4%, respectively). Some studies considered male sex to be an independent risk factor (10,11). The high prevalence of antibody positivity in the female sex in our study may have been mostly due to women preparing meals and more encounters with raw meat and raw vegetables (12).

It has been suggested that 1.1% of adults and 1.3% of women between the ages of 18 and 49 experience seroconversion each year (11). A recent study in Iraq reported that the IgM positivity was higher between the ages of 15 and 44 than other ages (10). In our study, the IgM positivity was higher in the 19-49 age interval than in other age groups. Especially in female patients,

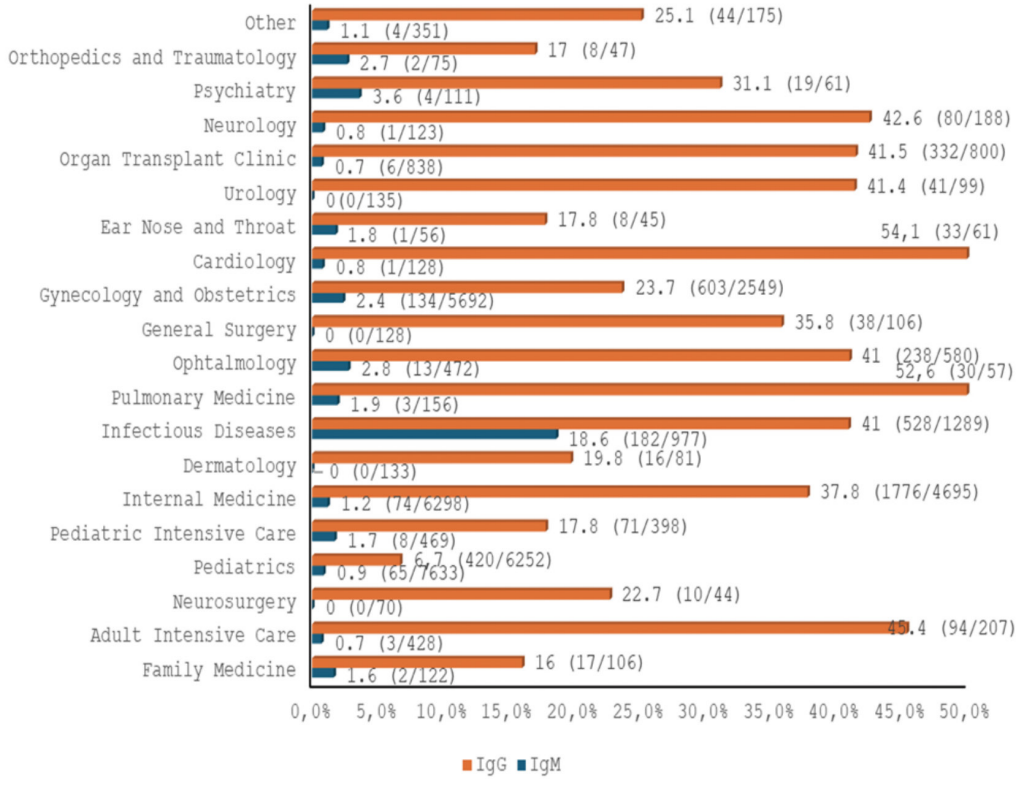

Figure 1. Anti-*T. gondii*-IgM and anti-*T. gondii*-IgG positivity rates in patient samples sent from different clinics % (positive/total)
Ig: Immunoglobulin, Other (Emergency Service, Anesthesia, Physical Therapy and Rehabilitation, Cardiovascular Surgery, Chest Surgery, Plastic Surgery, Pediatric Surgery, Nuclear Medicine, Genetics, Pharmacology, Pathology outpatient clinics), Adult Intensive Care (Anesthesia, Brain Surgery, Internal Diseases, Cardiovascular Surgery, Chest Diseases, Cardiology, Neurology), Pediatric Intensive Care (Newborn, Pediatric Surgery, Child)

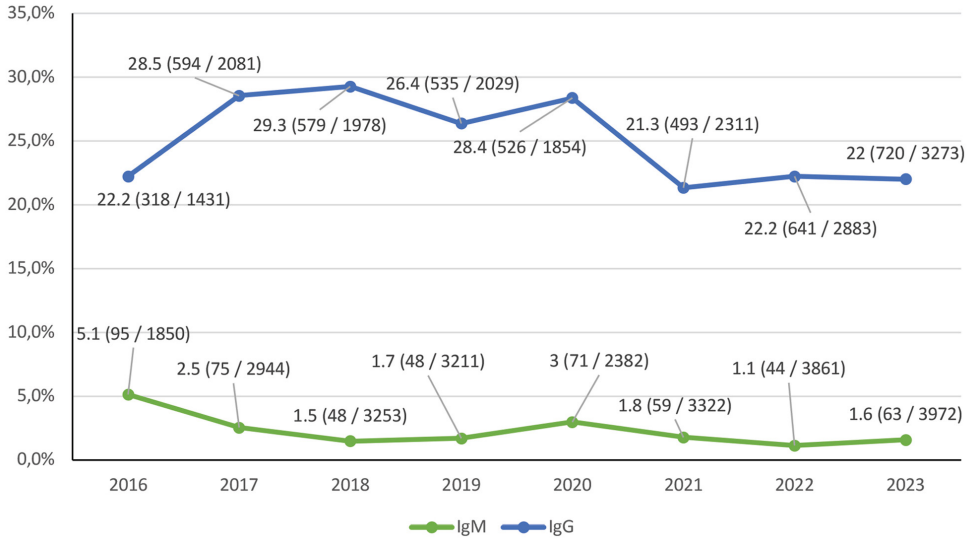

Figure 2. Anti-*T. gondii*-IgM and anti-*T. gondii*-IgG positivity rates per year % (positive/total)
Ig: Immunoglobulin

Table 3. *T. gondii*-avidity results n (%)

***T.gondii*-avidity**	**IgM**		
	Positive	**Interpolation**	**Negative**
Low	96 (38.1)	4 (19.1)	24 (7.8)
Gray zone	44 (17.5)	2 (9.5)	15 (4.9)
High	112 (44.4)	15 (71.4)	270 (87.3)
Total	252 (100)	21 (100)	309 (100)

Ig: Immunoglobulin

IgM positivity at this age interval (fertility age) is very important as it can cause congenital toxoplasmosis in the case of pregnancy. Seroconversion monitoring and avidity testing are recommended in the case of pregnancy (13).

The *T. gondii*-avidity test can predict when the infection has been acquired. A low avidity index indicates a recent infection, while a high avidity index excludes a recent infection (before four months) (14). In our study, avidity test results were analyzed in all IgM-positive or negative patients with an avidity test request. Similarly, the Toxoplasmosis Reference Centre of France, one of the few countries that has long had a mandatory screening program for pregnant women, recommends that all patients with anti-*T. gondii*-IgG positive be screened for avidity (13). In our study, it was determined that 24 (7.8%) of the patients who were negative for anti-*T. gondii*-IgM had low avidity, and thus the primary infections in these patients were not missed. In addition, it was thought that the reason for the high avidity observed in anti-*T. gondii*-IgM positive patients in our study could be the IgM positivity that can persist for months in some patients (14).

In the present study, it was understood that IgG positivity increases with age as reported in some studies (11,15,16). Hypothetically, it may be suggested that increased age and prolonged exposure to infection and risk factors in individuals lead to a higher prevalence (15,16).

It is seen that all clinics of the hospital are requested for diagnosis. In addition, it has been determined that there were more requests from children's health and diseases (32.9%), internal diseases (28.4%), obstetrics and gynecology (19.5%), infectious diseases (5.4%), organ transplant clinic (3.9%) and eye diseases clinic (2.5%) compared to other departments. More requests from these departments by physicians may indicate that the organ involvement of the risk groups for *T. gondii* infection (congenital toxoplasmosis, pregnancy, immunosuppressive patient groups) and the clinical picture are fully understood. Some studies suggest that routine monitoring of risk groups for this parasite is also valuable in terms of reactivation/reinfection (17).

As a result of the public health practices of countries, changes have occurred in the prevalence of toxoplasmosis over the years. A study in the Netherlands showed no decrease in prevalence in the last 10 years (18). However, a study in Portugal found that the prevalence decreased over the years (16). In our study, the *T. gondii* IgG and IgM test positivity rate was significantly lower in the last four years (2020-2023) than in the first four years (2016-2019) (p=0.0001; p=0.0001, respectively). The main reasons for this decrease in the positivity rate include that, as in the whole world, more importance has been given to the hygiene rules in our country with Coronavirus disease-2019, after which hygiene habits has become permanent.

For an effective fight against toxoplasmosis, it is crucial to evaluate environmental, animal and human health from a holistic perspective (19). Recommended methods for the control of toxoplasmosis include training on the subject, prevention of

contact with contagious materials, food and water sanitation, vaccinations to prevent the spread of oocysts to cats, and the use of immunotherapy and chemotherapy for the treatment of diseases (5).

Study Limitations

The limitations of this study include the fact that the data reflects a single center and risk factors could not be evaluated due to the retrospective nature of the study. But that it covers a long period of eight years and has a high number of samples represent its strengths.

CONCLUSION

In conclusion, *T. gondii* seroprevalence was found to be similar to the global average, with female sex and age among the risk factors. It has also been observed that seroprevalence has significantly decreased in recent years.

*Ethics

Ethics Committee Approval: This study was approved by the Ethics Board for Non-Invasive Clinical Research at Pamukkale University (date: 06/03/2024, number: E.501178).

Informed Consent: Retrospective study.

*Footnotes

Authorship Contributions

Concept: S.Z.Ö., A.Ç., M.D., Ç.E., İ.K., Design: Ü.Ç., M.G.Ö., Data Collection or Processing: S.Z.Ö., Ç.E., M.D., E.M., Analysis or Interpretation: S.Z.Ö., M.D., A.Ç., H.Ş., İ.K., Literature Search: S.Z.Ö., A.Ç., M.D., Ç.E., Writing: S.Z.Ö.

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

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A Parasite That Should not be Neglected in Patients with Ulcerative Colitis: *Entamoeba histolytica*

Ülseratif Kolit Hastalarında İhmal Edilmemesi Gereken Bir Parazit: Entamoeba histolytica

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ABSTRACT

Objective: To determine the prevalence of amoebiasis, which has been neglected in recent years according to the World Health Organization, in ulcerative colitis patients and investigate the relationship between amoebiasis and ulcerative colitis.
Methods: The study included 150 individuals, including 100 ulcerative colitis patients and 50 healthy individuals without gastrointestinal complaints. The samples collected were first analyzed macroscopically and then using native-Lugol, trichrome staining, and enzyme-linked immunosorbent assay (ELISA).
Results: In the microscopic examination, *Entamoeba* spp. cysts were found in 22% of the ulcerative colitis patients and 2% of those in the control group. *Entamoeba histolytica* (*E. histolytica*) adhesin antigen was detected by ELISA in 31% of the ulcerative colitis patients and 4% of those in the control group. A significant correlation was found between the incidence of *E. histolytica* and ulcerative colitis in the statistical evaluation.
Conclusion: *E. histolytica* should not be neglected in ulcerative colitis patients and should be investigated in the presence of diarrhea, bloody diarrhea, and abdominal pain.
Keywords: Amoeba, dysentery, *E. histolytica*, ulcerative colitis

ÖZ

Amaç: Bu çalışma, Dünya Sağlık Örgütü'ne göre son yıllarda ihmal edilen amoebiasisin, ülseratif kolit hastalarındaki yaygınlığını belirlemek ve amoebiasis ile ülseratif kolit arasındaki ilişkiyi araştırmak amacıyla yapıldı.
Yöntemler: Çalışmaya ülseratif kolit tanısı konmuş 100 hasta ve gastrointestinal şikayeti olmayan 50 sağlıklı birey olmak üzere toplam 150 kişi dahil edildi. Çalışmaya dahil edilen örnekler önce makroskobik olarak, sonrasında nativ-Lugol, trikrom boyama ve enzime bağlı immünosorbent deneyi (ELISA) yöntemleri kullanılarak incelendi.
Bulgular: Mikroskobik bakı ile ülseratif kolitli hastaların %22'sinde, kontrol grubunun %2'sinde *Entamoeba* spp. kisti saptandı. ELISA yöntemi ile ülseratif kolitli hastaların %31'inde, kontrol grubunun ise %4'ünde *Entamoeba histolytica* (*E. histolytica*) adezin antijeni saptandı. *E. histolytica* görülme sıklığı ile ülseratif kolit arasında yapılan istatistiksel değerlendirmede anlamlı bir ilişki saptandı.
Sonuç: Ülseratif kolit hastalarında *E. histolytica*'nın ihmal edilmemesi ve hastalarda ishal, kanlı ishal ve karın ağrısı varlığında mutlaka *E. histolytica*'nın ayırıcı tanı ile araştırılması gerektiği kanaatine varıldı.
Anahtar Kelimeler: Amip, dizanteri, *E. histolytica*, ülseratif kolit

INTRODUCTION

Inflammatory bowel disease (IBD) is a general term used for chronic inflammatory diseases involving the gastrointestinal system caused by immunological, genetic, and environmental factors, the etiology of which is not yet well understood. Ulcerative colitis, categorized in IBD, is a chronic disease characterized by diffuse inflammation of the mucosa of the colon and rectum. The exact cause of ulcerative colitis is

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Türkiye PARAZİTOLOJİ Dergisi

unknown. However, it is thought that the risk of developing the disease may increase in the presence of bacterial, viral, or parasitic infections (1,2).

Clinical symptoms of ulcerative colitis include abdominal pain and diarrhea with or without blood. There are no pathognomonic symptoms, signs, or tests for its diagnosis. Diagnosis can be made by evaluating the clinical symptoms in detail and excluding other diseases that may simulate the disease. *Entamoeba histolytica*, *Salmonella*, *Shigella*, *Escherichia coli*, *Campylobacter*, *Mycobacterium tuberculosis*, *Clostridium difficile*, Norovirus, Adenovirus, Rotavirus, and Cytomegalovirus should be excluded, especially in differential diagnosis (3-5).

Amoebiasis is a common parasitic disease caused by *E. histolytica*, affecting approximately 10% of the world's population. Transmission of the disease occurs through food and drinks contaminated with the cyst form of the parasite. The disease is usually asymptomatic but can cause clinical symptoms ranging from cramping abdominal pain, watery or bloody diarrhea, weight loss, and amebic colitis. In addition, *E. histolytica* can colonize in the large intestine, cross the mucosal epithelial barrier, spread to extra-intestinal organs, and cause abscesses. In particular, abscesses may occur in the liver, lungs, brain, skin, or perianal region. Acute amoebic colitis is clinically similar to IBD. Amoebiasis can exacerbate IBD symptoms or have a negative impact on the course and treatment of the disease (6-9).

The aim of the current study was to determine the prevalence of amoebiasis, which has been neglected in recent years according to the World Health Organization (10), in ulcerative colitis patients and investigate the relationship between amoebiasis and ulcerative colitis.

METHODS

Study Design

This cross-sectional study was conducted between July 2020 and October 2021 at the Van Yüzüncü Yıl University Faculty of Medicine Parasitology Laboratory. The study was initiated after patient consent was obtained. Prior to the research, permission was obtained with the decision of Van Yüzüncü Yıl University Faculty of Medicine, Non-Interventional Clinical Research Ethics Committee (2021/06-11). The study was designed with a patient group and a control group. Patients who applied to the Gastroenterology Outpatient Clinic of Van Yüzüncü Yıl University Dursun Odabaş Medical Centre and were diagnosed with ulcerative colitis based on endoscopic, radiological, histopathological, and clinical findings were included in the patient group, and individuals without gastrointestinal complaints were included in the control group.

Sample Size

The sample size of this prospective study was calculated using G*Power 3.1.9.7 statistical software within the scope of chi-squared (χ^2) goodness-of-fit tests (9). In the calculations, a minimum of 50 samples in each group was determined when the power was 0.80, the effect size was 0.4 (χ^2 test effect size interval value) and the type 1 error (α) was 0.05.

Study Population and Sample Collection

In the study, patients diagnosed with ulcerative colitis were included in the patient group, and healthy individuals without any chronic disease were included in the control group. The age and clinical findings such as diarrhea and abdominal pain of each study participant were recorded. Stool samples were collected and brought to Van Yüzüncü Yıl University Faculty of Medicine, Department of Parasitology Research Laboratory, and stored in the refrigerator at +4 °C.

Examining Stool Samples

Stool samples were first examined macroscopically (shape, consistency and color of the stool) and then microscopically. The stool samples were analyzed using both native-Lugol and trichrome staining to evaluate cystic and trophozoitic forms of of *Entamoeba* species. Trichrome staining was performed using Wheatley's trichrome staining kit (Gul Biology Laboratory, İstanbul, Türkiye), following the manufacturer's instructions.

Adhesin antigen was detected for *E. histolytica* seropositivity in the stool samples using an enzyme-linked immunosorbent assay (ELISA) kit (TechLab Systems Inc., Blacksburg, VA, USA), following the manufacturer's instructions.

Statistical Analysis

The χ^2 test, 2-ratio Z test of the ratios, and Fisher's Exact test were used in the statistical the analyses. In the calculations, statistical significance was accepted as $p<0.05$. IBM SPSS Statistics for Windows 26.0 (IBM Corp., Armonk, NY, USA) and MINITAB 14.0 were used for the calculations.

RESULTS

The study included 100 ulcerative colitis patients. Since it was difficult to reach healthy volunteers, only 50 individuals were included in the control group. The mean age of the 100 ulcerative colitis patients was 41.5±14.7 (range: 18-79) years and that of the 50 individuals in the control group was 38.6±17.8 (range: 18-81) years. There was no statistical difference between the age distributions in the groups (p=0.548).

Macroscopic examination revealed that 71 (47.33%) stool samples were positive for diarrhea (type 7 according to Bristol stool scale) and 38 (25.33%) were positive for bloody diarrhea. *Entamoeba* spp. cysts were detected in 15.3% of the 150 stool samples using native-Lugol (Figure 1) and trichrome (Figure 2) staining. No trophozoite forms were observed in the microscopic examination of the stool samples. With trichrome staining revealed *Entamoeba* spp. cysts in 22 (22%) stool samples of the ulcerative colitis patients and one (2%) of those in the control group. There was a significant difference between the frequency of *Entamoeba* spp. in the ulcerative colitis patients and the control group (p=0.001).

E. histolytica adhesin antigen was detected in 33 (22%) of the 150 stool samples via ELISA. *E. histolytica* was detected in 31 (31%) of the ulcerative colitis patients and two (4%) of those in the control group (Table 1). A statistically significant difference was found between the ulcerative colitis patients and control group in terms of *E. histolytica* positivity (p=0.001).

E. histolytica was detected in 23 (32.39%) of 71 patients with diarrhea and eight (27.58%) of 29 patients without diarrhea (Table 1). No statistically significant difference was found between *E. histolytica* positivity and diarrhea (p=0.63). In addition, *E. histolytica* was detected in 11 (28.95%) of 38 patients with bloody diarrhea and 20 (32.25%) of 62 patients without bloody diarrhea (Table 1). No significant difference was found between in terms of

E. histolytica positivity between patients having bloody diarrhea and not (p=0.726).

Abdominal pain was present in 45 (54%) patients with ulcerative colitis. *E. histolytica* was detected in 18 (40%) of 45 patients with abdominal pain and 13 (14.29%) of 55 patients without abdominal pain (Table 1) (p=0.072).

DISCUSSION

It was reported that the distribution of *E. histolytica* worldwide varies between 1% and 10% and in some regions, this rate is as high as 50%. In Türkiye, the prevalence of amoebiasis is between 0.4% and 18.4% and it is endemic in the southeastern region (11). The incidence of ulcerative colitis is quite high in European countries, but recent studies have shown that the incidence has plateaued or even decreased in these countries. However, a significant increase in the incidence of the disease was reported in South America, Asia, Middle East, and Africa, especially in developing countries (1,12).

In studies investigating the prevalence of amebiasis in ulcerative colitis patients in different countries, *E. histolytica* positivity was stated as 1.4-14.3% (2,9,13,14). In Türkiye, *E. histolytica/E. dispar* was detected at rates as high as 10-31.5% in studies conducted on ulcerative colitis patients (4,6,15,16). In the current study, *E. histolytica* positivity was found in 31% of the ulcerative colitis patients. While the western part of Türkiye is similar to the socio-economic structure of developed countries, the eastern and southeastern regions have a lower socio-economic structure. We believe that the high amebiasis rate found in the ulcerative colitis patients in the current study may be due to the socio-economic or hygiene habits of the region. However, since the main aim of the study was to investigate whether there is a relationship between ulcerative colitis and amoebiasis, the *E. histolytica* positivity rate in ulcerative colitis patients on its own is insufficient. Therefore, positivity rates in control groups are important. In the current study, *E. histolytica* positivity rate in the control group was 4%. When this positivity rate was compared with that in the ulcerative colitis patients, the difference was statistically significant (p=0.001). This indicates that there may be a relationship between *E. histolytica* and ulcerative colitis. In previous studies, the *E. histolytica* positivity rate was higher in the ulcerative colitis patients compared to the control groups (9,15,16). However, the relationship between ulcerative colitis and amoebiasis is not clearly known because the endoscopic appearance of amebic colitis may be confused with that of ulcerative colitis. The diagnosis of ulcerative colitis is primarily based on endoscopic findings showing inflammation from the rectum to the colon and is confirmed by biopsy specimens showing chronic colitis. It is usually diagnosed by sigmoidoscopy without parasitological examination, and therefore, amoebic colitis is often overlooked. Histopathological findings of amoebic colitis and ulcerative colitis may also resemble crypt abscess (17). Therefore, we believe that one of the reasons for the high rate of *E. histolytica* in ulcerative colitis patients is the lack of a clear distinction between amoebic colitis and ulcerative colitis. Hence, since misdiagnosis of amoebic colitis as ulcerative colitis and subsequent treatment with corticosteroids can be fatal, a differential diagnosis for *E. histolytica* should definitely be made in patients diagnosed with ulcerative colitis.

In the present study, no statistically significant relationship was found between abdominal pain (p=0.072), diarrhea with blood (p=0.726), and diarrhea (p=0.63) and *E. histolytica* positivity. The clinical symptoms of ulcerative colitis include abdominal pain and diarrhea with or without blood (2). The same symptoms

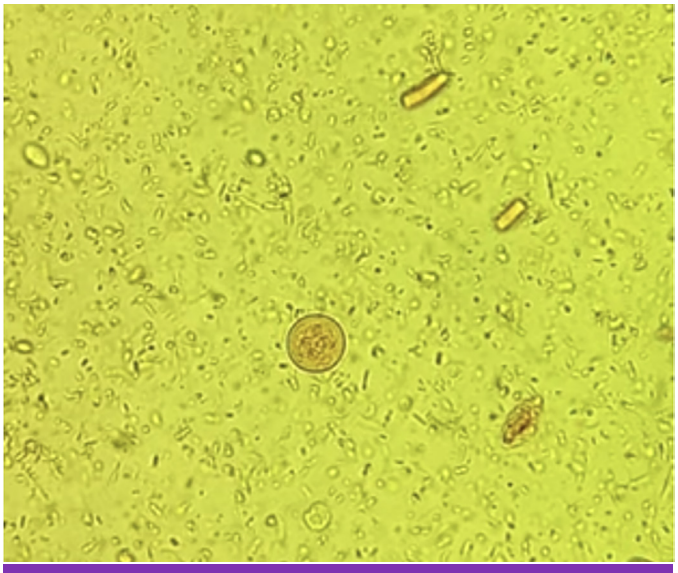

Figure 1. *Entamoeba* spp. cyst detected on a Lugol-stained preparation in a patient

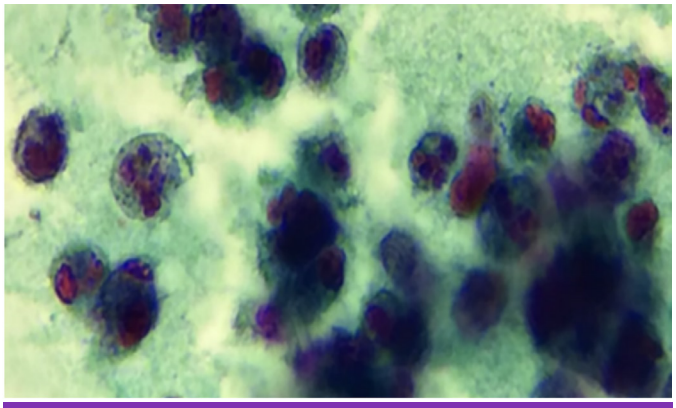

Figure 2. *Entamoeba* spp. cysts detected in a trichrome-stained preparation in a patient

Table 1. Comparison of the *E. histolytica* positivity rates

Variable		*E. histolytica* adhesin antigen		p-value
		Positive n (%)	Negative n (%)	
Working group	Ulcerative colitis patients (n=100)	31 (31.00)	69 (69.00)	**0.001**
	Control group (n=50)	2 (4.00)	48 (96.00)	
Diarrhea	Yes (n=71)	23 (32.39)	48 (67.61)	0.63
	No (n=29)	8 (27.58)	21 (72.42)	
Bloody diarrhea	Yes (n=38)	11 (28.95)	27 (71.05)	0.726
	No (n=62)	20 (32.25)	42 (67.75)	
Abdominal pain	Yes (n=45)	18 (40.00)	27 (60.00)	0.072
	No (n=55)	13 (23.63)	42 (76.36)	

are observed in symptomatic intestinal amoebiasis (18). For this reason, ulcerative colitis and amebiasis may coexist and be misdiagnosed due to the similar clinical course of both diseases. In addition, it should be kept in mind that the steroids used in the treatment of amebiasis misdiagnosed as ulcerative colitis may cause exacerbation of the disease and the formation of multiple liver amoebic abscesses (19).

In developing countries, the microscopic method is more frequently used in the diagnosis of the disease. However, the reliability of this method is debated. In the microscopic method, the presence of food residues and/or leucocytes in the feces, deformation of the parasite in stored feces, insufficient sample quantity, and inadequate experience of the person performing the microscopic examination result in misdiagnosis of *E. histolytica* (4,18). Furthermore, microscopic methods cannot distinguish between *E. histolytica* and *E. dispar* and *E. moshkovskii* and *E. bangladeshi*, which are morphologically similar to *E. histolytica*. In particular, the native Lugol method is the most commonly used method in microscopic examination. However, this method alone is insufficient to diagnose the parasite and must be supported by other methods. In the differential diagnosis of *E. histolytica*, serological methods or molecular methods based on the principle of detecting *E. histolytica*-specific antigens in the feces or parasite-specific antibodies in the serum are used. ELISA is the most preferred serological method in routine diagnosis due to its easy applicability, and high diagnostic sensitivity and specificity (20-22). In studies comparing the methods used in the diagnosis of the parasite (23-25), different results were obtained. In the current study, *Entamoeba* spp. cysts were detected in 15.3% of the stool samples under microscopic examination, while *E. histolytica* was detected in 22% of the samples via ELISA. Therefore, since microscopic methods may give misleading results in the differential diagnosis of amoebiasis in ulcerative colitis patients, we believe that the results should be supported by serological methods. Ayrıca Because of the similarity of clinical symptoms between ulcerative colitis and amebiasis, amebiasis should be distinguished from ulcerative colitis. Microscopic methods should not be evaluated alone because they are both misleading and inadequate when used alone. Although it is stated that the ELISA method and the adhesin antigen examination provide a definitive diagnosis for *E. histolytica*, it should not be forgotten that *E. histolytica* will not cause infection while continuing to live in the intestinal cavity. It was concluded that the most reliable evaluation should be the use of advanced methods together with the clinical picture of the disease and that a single method should not be used in diagnosis.

CONCLUSION

In conclusion, *E. histolytica* was detected in 31% of the ulcerative colitis patients. It was concluded that *E. histolytica* should not be neglected in ulcerative colitis patients. It should be excluded by differential diagnosis in the presence of diarrhea, bloody diarrhea, and abdominal pain in this patient group, and the result should be supported by serological methods since microscopic methods can give misleading results in diagnosis.

*Ethics

Ethics Committee Approval: Prior to the research, permission was obtained with the decision of Van Yüzüncü Yıl University Faculty of Medicine, Non-Interventional Clinical Research Ethics Committee (2021/06-11).

Informed Consent: The participants were informed about the research and their consent was obtained.

Footnotes

This article is an abridged version of Murat Soylu's Master Thesis titled "Investigation of *Entamoeba histolytica* Prevalence in Ulcerative Colitis Patients".

*Authorship Contributions

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

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Two Imported Malaria Cases with Delayed Response to Treatment in Hatay

Hatay'da Yurt Dışı Kaynaklı Tedaviye Geç Yanıt Veren İki Sıtma Olgusu

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ABSTRACT

The study presents two imported malaria cases with a history of travel to malaria-endemic areas and replied late response to treatment. In the blood preparations of the first case, dot-shaped nucleus structures were identified in the erythrocytes, which looked different from the classical erythrocytic forms. In the SD-Pf/Pan test, bands were obtained for both P.f and Pan; while in the SD-Pf/Pv test, a band was obtained for P.f. The *P. falciparum 18S rRNA* gene was detected using real-time polymerase chain reaction. Artemether-lumefantrine treatment protocol was started. Due to deterioration in general condition on the third day, artemether-lumefantrine treatment was extended to six days, and primaquine phosphate was added. Discharge was on the 16th day of treatment. In the second case, young trophozoites were identified in blood smears. Bands in P.f were obtained in both the SD-Pf/Pan and SD-Pf/Pv tests. Artemether-lumefantrine treatment protocol was started. On the third day of treatment, banana-like gametocytes were observed in blood smears. The patient was discharged at his own request and two days later, upon follow-up, gametocytes were still observed in blood smears. Artemether-lumefantrine treatment was restarted. Gametocytes continued to be observed in the following days. Primaquine phosphate was added to the treatment protocol. The patient was discharged after a 3-week follow-up. The study is presented to draw attention to the increasing cases of imported malaria in Hatay and the increase of malaria cases that respond late to treatment in recent years.
Keywords: Imported malaria, *Plasmodium falciparum*, Hatay

ÖZ

Çalışmada, sıtma endemik bölgeye seyahat öyküsü bulunan ve tedaviye geç yanıt veren iki importe sıtma olgusu sunulmaktadır. Birinci olgunun kan preparatlarında, eritrositlerde klasik eritrositer formlardan farklı görünümde nokta tarzında nükleus yapıları saptandı. SD Bioline Malaria Ag P.f/Pan (SD-Pf/Pan) testinde P.f ve Pan'da; SD Bioline Malaria Ag P.f/P.v (SD-Pf/Pv) testinde P.f'da band elde edildi. Gerçek zamanlı polimeraz zincir reaksiyonunda *P. falciparum 18S rRNA* geni saptandı. Artemether-lumefantrine tedavi protokolü başlanıldı. Genel durumunun üçüncü gününde kötüleşmesi nedeniyle artemether-lumefantrine tedavisi altı güne uzatıldı ve primaquine phosphate eklendi. Tedavisinin 16. gününde taburcu edildi. İkinci olgunun kan preparatlarında genç trofozoitler saptandı. SD-Pf/Pan ve SD-Pf/Pv testlerinde P.f'da band elde edildi. Artemether-lumefantrine tedavi protokolü başlanıldı. Tedavinin üçüncü gününde, kan preparatlarında muz şeklinde gametositler görüldü. Hasta kendi isteği ile taburcu oldu ve iki gün sonra kontrole geldiğinde kan preparatlarında gametositler görülmeye devam etti. Artemether-lumefantrine tedavisi tekrar başlanıldı. Sonraki günlerde gametositler görülmeye devam etti. Tedavi protokolüne primaquine phosphate eklendi. Üç haftalık takip sonunda hasta taburcu edildi. Çalışma Hatay'da artan importe sıtma olgularına ve tedaviye geç cevap veren sıtma olgularının son yıllardaki artışına dikkat çekmek amacıyla sunulmuştur.
Anahtar Kelimeler: İmporte sıtma, *Plasmodium falciparum*, Hatay

INTRODUCTION

Malaria is a disease transmitted to humans by female Anopheles mosquitoes infected with parasites of the *Plasmodium* genus. According to World Health Organization data, approximately half of the world's population is at risk, with approximately 247 million people infected with malaria in 85 countries in 2021, resulting in approximately 619,000 deaths. (1) Since 2010, no indigenous malaria cases have been reported in Türkiye, but imported cases are observed (2).

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***This case presentation was presented at the 23rd Parasitology Congress held in Antalya between October 30th and November 3rd.**

Türkiye PARAZİTOLOJİ Dergisi

Malaria diagnosis is made by observing the parasite under a microscope during the examination of blood preparations prepared from patient samples. Additionally, rapid diagnostic tests prepared to detect *Plasmodium* antigens and serological and molecular tests are also used for diagnosis (3).

Quinine is the first drug used in malaria treatment. Resistance developed 278 years after its use as an antimalarial agent, leading to the use of chloroquine in malaria treatment. After resistance also developed against chloroquine 12 years later, proguanil, sulfadoxine-pyrimethamine, mefloquine, and atovaquone began to be preferred in malaria treatment as antimalarial drugs. However, resistance to these agents has been reported (4).

The aim of the study was to draw attention to the diagnosis, effective treatment protocol, and importance of patient follow-up in malaria, following the identification of two cases of malaria from foreign sources traveling to malaria-endemic areas and showing delayed response to treatment.

CASE REPORT

Case 1

A 50-year-old patient who presented to the Infectious Diseases Outpatient Clinic of Hatay Mustafa Kemal University Faculty of Medicine with complaints of fever, chills, and tremors for the past three days revealed, upon history-taking, that he had traveled to Ghana for work and returned to Hatay three days ago. It was learned that he had received malaria treatment in Ghana but sought medical attention due to ongoing complaints.

On physical examination, the patient's general condition was moderate, vital signs were stable, with a temperature of 38 °C, and hepatosplenomegaly was present. Laboratory findings showed: white blood cell (WBC); 13230 µ/L, hemoglobin (Hgb); 13.5 g/dL, platelets; 90,000 µ/L, aspartate transaminase (AST): 214 U/L, alanine transaminase (ALT): 92 U/L, T. bil: 2.17 mg/dL, D. bil: 1.23 mg/dL, lactate dehydrogenase: 1086 U/L, C-reactive protein (CRP): 126 mg/dL, prothrombin time-international normalized ratio (PTZ-INR): 1.16.

Since the clinical symptoms were consistent with malaria and due to the history of international travel, blood was drawn from the patient and sent to the parasitology department. Thick and thin blood smears were prepared and stained with Giemsa staining method. During the examination of thin blood smears under the immersion objective, nucleus structures resembling dot-like formations, different from the classic erythrocytic forms, were observed in erythrocytes. Nucleus structures were also detected in the examination of thick blood smear preparations (Figure 1). Two rapid diagnostic tests recommended by the manufacturer's protocol were used to distinguish *P. falciparum* and other *Plasmodium* species from the peripheral blood sample taken from the patient: SD Bioline Malaria Ag P.f/Pan (SD-Pf/Pan) and SD Bioline Malaria Ag P.f/P.v (SD-Pf/Pv) (Standard Diagnostics, Inc; Suwon City, Republic of Korea). In the SD-Pf/Pan rapid diagnostic test, positive bands were obtained in both P.f and Pan, while in the SD-Pf/Pv test, a positive band was only obtained in P.f (Figure 2). DNA isolation was performed from the blood sample obtained from the patient using a commercial kit (QIAamp DNA Isolation kit, Qiagen, Germany), and species-specific real-time polymerase chain reaction (RT-PCR) was performed using a commercially prepared kit (Genesig® Std Real-time PCR detection kit for *P. falciparum, P.vivax, P. ovale*, *P. malaria*, Primerdesign™ Ltd., Chandler's Ford, UK). The PCR mixture and amplification protocol were applied according to the manufacturer's protocol. The *P. falciparum 18S rRNA* gene was detected.

The patient was treated with Artemether-lumefantrine therapy according to the protocol: Four tablets every 8 hours on the first day, followed by 4 tablets every 12 hours for the next two days. Due to shortness of breath observed during patient follow-ups, consultations with chest diseases and cardiology were sought, and their recommendations were followed. Additionally, on the third day of treatment, as the patient's condition worsened significantly and the presence of atypical morphology with dot-like nucleus structures continued to be observed in blood smears, the Artemether-lumefantrine treatment was extended to six days, and primaquine phosphate was added to the treatment protocol.

Peripheral blood samples were taken from the patient daily, and thin and thick blood smears were prepared and examined. On the third day of treatment, dot-like nucleus structures were still observed intensely. However, on the 10th day, there was a decrease in the atypical morphology observed, and the patient was discharged on the 16th day of follow-up (Figure 3, 4). The patient, whose condition improved clinically, was discharged with a follow-up appointment recommended 10 days later.

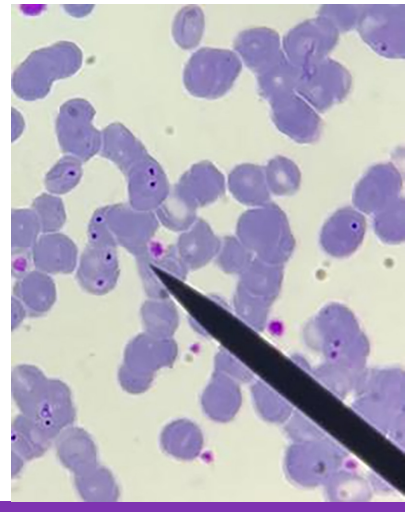

Figure 1. Point-like nuclear structures in the thin blood smear (100X magnification)

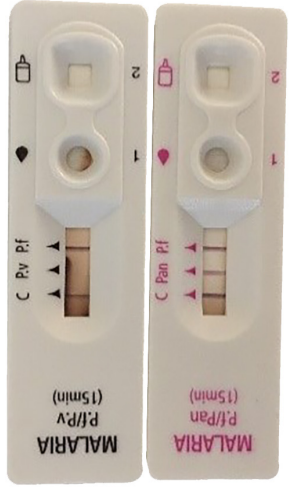

Figure 2. Rapid diagnostic tests for case 1

Case 2

A 54-year-old male patient presented to the Infectious Diseases Outpatient Clinic of Hatay Mustafa Kemal University Faculty of Medicine with complaints of high fever, chills, and tremors for the past 20 days. It was learned that the patient had been working in Sudan, had come to Hatay on leave, and had received malaria treatment in Sudan.

On physical examination, the patient's general condition was moderate, vital signs were stable, with a temperature of 39 °C, and hepatosplenomegaly was present. Laboratory findings showed: WBC; 624 µ/L, Hgb; 11.4 g/dL, platelets; 339,000 µ/L, AST: 29 U/L, ALT: 25 U/L, T. bil: 1.73 mg/dL, D. bil: 0.66 mg/dL, LDH: 345 U/L, CRP: 148 mg/dL, PTZ-INR: 1.12.

Due to clinical suspicion of malaria based on the patient's symptoms, laboratory findings, and history of working abroad, blood was drawn from the patient and referred to the parasitology department. Young trophozoites were detected in erythrocytes in thin smear blood preparations every 3-4 fields (Figure 5). Two rapid diagnostic tests, SD-Pf/Pan (Standard Diagnostics, Inc; Suwon City, Republic of Korea) and SD-Pf/Pv (Standard Diagnostics, Inc; Suwon City, Republic of Korea), were performed, and positive bands were obtained in P.f in both rapid diagnostic tests (Figure 6). The patient was admitted to the infectious diseases ward. Upon admission, the patient, who was in good general condition, was treated with the Artemether-lumefantrine therapy protocol.

Blood was drawn from the patient daily, and thick drop and thin smear preparations were examined in the parasitology department. On the third day of treatment, 1-2 banana-like gametocytes were detected in each preparation of the blood sample taken from the patient (Figure 7). The patient was advised to continue treatment. However, against medical advice, the patient chose to discharge themselves. Two days later, when the patient returned for a follow-up, gametocytes were still observed in preparations made from their blood samples. The Artemether-lumefantrine treatment protocol was restarted. Due to shortness of breath and low saturation observed during patient follow-ups, the patient was admitted to the intensive care unit and closely monitored. As gametocytes continued to be observed in subsequent blood samples, primaquine phosphate 1x1 was added to the treatment protocol for 14 days.

After approximately three weeks of follow-up, as gametocytes were no longer observed in blood preparations and the patient's clinical condition improved, they were discharged (Figure 8).

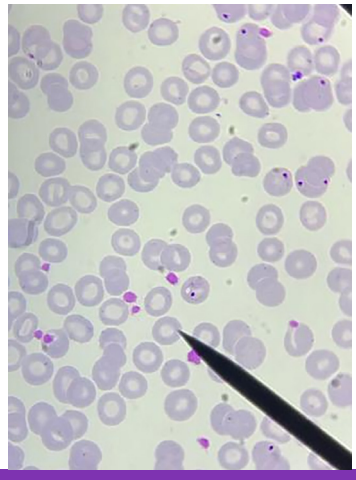

Figure 3. Point-like nuclear structures in the thin blood smear prepared on day 10 (100X magnification)

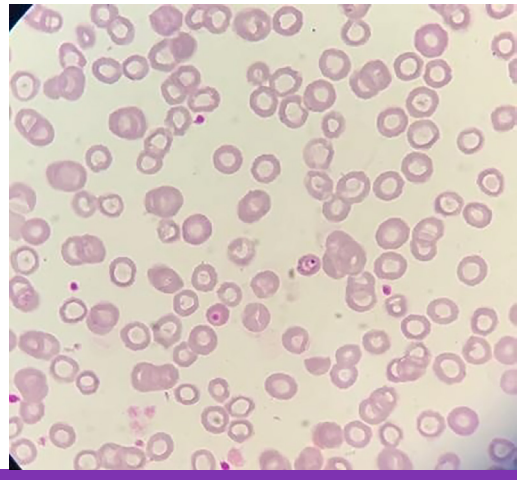

Figure 5. Young trophozoites in the thin blood smear (100X magnification)

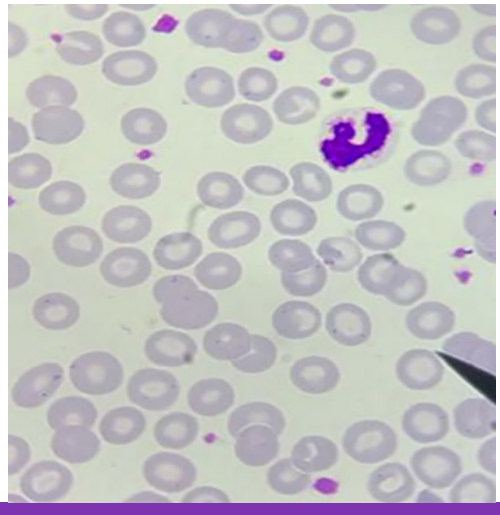

Figure 4. Thin blood smear prepared on day 16 (100X magnification)

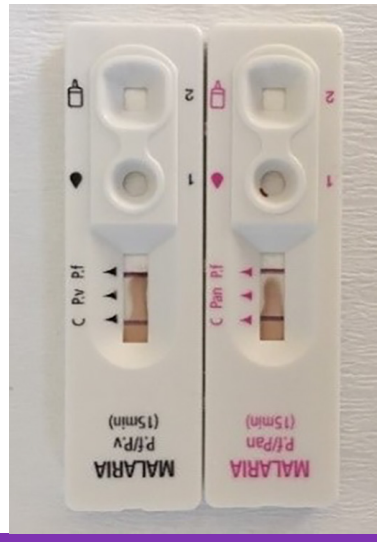

Figure 6. Rapid diagnostic tests for case 2

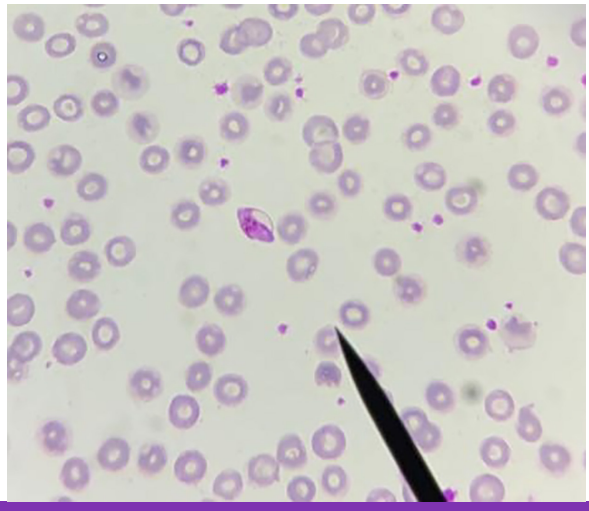

Figure 7. Gametocyte in the thin blood smear (100X magnification)

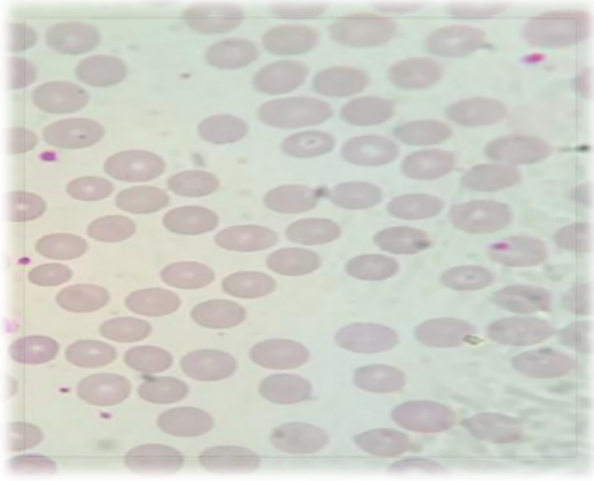

Figure 8. Thin blood smear after treatment (100X magnification)

In both cases, the causative species was identified as *P. falciparum*. However, as the artemether-lumefantrine combination therapy protocol did not yield sufficient response in both cases, primaquine phosphate was added to the treatment protocols to address possible mixed infections.

DISCUSSION

Malaria is a treatable disease when diagnosed. However, reports indicate resistance to drugs used in malaria treatment over the past decade. Resistance has been reported in three of the five malaria species that cause disease in humans, namely *P. falciparum, P. vivax*, and *P. malaria* (1).

Quinoline derivatives (quinine, chloroquine, mefloquine, primaquine), antifolates (sulfadoxine/pyrimethamine), artemisinin, and atovaquone are agents used in malaria treatment (4). Chloroquine is the preferred first-line antimalarial agent for treatment. However, in cases of resistance, other quinoline derivatives, along with doxycycline, atovaquone/proguanil, artemether/lumefantrine, artesunate, and sulfadoxine/pyrimethamine combinations, are used in treatment (4-7). Resistance to these agents varies according to geographical regions. In some areas where malaria is endemic, *P. vivax* infection has shown resistance to chloroquine and primaquine, while *P. falciparum* infection has developed resistance to many of the currently used antimalarial drugs (6).

Artemether and other artemisinin derivatives act on both the asexual and sexual stages of the parasite. However, when used alone in treatment, the rate of recurrence is high. Therefore, combination therapy with agents like lumefantrine is recommended (8,9). In the combination of artemisinin and lumefantrine, the different half-lives of these agents are utilized to achieve effective treatment (8). In a study by Ural et al. (10), a case of a patient who traveled to cameroon for a business trip and presented with symptoms of high fever, chills, tremors, and weakness a week after returning to Türkiye was reported. Upon examination of thin and thick blood smears, the patient was diagnosed as *P. falciparum* and treated with artemether 20 mg/lumefantrine 120 mg tablets. The study reported that artemisinin-based combination therapies are the best treatment option for *P. falciparum* cases (10). In the study, two cases returning from business trips to Ghana and Sudan to Hatay were diagnosed with malaria and typed as *P. falciparum*. Both cases were treated with Artemether-lumefantrine combination therapy, but due to inadequate response, primaquine phosphate was added to the treatment protocols.

Due to the fact that some of the population living in Hatay work abroad, especially in African countries and Saudi Arabia, where malaria is endemic, imported malaria cases are frequently encountered. Şahin et al. (11) reported 75 imported malaria cases between January 2008 and December 2017 and emphasized the significance of malaria in our region. In the study, two cases of imported malaria, which were caused by *P. falciparum* and showed delayed response to treatment, were presented.

CONCLUSION

It is believed that rapid diagnosis of suspected malaria cases, species identification, implementation of effective treatment protocols, and elimination of transmission, along with providing education on malaria disease and vector, individual control methods, and chemoprophylaxis before traveling to malaria-endemic countries, would contribute to reducing resistant parasites. In addition, the study aimed to draw attention to the importance of learning the region where the patients come from in imported malaria cases and applying the appropriate treatment protocol by looking at the antimalarial drug resistance of that region.

*Ethics

Informed Consent: In this cases, written consent of the patient has been obtained.

Footnotes

*Authorship Contributions

Data Collection or Processing: T.K., M.Ç., G.Ç., C.Ü., Analysis or Interpretation: T.K., M.Ç., G.Ç., C.Ü., Literature Search: T.K., G.Ç., C.Ü., Writing: T.K., M.Ç., G.Ç.

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

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New Data on Ectoparasites of the Caucasian Squirrel *Sciurus anomalus* (Rodentia: Sciuridae) in Türkiye: A Case Report

Türkiye'deki Kafkas Sincabı Sciurus anomalus'un (Rodentia: Sciuridae) Ektoparazitleri Hakkında Yeni Veri: Olgu Sunumu

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ABSTRACT

Sciurus anomalus Güldenstädt, 1785, known as the Caucasian squirrel, is a rodent distributed in all geographical regions of Türkiye. The material of this study consists of ectoparasites collected from male *S. anomalus* found dead on the highway (Karasu, Sakarya, Türkiye). As a result of microscopic examination, the specimens were identified: ticks as *Ixodes ricinus* Linnaeus, 1758 (larvae and nymph), sucking louses as *Neohaematopinus syriacus* Ferris, 1923 (female), and fleas as *Monopsyllus sciurorum sciurorum* (Schrank, 1803) (female and male). In this study, the presence of *Ixodes ricinus* infestation on *Sciurus anomalus* is reported for the first time in Türkiye.

Keywords: *Sciurus anomalus, Ixodes ricinus, Neohaematopinus syriacus, Monopsyllus sciurorum sciurorum,* Türkiye

ÖZ

Kafkas sincabı olarak bilinen *Sciurus anomalus* Güldenstädt, 1785 Türkiye'nin tüm coğrafik bölgelerinde dağılım gösteren bir rodenttir. Bu çalışmanın materyalini kara yolunda (Karasu, Sakarya, Türkiye) ölü olarak bulunan erkek *Sciurus anomalus* üzerinden toplanan ektoparazitler oluşturmaktadır. Mikroskobik inceleme sonunda kene örnekleri *Ixodes ricinus* Linnaeus, 1758 (larva ve nimf), bit örnekleri *Neohaematopinus syriacus* Ferris, 1923 (dişi), pire örnekleri ise *Monopsyllus sciurorum sciurorum* (Schrank, 1803) (dişi ve erkek) olarak tanımlanmıştır. Bu çalışmayla birlikte Türkiye'de ilk kez *Sciurus anomalus*'da *Ixodes ricinus* enfestasyonu tespit edilmiştir.

Anahtar Kelimeler: *Sciurus anomalus, Ixodes ricinus, Neohaematopinus syriacus, Monopsyllus sciurorum sciurorum,* Türkiye

INTRODUCTION

Sciurus anomalus Güldenstädt, 1785, one of 21 species described within the genus *Sciurus*, is commonly known as the Caucasian squirrel (1). *Sciurus anomalus* is distributed in forests where the habitat is suitable in Iran, Armenia, Azerbaijan, Georgia, Greece, Iraq, Jordan, Lebanon, and Syria, as well as in Türkiye (2).

Apart from *S. anomalus*, *Sciurus vulgaris*, *Spermophilus citellus*, *S. xanthophrymnus* and *S. torosensis* species in the squirrel family (Sciuridae) are also distributed in Türkiye (2,3).

Studies on ectoparasites of squirrels (*Sciurus* spp. and *Spermophilus* spp.) are unfortunately neglected in the field of veterinary parasitology in Türkiye. In the studies, four of the five squirrel species found in Türkiye have been examined as ectoparasitic, and only two ticks, 11 fleas and one lice species have been reported.

In the Turkish flea list published by Keskin and Hastriter (4), it has been reported of the presence of species that *Chaetopsylla globiceps*, *Ctenocephalides canis*, *Ctenophthalmus turcicus*, *Monopsyllus sciurorum sciurorum*, *Nosopsyllus fasciatus* on the Caucasian squirrel (*Sciurus anomalus*); *Monopsyllus sciurorum sciurorum* on the Eurasian red squirrel (*Sciurus vulgaris*); *Citellophilus simplex simplex*, *Citellophilus transcaucasicus*, *Nosopsyllus fasciatus*, *Oropsylla*

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ilovaiskii, *Neopsylla setosa spinea*, and *Pulex irritans* on the Asia minor ground squirrel (*Spermophilus xanthoprymnus*). In the Turkish mammal lice list published by Dik (5), *Neohaematopinus syriacus* infestation was reported only on the Caucasian squirrel (*Sciurus anomalus*). Among the tick species, it has been reported the presence of infestation of *Ixodes ricinus* on the Eurasian red squirrel (*Sciurus vulgaris*) (6), *Ixodes laguri* on *Spermophilus citellus* (as *Citellus citellus* in the study) (7), *Haemaphysalis* sp. and *Ixodes* sp. on the Asia minor ground squirrel *Spermophilus xanthophrymnus* (as *Citellus citellus* in the study) (8).

CASE REPORT

The specimen of the male Caucasian squirrel (*Sciurus anomalus*) (Rodentia: Sciuridae) (Figure 1), found dead on the highway (geographical coordinates: 41.087834, 30.647195) (Karasu, Sakarya, Türkiye) on April 12, 2024, was subjected to ectoparasitic examination.

Using a flea comb and blunt-ended forceps during ectoparasite examination, the ectoparasite (louse, flea, and tick) specimens were collected from the carcass. After that, all specimens were stored in Eppendorf tubes containing 70% ethanol. In the light of the relevant literature specific to each parasite group [flea (9), ticks (10), and louse (11)] under the light microscope (MIC-B30/B Binocular 45 Economic Microscope-Led-Achromat, SOIF Optical Instruments Factory, China), tick specimens were identified as *Ixodes ricinus* Linnaeus, 1758, louse specimens as *Neohaematopinus syriacus* (Mjöberg, 1910), and flea specimens as *Monopsyllus sciurorum sciurorum* (Schrank, 1803) (Figure 2) (n_{louse}: two females; n_{ticks}: two larvae and four nymphs; n_{fleas}: two males and one female). In addition, all permanent glass slides of louse, tick, and flea species identified are deposited in G. Eren's personal collection.

Figure 1. Dorsal and ventral view of the Caucasian squirrel (*Sciurus anomalus*) from which ectoparasite specimens were collected (photographed by Furkan Eren)

DISCUSSION

It is noteworthy that studies on ectoparasites of wild mammals (ticks, lice, fleas, myiasis flies and keds) in Türkiye have increased compared to the past (5,12). When the studies are carefully examined, it is seen that good results are obtained in terms of host-parasite relationships in studies carried out jointly, that is, multidisciplinary, by biologists, veterinarians or parasitologists (13,14).

Although there are many reports in the world (1,15) about the diversity of ticks infesting *Sciurus anomalus* and *S. vulgaris*, members of the *Sciurus* genus known as tree squirrels or bushy-tailed squirrels, information for Türkiye is limited or insufficient (4-8). It can be considered that the diversity of the ectoparasitic fauna of the Caucasian squirrel (*S. anomalus*) has been revealed to a great extent in studies conducted around the world. As a result of these studies, it is known that the *Ixodes acuminatus* and *I. ricinus* from the tick species; *Chaetopsylla globiceps*, *Ctenocephalides canis*, *Ctenophthalmus turcicus,* and *Monopsyllus sciurorum sciurorum* from the flea species; and *Enderleinellus krochinae*, *E. nitzschi* and *Neohaematopinus sciurinus* from the louse species cause infestation on Caucasian squirrels (1,4). Similarly, in the study carried out in 4 biogeographic regions covering France and Italy, 356 red squirrels (*S. vulgaris*) carcasses specimens were collected from the highways; and many ectoparasite species including flea (*Monopsyllus sciurorum sciurorum*, *Tarsopsylla octodecimdentata octodecimdentata*, *Dasypsyllus gallinulae gallinulae*), louse (*Enderleinellus nitzschi* and *Neohaematopinus sciurinus*) and tick (*Ixodes acuminatus* and *I. ricinus*) have been reported on these carcasses (15). As mentioned in the introduction of the paper, only 3 of 5 the squirrel species found in Türkiye have been studied as ectoparasites. While flea (4) and lice (5) infestations were reported in previous studies on the Caucasian squirrel (*S. anomalus*) examined in the present study, there is no data on tick infestation. *Ixodes ricinus*, detected on the *Sciurus anomalus* in this study, is one of the most studied ticks in the Western Palearctic region due to its vectorial potential (tick-borne encephalitis, Borreliosis, and Babesiosis). While the larval and nymph stages prefer small mammals, birds and reptiles, the adult stages prefer large mammals (10).

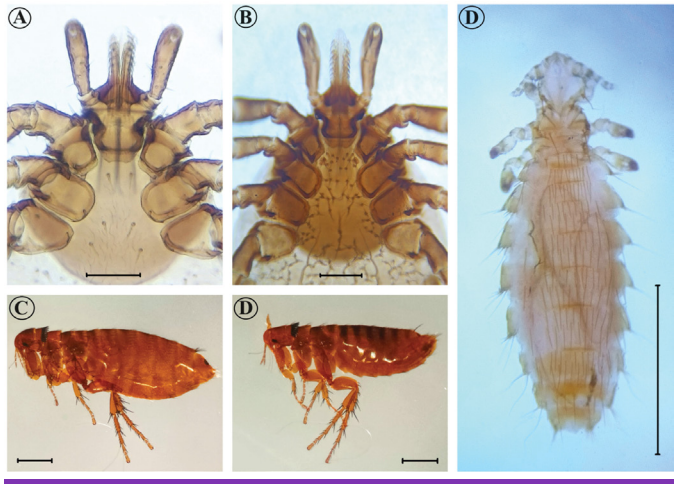

Figure 2. Species of ectoparasites identified: Larvae (A) and nymph (B) of *Ixodes ricinus* (scale bar: 100 µm); female (C) and male (D) of *Monopsyllus sciurorum sciurorum* (scale bar: 500 µm); female (E) of *Neohaematopinus syriacus* (scale bar: 1000 µm)

CONCLUSION

As a result of the literature review, it was found that *Ixodes ricinus* had previously been reported on Sciurus vulgaris in Türkiye (7) but not on *Sciurus anomalus*. Such case reports, according to the author, *Ixodes ricinus* species ticks can contribute to future studies by revealing the host-parasite relationships and vectorial potential.

This study aims to report *Ixodes ricinus* infestation on the *Sciurus anomalus* for the first time in Türkiye.

*Ethics

Informed Consent: Since ectoparasite specimens were collected from a dead squirrel carcass, neither owner consent forms nor ethics committee approval was required.

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Footnotes

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