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Türkiye Parazitoloji Dergisi

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Türkiye Parazitoloji Dergisi

TURKISH JOURNAL OF PARASITOLOGY

AMAÇ VE KAPSAM

Türkiye Parazitoloji Dergisi, 1976 yılından bu yana çıkan, Tıp, Veterinerlik ve Biyoloji alanlarında yapılan Parazitoloji konulu klinik ve deneysel çalışmaları, ilginç olgu bildirimlerini, davet edilmiş derlemeleri, Editöre mektupları yayınlayan; yayın dili Türkçe ve İngilizce olan, bağımsız ve önyargısız çift-kör hakemlik ilkelerine dayanan uluslararası bir dergidir.

Dergi, Türkiye Parazitoloji Derneği'nin bilimsel içerikli resmi yayın organı olup, Mart, Haziran, Eylül ve Aralık aylarında olmak üzere yılda 4 sayı yayınlanmakta ve Türkiye Parazitoloji Derneği tarafından finanse edilmektedir.

Derginin hedefi, klinik ve bilimsel açıdan uluslararası düzeyde nitelikli ve üst düzeyde özgün araştırmaları yayınlamaktır. Dergide ayrıca, tıp eğitimi ile ilgili temel yenilikleri kapsayan derlemeler, Editöryel yazılar, olgu sunumları ve özgün görüntüler de yayınlanmaktadır.

Derginin hedef kitlesi, tıbbi ve veteriner parazitoloji alanlarında ve biyoloji bilim dalının ilgili birimlerinde çalışan tüm bilim insanları ve bu alanlardaki yüksek lisans öğrencileridir. Bu kapsamda dergi, Türkiye Parazitoloji Derneği üyelerine ve yurt çapında parazitoloji'yle ilgili kişi ve kuruluşlara düzenli olarak ulaştırılmaktadır. Derginin tüm sayılarının içerikleri tam metin olarak www.tparazitolderg.org adresinde ücretsiz erişime açıktır.

Derginin Editöryel süreçleri ve yayın işleyişi ICMJE, WAME ve COPE standartları çerçevesinde yürütülmektedir.

Türkiye Parazitoloji Dergisi; Index Medicus/Medline/PubMed, BIOSIS-Zoological Record, BIOSIS Previews Biological Abstracts, CABI Abstracts and Bibliographic Databases, Index Copernicus, Tübitak/Ulakbim Türk Tıp Dizini ve Türkiye Atıf Dizini tarafından indekslenmektedir.

Abone İşlemleri/Baskı İzinleri ve Tekrar Baskılar/Reklam
Dergide basılan yazıların tam metinlerine ücretsiz olarak www.tparazitolderg.org adresinden ulaşılabilir. Basılı dergi aboneliği, baskı izinleri, tekrar baskılar ve reklam için Editör ofisine başvurulmalıdır.

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Yazarlara Bilgi
Yazarlara Bilgi sayfası derginin basılı versiyonunda ve www.tparazitolderg.org web sayfasında yayınlanmaktadır.

Materyal Sorumluluk Reddi
Türkiye Parazitoloji Dergisi'nde yayınlanan tüm yazılardaki görüş ve raporlar yazarların görüşüdür. Editörler ve Yayıncı bu yazılar için herhangi bir sorumluluk kabul etmemektedir.

Dergimiz asitsiz kağıda basılmaktadır.

Türkiye Parazitoloji Dergisi

TURKISH JOURNAL OF PARASITOLOGY

AIMS AND SCOPE

The Turkish Journal of Parasitology has been published since 1976. The journal publishes clinical and experimental studies, interesting case reports, invited reviews and letters to the editor on biological, medical and veterinary parasitology. The Turkish Journal of Parasitology is an international journal which is based on independent and unbiased double-blinded peer-review principles. The publishing language of the journal is Turkish and English.

The Turkish Journal of Parasitology is the scientific and the official publication of the Turkish Society for Parasitology and is published four times per year; in March, June, September and December, and is financed by the Turkish Society for Parasitology.

The aim of the journal is to publish original articles with highest clinical and scientific quality at the international level. The Turkish Journal of Parasitology also publishes reviews covering fundamental innovations in medical education, editorial articles, case reports and original images.

The target audience of the journal is scientists working on medical and veterinary parasitology, and relevant disciplines of biology, as well as PhD and MSc students studying on these topics. In this context, the journal is sent regularly to the members of the Turkish Society for Parasitology as well as to the organizations and individuals who are interested in parasitology countrywide. The contents of all issues in full text can be accessed free of charge through the web site www.tparazitolderg.org.

The editorial and publication processes of the journal are conducted in accordance with the ICMJE, WAME and COPE standards.

The Turkish Journal of Parasitology is indexed in Index Medicus/Medline/PubMed, BIOSIS-Zoological Record, BIOSIS Previews Biological Abstracts, CABI Abstracts and Bibliographic Databases, Index Copernicus, Tübitak/Ulakbim Turkish Medical Database and Turkiye Citation Index.

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The full texts of the published articles can be accessed free of charge through the web site www.tparazitolderg.org. Applications for subscriptions, permissions, reprints and advertisements should be made to the editorial office.

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Information for Authors
Information for authors is published in the journal and is available on the web site www.tparazitolderg.org.

Material Disclaimer
All opinions and reports in the articles published in the Turkish Journal of Parasitology are those of the authors. The editors and the publisher do not accept any responsibility for these articles.

The journal is printed on acid-free paper.

Türkiye Parazitoloji Dergisi

TURKISH JOURNAL OF PARASITOLOGY

YAZARLARA BİLGİ

Genel Kurallar
Türkiye Parazitoloji Dergisi, tıbbi ve veteriner parazitoloji alanlarında deneysel, gözlemsel araştırma, klinik denemeler, olgu sunumu ve derleme niteliğindeki, biyoloji bilim alanından ise parazitoloji konularını kapsayan makaleleri yayınlar.

Yazılar sadece www.tparazitolderg.org adresinden elektronik olarak gönderilmelidir.

Tüm yazarlar bilimsel katkılarını, sorumluluklarını ve çıkar çatışması olmadığını bildiren toplu imza ile yayına katılmalıdır.

Araştırmalara yapılan kısmi de olsa nakdi ya da ayni yardımların hangi kurum, kuruluş, ilaç-gereç firmalarınca yapıldığı dip not olarak bildirilmelidir.

Makalelerin formatı "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication (http://www.icmje.org/)" kurallarına göre düzenlenmelidir.

Deneysel, klinik ve ilaç araştırmaları için insan ve hayvan hakları ile ilgili uluslararası anlaşmalara uygun etik kurul raporu (Helsinki Declaration of 1975, revised 2002-http://www.wma.net/e/policy/b3.htm ve "Guide for the care and use of laboratory animals - www.nap.edu/catalog/5140.html) ve hastaların çalışma hakkında bilgilendirildiklerine ve olurlarının alındığına dair onay formu gereklidir.

Makale gönderim aşamasında, makalenin dergimizde yayınlanmasıyla ilgili bütün yazarların onayını belirten bir mektubun eklenmesi gereklidir. Ayrıca makalenin yayına kabul edilmesi halinde bütün yazarların Yayın Hakkı Devir Formu'nu imzalayıp postayla dergi adresine göndermeleri gereklidir.

Etik kurul kararı gereken çalışmalarda onay belgesinin eklenmesi gerekmektedir.

Yazıların hazırlanması
Yazılar A4 boyutunda, iki satır aralıklı olarak ve tüm sayfalarda sayfa numarası bulunacak şekilde gönderilmelidir. Toplam sayfa sayısı resim ile şekiller dahil araştırma yazılarında 15'i, olgu sunumlarında ise 6'yı geçmemelidir.

Başlık sayfasında sadece makalenin Türkçe ve İngilizce tam ve kısa başlıkları ve varsa makalenin daha önce tebliğ edildiği toplantı ve kongreler yazılmalıdır. Yazar adları ve çalıştıkları kuruma ait bilgiler sadece makale derginin on-line sistemine yüklenirken girilmeli, makale ana metninde yazara ait bilgiler olmamalıdır.

İkinci sayfada yalnızca Türkçe ve İngilizce özetler ile anahtar sözcükler yer almalıdır. 200 kelimeyi geçmeyen özet kısmı, Amaç, Yöntemler, Bulgular, Sonuç şeklinde bölümlü olmalıdır. Anahtar sözcükler ise 5 kelimeyi geçmeyecek şekilde Türkçe özetin altına Türkçe, İngilizce özetin altına İngilizce olarak eklenmelidir.

Araştırma yazılarının tam metin bölümü Giriş, Yöntemler, Bulgular, Tartışma, Sonuç, Çıkar Çatışması Beyanı, Kaynaklar, Tablo, Şekil ve Resimleri (açıklama yazılarıyla birlikte) içerecek şekilde düzenlenmelidir. Olgu sunumlarında ise Giriş, Olgu(lar), Tartışma, Sonuç, Kaynaklar, Tablo, Şekil ve Resimler (açıklama yazılarıyla birlikte) şeklinde olmalıdır.

Tablo, şekil ve resimler ayrı bir sayfada olmalı ve yazının içinde geçmesi gereken yeri cümlenin sonuna parantez içinde yazılmalıdır.

Siyah-beyaz veya renkli fotoğrafların yüksek çözünürlüklü jpg formatında gönderilmesi gerekmektedir.

Makale içinde ve kaynaklarda geçen parazitlerin cins ve tür isimleri italik ve sadece cins isminin ilk harfi büyük olarak yazılmalıdır.

Kısaltmalar ilk kez kullanıldığında açık olarak yazılmalı daha sonra makale içinde hep aynı kısaltma kullanılmalıdır.

Yazı içinde belirtilen tüm kaynaklar makale içindeki geçiş sırasına göre liste halinde numaralandırılarak verilmelidir. Kaynaklar yazılırken noktalama işaretlerine aşağıdaki örneklerde gösterildiği şekilde dikkat edilmeli ve yazı içinde her kaynağa ait numara ilgili cümlenin sonunda parantez içinde mutlaka belirtilmelidir. Dergi kısaltmaları Index Medicus tarafından gösterildiği şekilde yapılmalıdır. Altı ve daha az yazarlı olan kaynaklarda tüm isimler yazılmalı, yedi ve daha fazla yazarlı kaynakların ise ilk altı yazar ismi yazılıp Türkçe makalelerde "ve ark." , İngilizce makalelerde "et al" ilave edilmelidir.

Kaynak yazımı için örnekler

Süreli Yayınlar
Githeko AK, Service MW, Mbogo CM, Audi FK, Juma P0, Mousier WJ, et al. Plasmodium falciparum sporozoite and entomological inoculation rates at the Ahero rice irrigation scheme and the Miwani sugar belt in Western Kenya. Ann Trop Med Parasitol 2002; 52: 561-79.

Editörlü Kitapta Bölüm
Hornbeck P. Assay for antibody production. Colign JE. Kruisbeek AM, Marguiles DH, editors. Current Protocols in Immunology. New York: Greene Publishing Associates; 1991. p. 105-32.

Tek Yazarlı Kitap
Fleiss JL. Statistical Methods for Rates and Proportions. Second Edition. New York: John Wiley and Sons; 1981.

Yazar olarak Editörler
Balows A. Mousier WJ, Herramaflfl KL, editors. Manual of Clinical Microbiology. Fifth Edition. Washington DC: IRL Press.; 1990.

Kongre Bildirileri
Entrala E, Mascaro C. New stuructural findings in Cryptosporidium parvum oocysts. Eighth International Congress of Parasitology (ICOPA VIII); October,10-14; Izmir-Turkey: 1994. p. 1250-75

Tezler
Erakıncı G. Donörlerde parazitlere karşı oluşan antikorların aranması. İzmir: Ege Üniversitesi Sağlık Bilimleri Enstitüsü. 1997.

Elektronik Formatta Makale
Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) l995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodlElD/cid.htm.

Yayın Kurulu, gönderilen yazılarda bu kurallara uymayan yerlerin bulunması durumunda bilimsel içeriğe dokunmadan teknik açıdan gerekli değişiklikleri yapmaya yetkilidir.

Derleme yazılar, sadece yayın kurulu tarafından davet edilen yazarlar tarafından hazırlanır ve yayınlanır. Davetsiz olarak dergiye gönderilen derleme yazıları dikkate alınmayacaktır.

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Türkiye Parazitoloji Dergisi

TURKISH JOURNAL OF PARASITOLOGY

INSTRUCTIONS TO AUTHORS

General Rules
The Turkish Journal of Parasitology publishes experimental and observational research articles, clinical reviews, case reports and review articles on medical and veterinary parasitology, and publishes articles on parasitology in the biology field.

Manuscripts must be submitted online at www.tparazitolderg.org.

All submissions must be accompanied by a signed statement of scientific contributions and responsibilities of all authors and a statement declaring the absence of conflict of interests.

Any institution, organization, pharmaceutical or medical company providing any financial or material support, in whole or in part, must be disclosed in a footnote.

Manuscripts must be prepared in accordance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication (available at http://www.icmje.org/).

An approval of research protocols by an ethical committee in accordance with international agreements (Helsinki Declaration of 1975, revised 2002 - available at http://www.vma.net/e/policy/b3.htm <http://www.vma.net/e/policy/b3.htm>, "Guide for the care and use of laboratory animals - www.nap.edu/catalog/5140.html/) is required for experimental, clinical and drug studies. A form stating that the patients have been informed about the study and consents have been obtained from the patients is also required for experimental, clinical and drug studies.

All submissions must be accompanied by a letter that states that all authors have approved the publication of the paper in the Turkish Journal of Parasitology. Upon acceptance, all authors must sign the Copyright Transfer Form, and send this form to the editorial office through mail.

Submission of the studies requiring ethical committee decision must be accompanied by a copy of the submission to the ethical committee.

Preparation of the Manuscript
Manuscripts should be typed double-spaced on A4 size paper and pages should be numbered consecutively. The total number of pages should not exceed 15 for research articles and 6 for case reports; including figures and illustrations.

The title page should include full and short title in Turkish and English, and meeting and congress presentations of the manuscript must be stated, if any. Authors' names and their institutional affiliations must only be provided at the submission stage, author information must not be included in the main text.

The second page should include abstracts written both in Turkish and English, and key words. Structured abstracts, not to exceed 200 words, should consist of four sections, labeled as Objective, Methods, Results and Conclusion. No more than five key words in Turkish language should follow the Turkish abstract, as well as keywords in English should follow the English abstract.

For research articles main text should include Introduction, Methods, Results, Discussion, Conclusion, Conflict of Interest Disclosure, References, Tables, Figures and Illustrations (with legends) sections. Case reports should be divided into the following sections: Introduction, Case(s), Discussion, Conclusion, References, Tables, Figures and Illustrations (with legends).

Tables, figures and illustrations must be provided on a separate page and must be cited at an appropriate point in the text at the end of the sentence in parenthesis.

Both black and white and color figures must be uploaded in high resolution .jpg format.

When mentioning parasites in the main text and references, the genus and species names must be italicized and the genus name must be written with an initial capital letter.

Abbreviations should be expanded at first mention and used consistently thereafter.

All references cited in the text should be listed in numerical order in which they appear in the text. Attention should be paid to punctuation as shown in examples below. In text, each reference should be given in parenthesis at the end of the relevant sentence. Abbreviation of journal names must conform to Index Medicus style. All author names should be listed if there are six or fewer. In case of more than six authors, only the first six should be listed, followed by "ve ark." in articles in Turkish and followed by "et al." in articles in English.

Examples

Periodicals
Githeko AK, Service MW, Mbogo CM, Audi FK, Juma P0, Mousier WJ, et al. Plasmodium falciparum sporozoite and entomological inoculation rates at the Ahero rice irrigation scheme and the Miwani sugar belt in Western Kenya. Ann Trop Med Parasitol 2002; 52: 561-79.

Chapter in Edited Book
Hornbeck P. Assay for antibody production. Colign JE. Kruisbeek AM, Marguiles DH, editors. Current Protocols in Immunology. New York: Greene Publishing Associates; 1991. p. 105-32.

Book with a Single Author
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Editor(s) as Author
Balows A. Mousier WJ, Herramaflfl KL, editors. Manual of Clinical Microbiology. Fifth Edition. Washington DC: IRL Press.; 1990.

Conference Paper
Entrala E, Mascaro C. New structural findings in Cryptosporidium parvum oocysts. Eighth International Congress of Parasitology (ICOPA VIII); October,10-14; Izmir-Turkey: 1994. p. 1250-75

Thesis
Erakıncı G. Donörlerde parazitlere karşı oluşan antikorların aranması. İzmir: Ege Üniversitesi Sağlık Bilimleri Enstitüsü. 1997.

Article in Electronic Format
Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) l995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodlElD/cid.htm.

The editorial board has the authority to make necessary revisions in the format of the manuscript (without making any revision in the context) that does not comply with the above-mentioned requirements.

Review articles are only prepared and published by authors invited by the editorial board. Review articles that are submitted to the journal without an invitation of the editorial board will not be taken to consideration for publication.

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Türkiye Parazitoloji Dergisi

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EDİTÖRDEN

Dergimizin 2012 yılı son sayısında, 11 araştırma makalesi ve 5 olgu sunumuna yer verilmiştir. Dergimize gönderilen tıbbi parazitoloji alanındaki olgu sunumları yanı sıra veteriner parazitoloji alanında da olgu sunumlarının sayısının gittikçe arttığı memnuniyetle izlenmektedir. Tıbbi parazitoloji alanındaki bu artışa paralel olarak, sadece farklı özellikler gösteren olguların incelenmek üzere kabul edilmesi, olağan durumları yansıtan olguların yönetici aşamasında kapsam dışında bırakılması konusunda karar alınmıştır.

Bu yıl içinde yayınlanan 4 sayımızda; 43 araştırma makalesi, 15 olgu sunumu, 2 derleme ve 2 adet de Editöre mektup olmak üzere toplam 62 makaleye yer verilmiştir. Toplamda bundan daha fazla sayıda makale de şu anda değerlendirme aşamasındadır. Makale göndererek dergimize destek olan, bilim alanımızın tarihinin yazılmasına katkıda bulunan bütün arkadaşlarımıza ve titizlikle değerlendirme yapan hakem hocalarımıza Yayın Kurulumuz adına çok teşekkür ederim.

Gelen makale sayısının artması, her makale için en az iki hakem hocamızın görüşlerinin alınması ve akademik yükseltme alan meslektaşlarımızın sayısının artması nedeniyle önümüzdeki yılda daha zengin bir hakem listesi oluşturmayı ve böylelikle makalelerin inceleme sürecini mümkün olduğunca azaltmayı amaçladığımızı da belirtmek isterim.

Dergimizin en önemli hedeflerimizden biri olan "Science Citation Index-Expanded" kapsamına alınmak için 2013 yılında yeniden başvuru yapma imkanı bulunmaktadır. Bu nedenle başvurunun 2013 yılı Mart ayına kadar tamamlanması planlanmıştır. Dergimizin indekslendiği en önemli indeks olan PUBMED'de ise 2012 yılındaki ortalama tıklanma sayısı ayda 1000 civarında olmuştur. Bu durum dergimizin serbest erişime açık olmasından da kaynaklanmakta, yerli ve yabancı makalelerde atıf alma sayımızı arttırmaktadır.

Bu sayımızın da bilimsel çalışmalarınıza ve birikimlerinize yararlı olması umuduyla saygılar sunarım.

Prof. Dr. Yusuf ÖZBEL
Baş Editör

Türkiye Parazitoloji Dergisi

TURKISH JOURNAL OF PARASITOLOGY

EDITORIAL

We have included 11 research articles and 5 case reports in this latest issue of our journal of 2012. We are glad to see that the number of case reports submitted to our journal on medical parasitology field is increasing as well as the number of case reports submitted in veterinary parasitology field. In parallel to this development in medical parasitology field, we have decided to only evaluate extra ordinary case reports for publication and leave the ordinary cases out of scope during the system admin stage.

In total we have included 62 manuscripts - 43 research articles, 15 case reports, 2 review articles and 2 letters to the editor - in 4 issues we have published this year. A greater number of articles are being evaluated for publication at the moment. On behalf of the editorial board I would like to thank all of our colleagues who supported us and helped us shape the history of our science field by submitting manuscripts and by evaluating manuscripts rigorously.

Due to the increasing number of manuscripts submitted, the increasing number of our colleagues receiving academic promotion and our need to receive the opinions of at least reviewers on each manuscript we are planning to create a richer reviewer list next year so we can keep the evaluation process of each manuscript as short as possible.

One of our journals most important goals is being accepted for coverage by Science Citation Index-Expanded and the Turkish Journal of Parasitology will be eligible to re-apply for evaluation in 2013. For this reason we are aiming to complete out application by March 2013. The monthly average number of clicks received by our journal on PubMed – the most important database our journal is indexed in- is around 1000. Our open-access policy is the reason of this situation and it increases the number of citations our journal receives both nationally and internationally.

I hope this latest issue will be usefull for your studies and archives.

Best regards.

Prof. Dr. Yusuf ÖZBEL
Editor-in-Chief

Türkiye Parazitoloji Dergisi

TURKISH JOURNAL OF PARASITOLOGY

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The cytopathic Effects of *Trichomonas vaginalis* on Fibroblast Cell Culture Alone and with *C. albicans* and *E. coli*

Klinik Örneklerden İzole Edilen *Trichomonas vaginalis*'in Tek veya *Candida* ve *E. coli* ile Birlikte L929 Fare Fibroblast Hücre Kültür Serileri Üzerine Sitopatik Etkisi

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ABSTRACT

Objective: In this study, the cytopathic effects of *Trichomonas vaginalis* were investigated in L929 mouse fibroblast cell cultures (FCC) under different conditions: only parasite, or coexistence with *Candida albicans* and *Escherichia coli*.
Methods: The parasite was isolated from a symptomatic patient and cultured in Cysteine-Peptone-Liver infusion Maltose medium (CPLM). *C. albicans* strain 10235 and *E. coli* strain 25922 were used in the experiments. Five groups were created and inoculated on FCC. The groups were as follows; only *T. vaginalis, C. albicans, E. coli, T. vaginalis+C. albicans* and *T. vaginalis+E. coli*. The plates were incubated for 24 hours and cell viability was examined under an inverted microscope. Each experiment was repeated 11 times.
Results: The fibroblast death rate was 19.1%, 21%, 40.9%, 96.5% and 89.6% in the five groups, respectively.
Conclusion: All fibroblasts were alive in the control group. *T. vaginalis* showed almost 100% cytopathic effects on FCC with *C. albicans* and parasites were very motile in this coexistence. *(Turkiye Parazitol Derg 2012; 36: 193-7)*

Key Words: *Trichomonas vaginalis*, fibroblast cell culture, *Candida albicans*, *Escherichia coli*, cytopathic effect

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ÖZET

Amaç: Çalışmada, *T. vaginalis*'in fibroblast hücre kültürlerinde hem tek başına hem de *E. coli* ve *Candida* ile oluşturabilecekleri sitopatik etkinin araştırılması amaçlanmıştır.
Yöntemler: Bu amaçla, L929 fare fibroblast hücre serisi ve *T. vaginalis*'in kültüründe ise CPLM besiyeri kullanılmıştır. Deneylerde semptomatik klinik örneklerden izole edilen yerel bir *T. vaginalis* suşu, *Candida* 10235 suşu ve *E. coli* 25922 suşu ile çalışılmıştır. Deneysel çalışmada; altı grup oluşturulmuş ve fibroblast kültürü üzerine inoküle edilmişlerdir. Bu gruplar; 1. *T. vaginalis*, 2. *Candida*, 3. *E.coli*, 4. *T. vaginalis+Candida*, 5. *T. vaginalis+E. coli* ve 6. Kontrol (fibroblast kültürü)'dür. Plaklar %5 CO_2'li etüvde 37°C'de 24 saat inkübe edildikten sonra inverted mikroskop altında gözlenerek canlılık sayımları yapılmıştır. Çalışmada grupların herbiri 11 kez çalışılmıştır. Kruskall-Wallis, Mann-Whitney U testi kullanılarak sonuçlar değerlendirilmiştir.
Bulgular: Kontrol grubundaki L929 fare fibroblast hücrelerinin tamamı canlı iken, 1. grupta bulunan yalnız *T. vaginalis*'in inoküle edildiği hücre kültürlerinde fibroblastların %19.09'unun, 2. grupta bulunan yalnız *Candida*'nın %21.00'inin, 3. grupta *E. coli*'nin tek başına olduğu kültürlerde %40.91'inin, 4. grupta *T. vaginalis* ve *Candida*'nın birlikte ekildiği hücre kültüründe %96.55'inin, 5. grupta *T. vaginalis* ve *E. coli*'nin birlikte inoküle edildiği godelerde ise hücrelerin %89.64'ünün öldüğü saptanmıştır.
Sonuç: *T. vaginalis*'in fibroblast hücre kültüründe patojen etkisi özellikle *Candida* varlığında çok daha belirgin olmaktadır.
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Anahtar Sözcükler: *Trichomonas vaginalis*, fibroblast cell culture, *Candida albicans*, *Escherichia coli*, sitopatik etki

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INTRODUCTION

Trichomoniosis is the most common non-viral sexually-transmitted disease around the world. Each year, approximately 170 million people are infected with the parasite (1). The most prominent complaint in trichomoniosis is vaginal discharge. The patients mostly complain of burning and itching. Upon examination of vaginal mucosa with a speculum, common hyperaemic, bright red lesions can be observed. The most common urinary symptom is dysuria; cystitis can also be seen in very few cases (2). Cervical carcinoma has been reported to show a relationship with *T. vaginalis* (3, 4). The infection is usually asymptomatic in men. *T. vaginalis* may play an important role as a cofactor in the transmission of the HIV virus. In some studies in Africa, levels of HIV positivity in *T. vaginalis* positive individuals were found to be more than two-fold (5). To touch on *in vitro* studies, in cultured mammalian cells *T. vaginalis* showed cytopathic effects (6-8). Parasites kill the target cells with only direct contact (9). Four different trichomonas surface proteins were identified adhering to cells easily (10). Also, *T. vaginalis* cell separation factor (cell-detaching-factor, CDF), mammalian cell culture cells leads to leave. A correlation was found between the severity of the infection and CDF in the pathogenesis of *T. vaginalis* (7). For many years, *T. vaginalis* was accepted as an apathogenic microorganism; however, both *in vivo* and *in vitro* studies have revealed that it is actually pathogenic (10-17). *T. vaginalis* was first grown by cell culture in the 1940s; Houge investigated the effect of the parasite on fibroblast cells in 1943. In those studies, it was attempted to determine the parasite's effect on cells, and whether there are any mechanical effects, toxins or enzymatic reactions (16). Heath worked on the effect of pathogenesis of the parasite on a single layer of vaginal epithelial cells in 1981 and found that approximately 10% of cells died in cultures (15).

However, there has been no study regarding the damage caused by *T. vaginalis* on cell lines with *C. albicans*. Therefore, this study investigated whether *T. vaginalis*, both alone and with *E. coli* and *C. albicans,* can play a role in the cytopathic effects on FCC.

METHODS

Test Microorganisms

T. vaginalis was isolated from a female patient with clinical symptoms of urogenital disease and cultured in CPLM. *C. albicans* 10231 and *E. coli* 25922 strains were used in the assays.

Cultivation of *T. vaginalis*

The medium was renewed every three or four days to keep the parasites alive. Before inoculation, the medium was heated at 37°C for a few minutes and 1 mL of inoculum was transferred to fresh medium. 20% inactivated human serum (heat inactivated at 56°C for 30 min and cooled), Penicillin G, streptomycin and Triflucan was added to each tube.

Fibroblast Cell Culture

The L929 mouse fibroblast cell line was used in this study. FCC passages were continued in order to ensure its sustainability and viability. Cells in the flasks were washed with PBS, and then rinsed with a trypsin/EDTA solution (0.05% trypsin+0.02 % EDTA). Trypsin was aspirated from flasks. The flasks were incubated 37°C in an incubator for 5 min and then cells were separated from the surface of flasks. The cell suspension was created by adding DMEM. The prepared cell suspension was divided into two flasks and was passaged. Cell proliferation was viewed in flasks. This process was repeated and continuity of cell culture was achieved.

Experimental Design

Six groups were formed in the experiments and were inoculated on FCC. The groups created were as follows**:**

1. *T. vaginalis* ($1.2x10^6$ parasite/mL)
2. *C. albicans*
3. *E. coli*
4. *T. vaginalis+C. albicans*
5. *T. vaginalis+E. coli*
6. Control FCC (no microorganism inoculation)

In the study, the cells collected from the L929 fibroblast cell series were placed in 24-well cell culture plates. The monolayer of fibroblast cells in the wells occurred within 24 hours. Solutions were inoculated in the wells: the first group contained 100 µL of *T. vaginalis*, the second group had 100 µL of *C. albicans*, and the third group contained 100 µL of *E. coli*. In the fourth group, both *T. vaginalis* and *C. albicans* (100 µL each) were inoculated, and the fifth group included both *T. vaginalis* and *E. coli* (100 µL). Cells in the last group formed the control group. Plates were incubated for 24 hours at 5% CO_2 and 37°C. Then, cells were examined under an inverted microscope (Eclipse TS 100, Nikon, Tokyo, Japan) and viability counts were performed with 0.01% neutral red. Each group was studied 11 times.

Statistical Analysis

SPSS 15.0 for Windows was used to analyse the statistical parameters. Significance between two means in an independent group was assessed by using Mann-Whitney U and Kruskal Wallis tests. The data was presented as mean±standard deviations and the p value was set at 0.01.

RESULTS

After incubation for 24 h, all of the control cells were attached and formed a normal monolayer (Figure 1A). In the inoculated *E. coli* group, a level of 40.91±8.03% was obtained for cell death (Figure 1B). At that time, the effects of *T. vaginalis* in the groups were clearly visible in L929 fibroblast cell cultures. In this first group of inoculated *T. vaginalis,* cell death was determined to be 19.09±4.74% (Figure 1C). The inoculated parasites were seen to attach to the monolayer and divide, producing a visible focal lesion. While the lesion was gradually expanded, the monolayer was destroyed and parasites became free-swimming.

When inoculated with *C. albicans* and *T. vaginalis,* there was almost no monolayer of fibroblasts and all cells were round. A large amount of *T. vaginalis* was observed and 96.55±2.91% fibroblast cells were counted as dead (Figure 1D). When *T. vaginalis* and *E. coli* were inoculated together in fibroblast cells, the cells left the base and had a round appearance; 89.64±4.38% of fibroblasts were dead (Figure 1E). In the group containing only *C. albicans,* cell death was determined to be 21.00±4.72% (Figure 1F). The percentage values of dead cells are presented in Table 1.

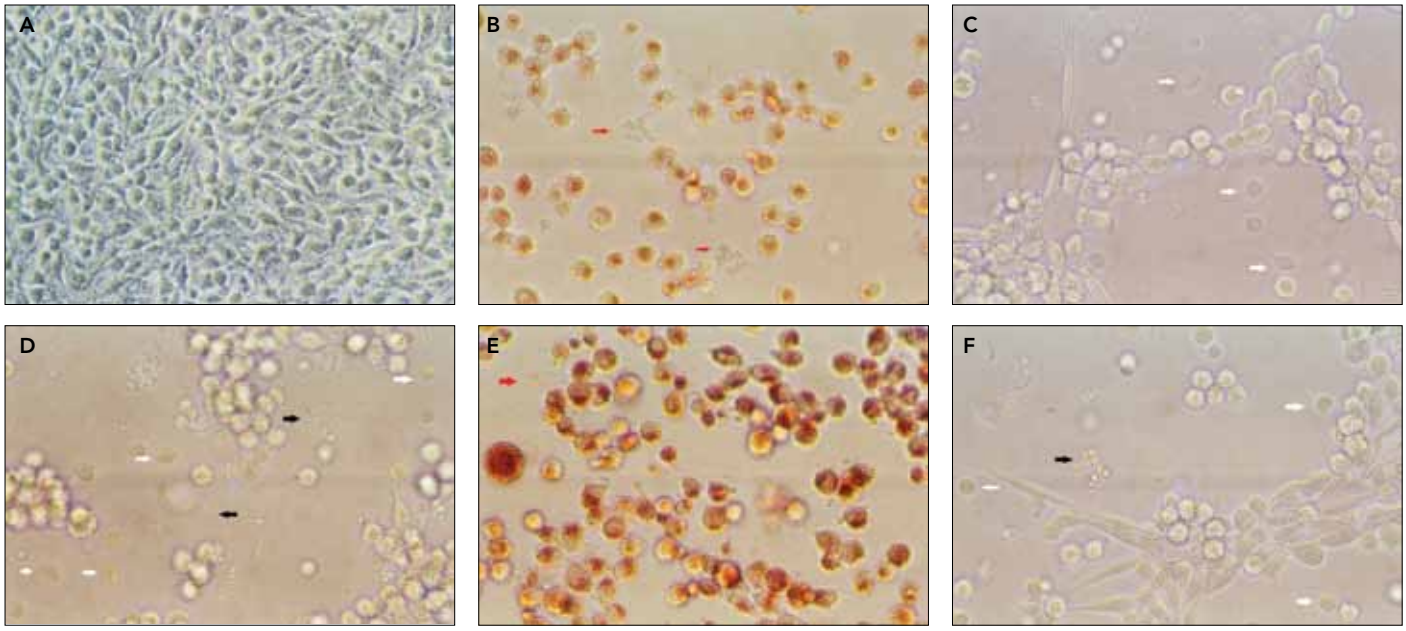

Figure 1. The changes in FCC under different conditions. A) Control-fibroblasts, B) *E. coli* and fibroblasts, C) *T. vaginalis*+fibroblasts, D) *T. vaginalis+C. albicans*+fibroblasts, E) *T. vaginalis+E. coli*+fibroblasts, F) *C. albicans*+fibroblasts (white arrows: *T. vaginalis*, black arrows: *C. albicans*, red arrows: *E. coli*)

Table 1. Cytopathic effects of infectious agents on fibroblast cells

Organisms	% Live cells (n=11)	% Dead cells (n=11)
T. vaginalis	79.09±5.15	19.09±4.74
C. albicans	78.91±4.78	21.00±4.73
E. coli	59.09±8.03	40.91±8.03
T. vaginalis+C. albicans	3.45±2.91	96.55±2.91
T. vaginalis+E. coli	9.45±3.61	89.64±4.38
Control	99.18±1.25	0.82±1.25

A significant difference between live and dead cell groups was determined by Kruskal-Wallis analysis of variance. The Mann-Whitney U test was used to determine which groups caused the difference. According to this test, while there was no significant difference between the first and second groups ($p>0.01$), significant differences were determined between all of the other groups ($p<0.01$). *T. vaginalis* and *C. albicans* showed a similar effect on the fibroblast cells; however, the two organisms together were found to make the cytopathic effect about 100%.

DISCUSSION

Trichomoniosis is one of the most important forms of protozoan parasitosis that is transmitted by sexual intercourse. Depending upon the social conditions, the methods used in diagnosis and the sexual habits of people, its prevalence varies from one country to another. Donne first described the parasite; the question of its pathogenicity has been subject to some degree of controversy. *T. vaginalis* causes vaginitis in women and urethritis and balanitis in men. During trichomoniosis, significant changes may be observed in the epithelial cell layers of the vagina (2). After Houge's observation that CPE was associated with *T. vaginalis* in embryonic human and chicken tissue explants, it was estimated that tissue cultures might become useful tools for the demonstration and identification of at least some aspects of the virulence of the organism (16).

To date, virulence factors such as adhesion molecules, proteolysis, haemolysis, cell separation factors and cytotoxicity factors were determined (6, 17-28). Adhesion of *T. vaginalis* to the vaginal epithelial cells was found to be effective. Parasite cell surface proteins and glycoproteins provide the host-cell adhesion. To date, four adhesion molecules have been identified (AP65, AP51, AP33 and AP23) (29). In the adhesion process of parasites, the target protein is laminin (28). Parasite surface carbohydrates, such as lectin-binding D-lactose and N-acetyl-D-glucosamine, also have important roles in virulence (30). Parasites are unable to synthesise lipids, so lipids are obtained from erythrocytes. Parasites can haemolyse erythrocytes via cysteine proteases; the effect of this activity in virulence has also been reported (21). Krieger et al. (31), who correlated beta-haemolytic activity by *T. vaginalis* with the symptoms of the patient and with the mouse assay, found that the pathogenic strains had higher haemolytic activity. The parasite has a maximum cysteine protease (23 pieces) (13, 32), which reduces immunoglobulin levels in the vagina (27). *In vitro* studies showed that the cell separation factor decreases in the presence of beta-oestradiol. The cell separation factor is capable of separation without killing the host epithelial cells. This effect was also observed in the present study. Cells separating from the monolayer fibroblast series took the vital dyes, but they became round and small. Other molecules that play a role in the pathogenesis of the parasite show a pore forming molecule perforin-like domain (9). In addition, some of *T. vaginalis* strains were a virus carried by double-stranded and that the virus was found in the majority of clinical isolates. This virus is thought to play a role in the pathogenesis of the parasite (33).

The cytopathic effects of *T. vaginalis,* both alone and with *C. albicans* and *E. coli,* on fibroblast cell culture series were stud-

ied, and the striking findings of this study were presented. In some earlier studies, the effect of *T. vaginalis* on the cell series was found to be 10% (15); in our study, this rate was higher (20%). This effect increased when *C. albicans* or *E. coli* was co-incubated with the parasite; in particular, *C. albicans* revealed more disruption of FCC, which was almost 100%. Additionally, the trophozoites were very active and motile in this situation. The findings showed that mixed vaginal infections due to *T. vaginalis* and *C. albicans* may result in more complicated clinical symptoms and require a different treatment. Candidiasis is reported as the most common cause of vaginitis in Europe and the second most common cause of vaginitis in the United States (34). According to the World Health Organization (WHO), the worldwide prevalence of trichomoniosis is 174 million and accounts for 10% to 25% of vaginal infections (35). The prevalence of trichomoniasis is reported to be approximately 5% in Turkey (36, 37). These two common infections are found together in many cases, which implies the importance of the present study. Alderete and Pearlman reported an extensive disruption in different monolayers (human urogenital and vagina, human epithelial, normal baboon testicular, and monkey kidney cells) with exposure to *T. vaginalis* (12). The explanation of the cellular disruption in cell monolayers is the key point for understanding the pathogenesis of the parasite. For instance, the presence of Zn^{2+} downregulates the transcriptional levels of a protein and has a negative effect on trichomonal cytotoxicity, while lipophosphoglycan mutants of *T. vaginalis* shows reduced adherence and cytotoxicity to human ectocervical cells (38, 39). Another factor that can affect the cytotoxicity is the source of the parasite; fresh isolates were more cytotoxic and can easily attach to cell layers than laboratory strains that have been cultivated for a long time in axenic cultures (40). In the study, the *T. vaginalis* strain was isolated from a symptomatic clinical case and freshly used, so the cytopathic effect was not decreased due to long-term cultivation.

CONCLUSION

The other microorganisms in the vagina may affect the cytotoxic potential of T. vaginalis; in particular, the presence of *C. albicans* may increase the disruption of epithelial cell layers.

Conflict of Interest

No conflict of interest was declared by the authors.

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The Prevalence, Isolation and Morphotyping of Potentially Pathogenic Free-Living Amoebae from Tap Water and Environmental Water Sources in Sivas

Sivas İlinde Potansiyel Patojen Serbest Yaşayan Amip Türlerinin Musluk Sularında ve Çevresel Su Kaynaklarında Yaygınlığı, İzolasyonu ve Morfotiplendirmesi

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ABSTRACT

Objective: To our knowledge, there is no study dealing with the prevalence of free-living amoebas (FLA) in water sources in Turkey, previous studies were mostly case presentations. The aim of the present study was to investigate the prevalence of FLA from tap water and natural water sources in different parts of the city.
Methods: In the study, 250 samples were collected from the city centre, districts and villages. Two litres of water was collected from each source and filtered through a vacuum filtration system. The filter papers were washed in "Page's Amoeba Saline (PAS)" solution and incubated overnight. Filter papers were removed from the tubes and centrifuged; the final pellet was inoculated on non-nutrient agar (NNA) plates. The growth rate of FLA was checked after three days of inoculation and the flagellation test was performed to determine the presence of *Naegleria* spp. Heat tolerance of isolated strains was checked at 37, 42 and 52°C for the presence of pathogenic *Acanthamoeba* species. The cyst and trophozoite morphology of amoebas were examined under a light microscope and the genera was identified according to morphotyping keys.
Results: FLA were found in 75 (30.0%) of examined water samples. Eleven (4.4%) were identified as *Acanthamoeba* spp., 25 (10.0%) as *Naegleria* spp. and 39 (15.6%) as *Hartmannella* spp. after microscopic examination.
Conclusion: Our study revealed that FLA are common inhabitants of household water as they are in the environment, so their own potential risks as well as transferring bacteria as other pathogens is important for human health. *(Turkiye Parazitol Derg 2012; 36: 198-203)*

Key Words: Free living amoeba, *Acanthamoeba, Naegleria*, isolation

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ÖZET

Amaç: Ülkemizde günümüze kadar su kaynaklarında serbest yaşayan amip (SYA) yaygınlığına yönelik kapsamlı bir çalışma yapılmamış, genellikle olgu sunumlarında SYA varlığı bildirilmiştir. Bu çalışmanın amacı Sivas ilinde musluk sularında ve çevresel su kaynaklarında SYA yaygınlığının belirlenmesidir.
Yöntemler: Çalışma kapsamında şehir merkezinden, ilçelerden ve köylerden toplam 250 örnek toplanmıştır. Her bir kaynaktan iki litre su alınarak vakumlu filtrelerden süzülmüştür. Filtre kağıtları steril "Page's Amoeba Saline (PAS)" solüsyonunda bir gece bekletilmiştir. İnkübasyon sonrası filtreler çıkarılıp tüp santrifüj edildikten sonra dip kısımdan alınan bir iki damla örnek, Besleyici-Değeri Olmayan Agar (BDOA) plaklarına inoküle edilmiştir. İnkübasyonun üçüncü gününden itibaren besiyerlerinde üreme kontrolleri yapılmıştır. *Naegleria* spp. belirlenmesi

This study is presented as a poster in the 17th National Congress of Parasitology and Parasitic diseases in the Caucasus and the Middle East symposium at 4-10 September, 2011, Kars, Turkey

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için kamçı deneyi yapılmış, patojenik *Acanthamoeba* türleri için de 37°C, 42°C ve 52°C de ısı tolerans testi uygulanmıştır. Amiplerin kist ve trofozoit morfolojileri ışık mikroskobu altında incelenmiş ve morfolojik anahtarlar kullanılarak cins ayrımları yapılmıştır.

Bulgular: İncelenen toplam 250 örneğin 75'inde (%30.0) SYA tespit edilmiştir. Bu türlerin mikroskobik olarak temel morfotiplendirme anahtarlarına göre incelemelerinde 11'inin (%4.4) *Acanthamoeba* spp., 25'inin (%10.0) *Naegleria* spp. ve 39'unun (% 15.6) *Hartmannella* spp., olduğu belirlenmiştir.

Sonuç: Su örneklerinde bu kadar yaygın SYA bulunması, hem amiplerin kendileri hem de taşıdıkları çeşitli bakteriler nedeniyle insan sağlığı açısından önemli risk oluşturmaktadır. *(Turkiye Parazitol Derg 2012; 36: 198-203)*

Anahtar Sözcükler: Serbest yaşayan amipler, *Acanthamoeba, Naegleria*, izolasyon

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INTRODUCTION

Acanthamoeba and *Naegleria* are the most common Free-living amoebas (FLA) that are associated with human and animal diseases (1-4). *Balamuthia mandrillaris, Hartmannella* and *Sappinia* species are also free-living amoebae but are less common causes of clinically significant infections (5, 6). The disease of the central nervous system by infection of *Naegleria* spp. was first documented in 1965 and the disease was called primary amoebic meningoencephalitis (PAM) (2). *N. fowleri* is the only species of *Naegleria* that causes human disease (7); it can be isolated from soil and fresh water (8, 9). *Acanthamoeba* is another important group of FLA that is commonly found in the environment. Previously, it was isolated from many different environment and clinical samples: soil, water, sewage water, tap water, thermal water mud, air, sea water, ear, lung secretions and nasopharyngeal mucosa samples (2, 3, 9-23). They are the causative agents of granulomatous amoebic encephalitis (GAE) and Acanthamoeba Keratitis (AK) (1, 24-27). In the subsequent years, *Acanthamoeba* were found to be responsible for some other lesions in eyes, ears, skin and innards (1). *B. mandrillaris* may also cause GAE, and was first isolated from mandrill monkeys in 1986; to date, more than one hundred cases have been reported (6). In the early 2000s, *Sappinia diploidea* was isolated from a patient with amoebic encephalitis (5); other species of *Sappinia* have since been isolated from faecal-contaminated soils. Another FLA genera is *Hartmannella* and some records are available regarding the potential pathogenicity of *H. vermiformis* in humans (13). However, to date, both experimental and clinical studies about FLA have been rather limited regarding the isolation of parasite from environment. In addition to their pathogenic potential, these amoebas may act as a "Trojan horse" of many different types of bacteria and virus. These pathogens can lead to severe human disease as complications of amoebic keratitis. For these reasons, the health importance and pathogenic potential of FLA has been better explained in recent years (28-30).

Free-living amoeba-associated diseases are relatively rare among people when compared with their environmental abundance. However, the illnesses caused by pathogenic FLA are severe, and often challenging to treat, so a better understanding of their ecologic distribution is necessary in the places where humans interact with FLA (31, 32). In Turkey, there is no study about the current prevalence of FLA in water sources and other environments. The aim of the present study was to investigate the prevalence of FLA in natural sources and domestic water systems in Centrum, districts and villages.

METHODS

Study Area

Sivas is located at the eastern part of the Central Anatolian region of Turkey; it is the second largest province in Turkey. According to the 2007 Turkish census, its population was 300,795. The city, which lies at an elevation of 1,278 m in the broad valley of the Kızılırmak river, is a moderately-sized trade centre and industrial city, although the economy has traditionally been based on agriculture.

The study sample size was determined at α: 0.05, d: ±0.06 according to the prevalence of previous studies. The sample size was approximately 250 samples.

Collection and Filtration of Water Samples

Specimens were collected from faucets in Centrum and fountains of the villages between June and December 2010. Twenty-five of the samples were surface water (streams) in rural areas (Divriği, Şarkışla, Kangal, Suşehri, Gemerek, Altınyayla, Gürün, Ulaş, Koyulhisar, and Akıncılar districts), 8 were from hot springs (Kangal, Yıldızeli, Hafik), 2 were from creeks (branches of Kızılırmak) and 4 were from wells. The distribution of 250 samples according to regions were as follows: 24 from Centrum (fountain and faucets), 43 (tap water) from districts and 144 (tap water-fountains) from villages. Water samples were collected with 2-litre sterile glass bottles and filtered. A vacuum filtration system with 0.45 µm pore size was used in the study (Sartorius AG, Goettingen, Germany). The specimens were transported and stored at ambient temperature and cultured for amoebae within 3 days. Filter papers were stored in sterile glass tubes until examination.

The Incubation of Samples and Growth

Filter papers were incubated overnight in 15 mL sterile buffer solution. In the following day, the tubes were centrifuged at 1500 rpm for 10 minutes. A few drops of pellet were inoculated on non-nutrient agar (NNA) with a lawn of inactive *Escherichia coli*. NNA was prepared with Page Amoeba Saline (PAS) (2.5 mM NaCl, 1 mM KH_2PO_4, 0.5 mM, Na_2HPO_4, 40 µm $CaCl_2$-$6H_2O$ and 20 µm $MgSO_2.7H_2O$). Agar was dissolved in PAS (1.5%), autoclaved and dispensed onto sterile plates. After inoculation, the plates were incubated at 30°C (8, 10).

Growth Control and Passages

After 3 days of incubation, the plates were monitored for the detection of trophozoites or cysts of amoeba daily until 15 days using light microscopy. In order to obtain fresh cultures, approximately 1 cm^2 of agar was taken from the grown culture and placed at the centre of new NNA plates. The strains were maintained by serial passages in this medium (8, 10).

Table 1. The source of water samples and the prevalence of FLA

Water Source	(+)		(-)		Total examined	
	No	%	No	%	No	%
Tap water (Drinking)	62	29.4	149	70.6	211	84.4
Environmental water samples (stream, hot spring, creek, well water)	13	33.3	26	66.7	39	15.6
Total	75	30.0	175	70.0	250	100.0
(χ^2: 0.24, p>0.05)						

Identification of FLA at Genera Level

In order to determine the genera of FLA, the movement and structural properties of amoebas were examined. Additionally, the flagellation test (FT) was used to identify *Naegleria*. After examination under a light microscope permanent smears were prepared and stained with Trichrome (33). A piece of agar was placed over a slide incubated in a humid environment for an hour. The transferred amoebas were fixed with Schaudinn at 37°C for a few minutes on the slides. Then, the slides were fixed in the same solution for an hour. For the morphological identification of isolates, we utilised from the study of Smirnov and Goodkov (34).

The Flagellation test

The organism is exposed to a hypotonic environment in the test. The amoebas were collected from plates and put into 1 mL distilled water. After 2 hours of incubation at 37°C, 100 µL of sample was transferred to slides and examined under light microscope for the presence of any free-swimming flagellates (12, 18).

Heat Tolerance Test

Previously cultured FLA cysts were inoculated in three fresh NNA, as described before. One of the plates was incubated at 37°C and the others at 42°C and 52°C. After two days of inoculation, the growth rate and cell motility of FLA at different temperatures were recorded daily under light microscope (12).

Axenic Culture of FLA

The isolates were axenically cultured with protease peptone, yeast extract, and glucose (PPYG) medium in 25 cm^2 Corning® flasks and incubated at 35°C. PPYG medium was prepared as described previously: 0.4% protease peptone, 0.2% yeast extract, 1.0% glucose. Before axenisation, amoebas were removed from NNA with a spatula and washed three times in PAS by centrifugation at 500 x g. The pellet was inoculated in PPYG, and gentamicine (50 µg/mL) was added to medium to inhibit bacterial growth (33).

Statistical Analysis

Data was analysed statistically with SPSS 14.0 for Windows software. The Chi-square test was used to compare results and the p value was set at 0.05.

RESULTS

Free-living amoebas were recovered from 75 out of 250 (30%) water samples. The prevalence of FLA in tap water (29.4%) and an almost identical proportion was recovered from samples from environmental sources (33.3%; Table 1). *Acanthamoeba* spp. were identified in 11 (4.4%), *Naegleria* spp. in 25 (10.0%) and *Hartmannella* spp. in 39 (15.6%) with morphotyping (Figure 1). The statistical comparison of different regions (Centrum, districts and villages) and more detailed representation of environmental sources are given in Tables 2, 3 and 4. The prevalence of FLA was higher in villages than in Centrum (χ^2=6.424, p<0.05). Interestingly, eight of the 11 *Acanthamoeba* isolates were from a district, Kangal.

In a heat tolerance test, 50 strains were grown at 37°C, 12 strains at 42°C and 3 strains at 52°C. Additionally, we observed that as the temperature increased the growth rate of FLA decreased.

Despite being repeated twice, the flagellated form of *Naegleria* spp. could not be observed in the flagellation test. The axenisation of strains was achieved only for five (10%) of the 50 samples. All of the strains that successfully axenised were *Acanthamoeba*. Genomic DNA of strains was isolated and stored for genotyping.

We examined the cyst and trophozoites morphology of isolated strains. The vegetative forms of amoebas resembled each other. However, the difference in pseudopods is important. *Acanthamoeba* were identified according to its hyaline lobopode and *Hartmanella* were identified according to its rod shaped trophozoites. Cyst forms of *Acanthamoeba* were very typical with a star-like shape, and were easy to differentiate as Group I and II. The differentiation of *Naegleria* and *Hartmannella* could be performed according to trophozoite forms.

DISCUSSION

Free-living amoebas are distributed worldwide and have been isolated from domestic tap water, drinking water, natural and treated water, sea water and bottle water (2). In the present study, we investigated the prevalence of FLA in stream, hot spring, creek and well water, as well as in domestic tap water systems in Sivas Centrum, districts and villages.

Free-living amoebas, such as the genera *Naegleria*, *Acanthamoeba,* and *Vahlkampfia,* have been commonly found in various environments all around the world and recognised as important pathogens of humans or animals (28). *N. fowleri* is the causative agent of PAME and the transfer of infection to healthy humans occurs via contaminated waters. *Acanthamoeba* spp. and *B. mandrillaris* are opportunistic pathogens of immunosuppressed people and mostly cause GAE. The pathology of disease can be observed in the lungs, sinuses and skin in immunodeficient patients (31, 35). Additionally, *Acanthamoeba* spp. invade the cornea of the eye and cause AK due to contact lens usage (3, 11, 32, 36, 37). Besides their pathogenicity, FLA may transfer some other pathogens to the human body (30).

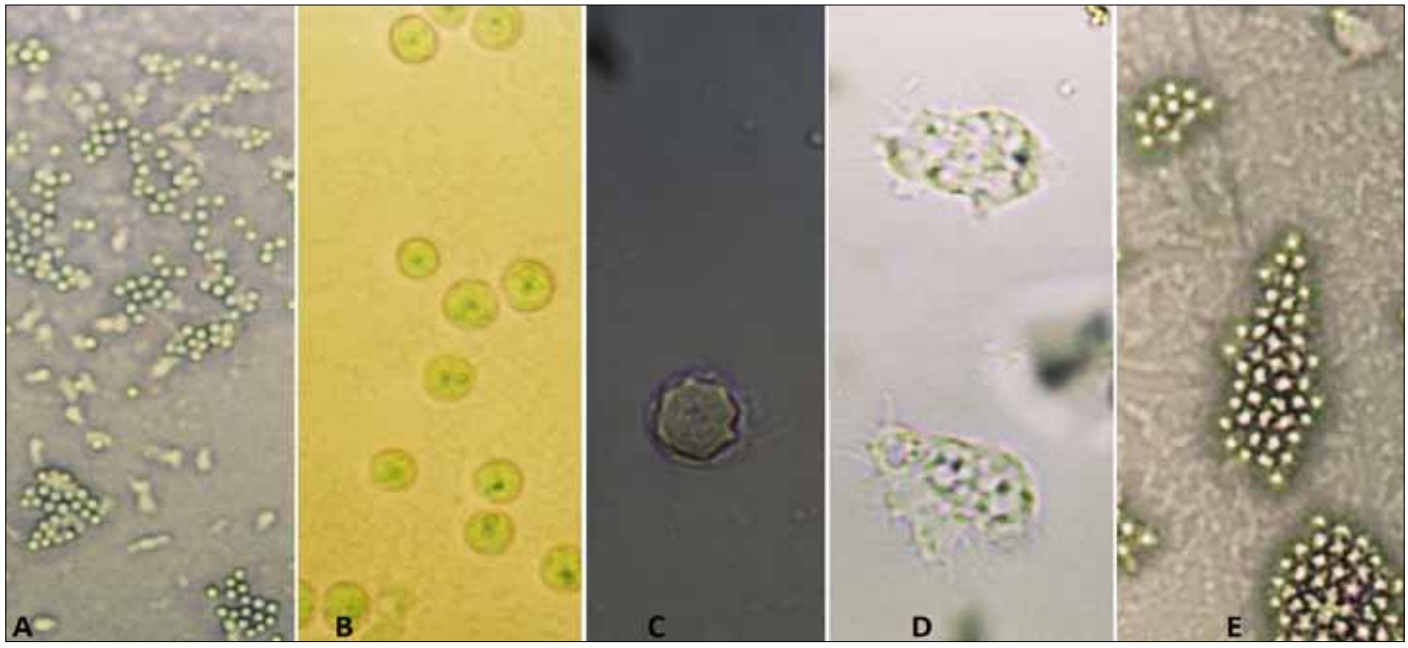

Figure 1. The isolated amoebas from water samples: A. The cysts and trophozoites of *Naegleria* spp. on NNA(x10), B. *Naegleria* spp. cysts on NNA(x40), C. *Acanthamoeba* spp. cysts in saline (x40), D. *Acanthamoeba* spp. trophozoites in saline (x40), and E. *Acanthamoeba* spp. cysts on NNA (x10)

Table 2. The regional distribution of tap water samples and the prevalence of FLA

Region	(+)		(-)		Total examined	
	No	%	No	%	No	%
Centrum	2	8.3	22	91.7	24	9.6
Districts	11	25.6	32	74.4	43	17.2
Villages	49	34.0	95	66.0	144	57.6
Total	63	30.0	175	70.0	211	100.0

Table 3. The statistical comparison of FLA prevalence according to regions

Districts-Villages	χ^2: 1.084	p>0.05
Districts-Centrum	χ^2: 2.93	p>0.05
Villages-Centrum	χ^2: 6.424	p<0.05*
*important		

Free-living amoebas species tolerate temperature ranges of 10-30°C. In the study, in the thermotolerance test, 50 strains were grown at 37°C, 12 out of the 50 clones were able to grow at 42°C after two days. Morphologically, 11 out of the twelve isolates displayed acanthapodia, and the presence of double-walled cysts was identified. While these eleven isolates were determined as belonging to the genus *Acanthamoeba* spp. one isolate was determined as *Hartmannella* spp. Three out of 50 clones were also able to grow at 52°C after two days. These isolates were also morphologically determined as *Acanthamoeba* spp. Three samples were taken from Kangal, Divriği and Suşehri town. The axenisation of strains were achieved only for five (10%) of the 50. All of the strains that successfully axenised in PPYG medium were *Acanthamoeba*.

The prevalence of FLA was reported to be between 23% and 89% from swimming pools, springs, lakes and tap water (2, 17, 20). In Germany, the following genera were identified from hot springs: *Acanthamoeba* (22%), *Naegleria* (22%), *Vahlkampfia* (20%), *Hartmannella* (15%), and *Vannella* (7%) (23). The most common was *Hartmannella* spp. in our study, which accounted for almost 50% of the isolated amoebas. FLA were detected in 80% of environmental water sources in Bulgaria and in 9.3% of tap water in USA, and 79% of river water in Germany (13, 18, 19). In our country, there has been no study dealing with the prevalence of FLA on a large scale, especially from water sources. In Kayseri, FLA were found in 5 (19.2%) samples of well water (38). In the present study, FLA were investigated in 250 samples, mostly comprising tap water. FLA were recovered from 75 out of 250 (30%) water samples. The prevalence of FLA in tap water (29.4%) and an almost identical proportion was recovered from samples from environmental sources (33.3%). *Acanthamoeba* spp. were identified in 11 (4.4%), *Naegleria* spp. in 25 (10.0%) and *Hartmannella* spp. in 39 (15.6%) via morphotyping. The prevalence of FLA obtained was higher in villages than in Centrum (χ^2=6.424, p<0.05). Interestingly, the highest *Acanthamoeba* isolates were established from one district, Kangal.

CONCLUSION

The results in this study show that potentially pathogenic FLA are widely distributed, even in drinking water. In particular in the

Table 4. The source of environmental water samples and the prevalence of FLA

	(+)		(-)		Total examined	
	No	**%**	**No**	**%**	**No**	**%**
Stream	6	24.0	19	76.0	25	64.1
Hot spring	4	50.0	4	50.0	8	20.6
Creek	1	50.0	1	50.0	2	5.1
Well water	2	50.0	2	50.0	4	10.2
Total	13	33.3	26	66.7	39	100.0

areas where tap water was possibly contaminated with soil, the prevalence of FLA was higher; the prevalence was low in Centrum, because municipal water purification systems use chlorine to remove harmful microorganisms from the water supply. However, environmental strains are more resistant to several chemicals than collection strains (14). This highlights the importance of effective disinfection in water supply systems for protection against FLA.

In the present study, FLA were recovered from a variety of ecological habitats using culture methods. It was clear that FLA were common anywhere that people can be found. The classification of FLA as potential pathogens or non-pathogens is not acceptable and knowledge of the prevalence of FLA in household water can provide a focus for the prevention of amoeba-associated illnesses.

Conflict of Interest

No conflict of interest was declared by the authors.

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Adıyaman'da 2000-2011 Yılları Arasında Aktif ve Pasif Sürveyans ile Saptanan Sıtma Olguları

Malaria Cases Detected by Active and Passive Surveillance in Adıyaman between 2000-2008

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ÖZET

Amaç: Sıtma, Adıyaman ve çevresinde önemini koruyan bir sağlık sorunudur. Sıtma Savaş Birimi'nin düzenli çalışmalarıyla sıtma hastalığı kontrol altında tutulabilmekte ancak bölgenin sosyo-ekonomik ve kültürel koşulları ve Adıyaman'dan mevsimlik işçi olarak çalışmaya giden tarım işçileri nedeni ile eradikasyon tam olarak başarılamamıştır. Bu çalışmada Adıyaman ilinde 2000-2011 yılları arasında Sağlık Müdürlüğü Sıtma Savaş Birimi'nce aktif ve pasif sürveyans çalışmaları ile saptanan sıtma olguları değerlendirilmiştir.
Yöntemler: Sıtmanın yaygın olduğu bölgelerde aktif ve pasif sürveyans yöntemi ile alınan 312.125 kan örneklerinde ince yayma ve kalın damla yöntemiyle sıtma paraziti aranmıştır.
Bulgular: İncelenen örneklerde 39'u (%21.1) aktif, 145'i (%78.8) pasif sürveyans yöntemiyle toplam 184 kişide sıtma olgusu saptanmıştır. İncelenen bütün kanlar içerisinde pozitif olguların oranı %0.05'dir. Hastaların 108'i (%58.6) erkek, 76'sı (%41.3) ise kadınlardan oluşmaktadır. Olguların 3'ü yerli, 181'i ise hariçten gelen olgu idi. Yurtdışı kaynaklı olan bir *Plasmodium falciparum* sıtması dışında olguların tamamının *Plasmodium vivax* olduğu görüldü. 2008-2011 yılları arasında sıtma vakası bildirilmemiştir
Sonuç: Adıyaman'da tarım işçilerinin fazla olması ve bu işçilerin özellikle sıtmanın endemik olduğu bölgelere çalışmaya gitmesi, bölge insanını sıtma açısından tehdit etmektedir. Sıtma hastalığı ile mücadelede, Sıtma Savaş Birimi ile Adıyaman Üniversitesi Tıp Fakültesi'nin işbirliği içinde planlı halk sağlığı eğitimleri yapmaları önemlidir. *(Turkiye Parazitol Derg 2012; 36: 204-7)*

Anahtar Sözcükler: Sıtma, aktif ve pasif sürveyans, Adıyaman

Geliş Tarihi: 31.05.2012 **Kabul Tarihi:** 20.11.2012

ABSTRACT

Objective: In this study, malaria cases were determined in Adıyaman with active and passive surveillance studies by Local Health Authority, Centre for Struggle against malaria between the years 2000-2011.
Methods: In 312.125 blood samples, obtained with the method of active and passive surveillance from the region where malaria is common, malarial parasite was investigated by the method of thin and thick blood smears.
Results: In the observed samples, 184 malaria cases were determined; 38 (21.1%) with active, 145 (78.8%) of them with passive surveillance method. The rate of positive cases among all the observations was 0.05%. 108 (58.6%) of the cases were male, 76 (41.3%) of the cases were female. It was stated that 3 of the cases were provincial cases and 181 of them originated from extra-provincial sources. It was observed that, apart from one *Plasmodium falciparum* case which was from a foreign-source; all of the cases were *Plasmodium vivax* positive. There were no cases of malaria between 2008 and 2011 years.
Conclusion: The fact that there are many farming workers in Adıyaman and that these workers work in regions where malaria is endemic, threatens the population in the region with malarial infection . It was considered important for the Centre for the Struggle against Malaria and Adıyaman University, Medical Faculty to cooperate in the struggle against malaria by offering planned training programs in public health. *(Turkiye Parazitol Derg 2012; 36: 204-7)*

Key Words: Malaria, active and passive surveillance, Adıyaman

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GİRİŞ

Sıtma bir protozoon olan *Plasmodium* türlerinden birinin veya daha fazlasının dişi *Anopheles* cinsi sivrisineklerin insanı sokması, organ transplantasyonu veya enfekte kan ile insana geçmesi sonucu gelişir (1, 2). Plasmodium'ların insanı enfekte eden dört türü (*P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*) bilinmekle birlikte, yapılan moleküler çalışmalar sonucunda insanı enfekte eden beşinci bir tür (*P. knowlesi*) bildirilmiştir. *P. vivax* Türkiye ve Dünya'da en fazla sıtma oluşturan türdür. *P. falciparum* daha çok tropikal bölgeler ve Afrika'da görülmekte olup Türkiye'de nadir olarak görülmektedir. Enfeksiyon genellikle insana, enfekte dişi Anopheles cinsi sivrisineklerin kan emmesi esnasında sporozoitleri enjekte etmesi ile bulaşmaktadır. Bunun yanı sıra enfekte kişiden kan transfüzyonu, kontamine iğnelerin kullanımı gibi yollarla da bulaş olabilmektedir (3-5).

Ülkemizde 1980'li yıllarda yerli olgular toplam olguların %60'ını, emporte olgular %27'sini oluştururken, 1998 yılında yerli olgular %83, emporte olgular %17 olarak saptanmıştır. Bunun nedeni, son yıllarda GAP'ın devreye girmesi ile 1980'li yıllarda Güneydoğu Anadolu Bölgesi'nden Çukurova-Amik Ovası Bölgesi'ne tarım alanında çalışmak için göçer işçilerin bu bölgeye daha az gelmeleridir (4).

Güneydoğu Anadolu Bölgesinde Adıyaman, Batman, Diyarbakır, Gaziantep, Kilis, Mardin, Siirt, Şanlıurfa ve Şırnak illerinin kapsadığı alan "GAP Bölgesi" olarak tanımlanmaktadır. Bu illerde sulama ve su kullanım hatalarından doğacak çevre sorunlarının yanında sıtma hastalığının görülme sıklığında artış olabileceği görülmektedir. Mevsimlik işçi, askerlik, göç gibi nedenlerle endemik bölgelerden endemik olmayan bölgelere sıtmanın taşındığı bilinmektedir. Adıyaman'da mevsimlik işçilerin fazla olması nedeniyle sıtma hastalığının kontrol altında tutulması gerektiği ve gerekli önlemler zamanında alınmadığı takdirde bu bölgede salgınların yaşanabileceği akıldan çıkarılmamalıdır (6). Bu çalışmada, 2000-2011 yılları arasında Adıyaman'da sıtmanın durumu çeşitli yönlerden incelenmiştir.

YÖNTEMLER

Bu araştırmada 2000-2011 yılları arasında Adıyaman İl Sağlık Müdürlüğü Bulaşıcı Hastalıklar Şube Müdürlüğü Sıtma Savaş Birimi tarafından yapılan aktif ve pasif sürveyans çalışma verileri retrospektif olarak değerlendirildi. Bireylerden alınan kan örneklerinden kalın damla ve ince yayma kan preparatları hazırlandı, Giemsa boyasıyla boyanıp ışık mikroskopunda X100 büyütmede immersiyon objektifiyle sıtma paraziti araştırıldı. Pozitif saptanan örneklerin tamamı ve negatif saptanan örneklerin %20'si Adana, Sıtma ve Tropikal Hastalıklar Eğitim ve Araştırma Merkezi Laboratuvarı'na gönderilerek kesin tanısı yapıldı. Sıtma saptanan olgular; yaş grupları, yerleşim merkezleri, cinsiyet, yerli veya dışarıdan gelen olgu oluşlarına göre incelendi.

BULGULAR

2000-2008 yılları arasında toplam 312.125 kan örneği incelenmiş, 39'u (%21.1) aktif, 145'i (%78.8) pasif sürveyans yöntemiyle toplam 184 (%0.05) sıtma olgusu saptanmıştır. 2008 yılından sonra ise Adıyaman ilinde sıtma olgusu saptanmadığı görülmüştür. İncelenen kanlar içerisinde pozitif olguların oranı %0.05'dir. Yıllara göre aktif ve pasif sürveyans yöntemiyle pozitif olguların dağılımı Tablo 1'de görülmektedir. Yaşlar 1-66 arasında değişen hastaların 108'i (%58.6) erkek ve 76'sı (%41.3) kadındır (Tablo 2). Sıtma olguları her yaş grubunda görülmekle beraber; en az olgu 0-11 aylık yaş grubunda %0.54 oranında, en fazla olgu ise 15-24 yaş grubunda %46.19 oranında görülmüştür. Bu oranları sırasıyla 65 yaş ve üstü (%1.63), 45-64 yaş (%8.69), 1-4 yaş (%3.26), 10-14 yaş (%13.04), 5-9 yaş (%7.06), 25- 44 yaş (%19.56), grupları izlemektedir (Tablo 3). Pozitif olguların %1.63'ü yerli, %97.81'i importe ve %0.54'ü yurtdışı importedir. 2005 yılında Kamerun'dan dönen Adıyamanlı bir hastada *P. falcipar*um olgusu dışında olguların tamamında *P. vivax* görüldü. Olgularda nüks saptanmadı (Tablo 4).

Hastaların çoğunlukla, Adıyaman merkezinden (Merkez İlçe) ve Kahta İlçesi'nden olduğu belirlenmiştir. 2000-2008 yılları arasında Adıyaman'da saptanan sıtma olgularının il, ilçe ve köylere göre dağılımı Tablo 5'de görülmektedir.

TARTIŞMA

Günümüzde dünya nüfusunun %41'inden fazlası (yaklaşık 2.3 milyar kişi) sıtmanın endemik olduğu bölgelerde yaşamakta olup

Tablo 1. 2000-2008 yılları arasında aktif ve pasif sürveyans yöntemiyle toplanan kanlarda saptanan sıtma olgu sayısı

	Toplam alınan kan			Olgu sayısı		
Yıl	**Aktif**	**Pasif**	**Toplam**	**Aktif**	**Pasif**	**Toplam**
2000	31.861	4.651	36.512	1	2	3
2001	38.072	2.152	40.224	-	11	11
2002	35.072	1.798	36.87	14	39	53
2003	33.872	2.627	36.449	11	66	77
2004	29.514	2.807	32.321	7	19	26
2005	27.577	2.758	30.319	2	8	10
2006	30.699	743	31.442	3	-	3
2007	31.839	754	32.593	-	-	-
2008	32.715	680	33.395	1	-	1
Toplam	291.221	19.047	312.125	39 (%21.1)	145 (78.8)	184

Tablo 2. Olguların yıllara ve cinsiyete göre dağılımı

Yıl	**Toplam alınan kan**	**Erkek**	**Kadın**	**Olgu sayısı**
2000	36.512	2	1	3
2001	40.224	5	6	11
2002	36.87	31	22	53
2003	36.449	49	28	77
2004	32.321	14	12	26
2005	32.319	5	5	10
2006	31.442	1	2	3
2007	32.593	-	-	0
2008	33.395	1	-	1
Toplam	312.125	108 (%58.6)	76 (%41.3)	184

Tablo 3. Sıtma olgularının yıllara ve yaş gruplarına göre dağılımı

Yaş Grupları	2000	2001	2002	2003	2004	2005	2006	2007	2008	Toplam	%
0-11 Ay				1						1	0.54
1-4 Yaş			4	1			1			6	3.26
5-9 Yaş			4	9						13	7.06
10-14 Yaş			8	11	3	2				24	13.04
15-24 Yaş	2	3	21	33	20	4	1		1	85	46.19
25-44 Yaş	1	6	10	15	1	3				36	19.56
45-64 Yaş			9	4	2	1				16	8.69
65 yaş ve üzeri				3						3	1.63
Toplam	3	9	56	77	27	10	2		1	184	100.

bunlar arasından her yıl 300 milyon yeni sıtma olgusu çıkmaktadır (7). Tüm eradikasyon çalışmalarına rağmen sıtma Güneydoğu Anadolu ve Çukurova bölgelerinde hala endemik olarak bulunmaktadır. Yurdumuzda sıklıkla rastlanan tür *P. vivax* olup *P. falciparum* olguları daha çok dış kaynaklıdır (4).

Adıyaman İli Strata IB bölgesinde olduğu bildirilmektedir. Strata I; sürekli yerli bulaşın olduğu ve sıtmanın bölge boyutunda endemik olduğu yer anlamına gelmektedir. Bu bölgenin A ve B olarak ikiye ayrılmasının en önemli nedeni, iki bölge arasındaki işçi hareketine bağlı olarak olgu yoğunluğu değişmesi görülmektedir. Olgu yoğunluğunun Strata IB bölgelerinde olduğu bildirilmektedir (4).

Adıyaman bölgesinden alınan 312.125 kan örneklerinde 184 (%0.05) kişide pozitif olgu bulunmuştur. Pozitiflik erkeklerde (%58.6), kadınlara (%41.3) göre daha yüksek bulunmuştur. Erkeklerde daha fazla görülmesi, sıtmanın endemik olduğu illere daha çok erkeklerin gittiği şeklinde açıklanmaktadır (mevsimlik işçi olarak). Pozitif olguların %1.6'sı yerli, %98.3'i dış kaynaklı olduğu tespit edilmiştir.

Türkiye'de tespit edilen sıtma olgularının %91'i GAP kapsamındaki illerde görülürken %9'u diğer illerimizde görülmektedir. Ülkemizde yapılan çalışmalarda, Şanlıurfa'da 1995-2000 yıllarını arasında saptanılan sıtma olgularının %64.5'inin yerli oluştuğu saptanmış (8). Malatya'da Güneş ve ark. (9) 1989-1998 yılları arasında 299 sıtma vakası tespit etmişlerdir. Malatya'da yapılan ikinci çalışmada Atambay ve ark. (10) 2003-2004 yılları arasında 66, Elazığ'da Kuk ve ark. (11) 1996-2004 yılları arasında 200, Diyarbakır'da Temiz ve ark. (12) 1999-2004 yılları arasındaki 22062 sıtma olgusu bildirilmiştir. 2000-2008 yılları arasında Adıyaman'da saptanan 184 olgunun 39'u (%21.1) aktif, 145'i (%78.8) pasif sürveyans yöntemiyle elde edilmiştir. Saptanan sıtma olgularının %30.4'ü (56 olgu) Adıyaman Merkez ve merkeze bağlı köylerde, %26'sı (48 olgu) Kahta ilçe merkezinde, %7'si (13 olgu) Kahta İlçesi'ne bağlı Köseler Köyü'nde ve %7'si (13 olgu) Sincik İlçesi'nin Dilektepe Köyü'nde saptanmıştır. Saptanan olguların %29.3'ü ise diğer ilçe ve köylerinde tespit edilmiştir. Kahta İlçesi'nin Erikli Köyü ve Sincik İlçesi'nin Dilektepe Köyü'nde yaşayanların hemen hemen hepsi geçimini tarımdan sağlamaktadır. Hastaların anket formunda tarım işçilerinin Batman, Mardin, Şanlıurfa ve Diyarbakır'ın Bismil ilçelerine çalışmaya gittikleri ve döndüklerinde hastalık belirtilerinin ortaya çıktığı tespit edilmiştir.

Tablo 4. Sıtma olguları sınıflamasının yıllara göre dağılımı

Yıl	Yerli	İmporte	Yurt dışı	Nüks
2000	1	2	-	-
2001	1	10	-	-
2002	1	52	-	-
2003	-	77	-	-
2004	-	26	-	-
2005	-	9	1	-
2006	-	3	-	-
2007	-	-	-	-
2008	-	1	-	-
Toplam	3 (%1.63)	180 (%97.82)	1 (%0.54)	184

Sıtma olguları en az 0-11 aylık yaş grubunda (%0.78), en fazla 15-24 yaş grubunda (%24.88) ve 24-44 yaş (%24.29) gruplarında görülmektedir. 0-11 aylık bebeklerde ve 1-4 yaş arası çocuklarda sıtmanın az oranda görülmesi, tarım işçilerinin çocuklarını sıtma hastalığından korumak için yanlarında götürmedikleri veya götürülenlerin çadırlar içerisinde kurulan cibinliklerin altında sivrisinekten korundukları tespit edildi.

SONUÇ

Çalışma sonunda Adıyaman ilinde 2008 yılından beri herhangi bir sıtma olgusuna rastlanmamıştır. Fakat Adıyaman'a 110 km uzaklıkta olan Şanlıurfa'da GAP'ın tam olarak sulamaya açılmasıyla birlikte sıtmada meydana gelebilecek salgın, Adıyaman'da yaşayan insanları doğrudan etkileyecektir. Sunulan sağlık hizmetlerindeki olası bir yetersizlik, yeni salgınlara sebep olabileceği kanısındayız.

Teşekkür

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Çıkar Çatışması

Yazarlar herhangi bir çıkar çatışması bildirmemişlerdir.

Tablo 5. Sıtma olgularının il, ilçe ve köylere göre dağılımı

İl	2000	2001	2002	2003	2004	2005	2006	2007	2008	Sayı
Adıyaman/Merkez	1		29	19	3	4				56
İlçe										
Kahta/Merkez			10	25	8	2	3			48
Erikli	2	11								13
Köseler			1							1
Doluca			1							1
Akdoğan			3							3
Narince			1							1
Alidam			1							1
Bağbaşı			2							2
Damlacık			1							1
Lilan			1							1
Çataltepe				5						5
Çaltılı				4						4
Girne				1						1
Dumlu				1						1
Bostanlı				5						5
Kılıç				2						2
Güzelçay				1						1
Geldibuldu				1						1
Teğmenli				1						1
Taşlıca				2						2
Kılıç köyü					2					2
Ziyaret köyü					2					2
Briman						1				1
Gölgeli						3				3
Şehbaba									1	1
Samsat/Merkez				4						4
Yarımbağ			1							1
Örentaş			1							1
Uzuntepe			1							1
Taşköyü				1						1
Sincik/Çamdere				1	2					3
Dilektepe				4	9					13
Toplam	3	11	53	77	26	10	3		1	184

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Ürolojik Kanserli Hastalarda *Demodex folliculorum* Araştırılması

Investigating *Demodex folliculorum* in Patients with Urological Cancer

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ÖZET

Amaç: Bu çalışmada, ürolojik kanserli hastalarda *Demodex folliculorum* sıklığının belirlenmesi amaçlandı.
Yöntemler: Çalışmaya Mustafa Kemal Üniversitesi Tıp Fakültesi Hastanesi Üroloji Kliniği'ne Mart 2011-Nisan 2012 tarihleri arasında başvuran 49 ürolojik kanserli hasta ve kontrol grubu olarak yaş ve cinsiyeti uyumlu 31 sağlıklı birey dahil edildi. Hastaların demografik özellikleri kaydedildi. Bireylerin perine bölgesinden standart yüzeyel deri biyopsisi yöntemiyle örnek alındı ve mikroskobik olarak incelendi. Her bir cm^2'de 5 ve daha fazla *D. folliculorum* görülmesi pozitif olarak değerlendirildi.
Bulgular: Çalışmaya dahil edilen hastaların yaş ortalaması 60.2±18.6 olarak belirlendi. Kanser hastalarının 11'inde (%22.4), kontrol grubunun ise 1'inde (%3.2) *D. folliculorum* pozitif olarak değerlendirildi. Kanser tanısı olan grupta *D. folliculorum* sıklığının kontrol grubuna kıyasla anlamlı oranda yüksek olduğu saptandı. Kanser grupları arasında *D. folliculorum* saptanma yönünden istatistiksel olarak anlamlı bir farklılık bulunmadı. Kanserli grupta *D. folliculorum* pozitif olan hastaların yaş ortalamasının negatif olanlardan anlamlı oranda yüksek olduğu belirlendi.
Sonuç: Kanser gibi immünsüpresyon durumlarında *D. folliculorum* görülme oranının artabileceği hatırda tutulmalıdır.
(Turkiye Parazitol Derg 2012; 36: 208-10)

Anahtar Sözcükler: *Demodex folliculorum*, kanser, immünsüpresyon, Hatay

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ABSTRACT

Objective: In this study, it was aimed to determine frequency of *Demodex folliculorum* infestation in patients with urological cancers.
Methods: This study evaluated 49 patients with urological cancers; 31 sex-matched healthy individuals as a control group were included in the study between March 2011 and April 2012 at the Hospital of Mustafa Kemal University, School of Medicine, Urology Clinic. The demographic characteristics of the patients were recorded. Samples from the perineal region of the subjects were taken by standard method of superficial skin biopsy and evaluated by microscopy. Presence of five or more *Demodex* sp. in a cm^2 was considered as positive.
Results: Mean age was found to be 60.2±18.6 years. *D. folliculorum* was found to be positive in 11 (22.4%) of the patients with cancer and in 1 (3.2%) of the subjects in the control group. It was found that *D. folliculorum* frequency was significantly higher in the cancer group compared to the control group. No significant difference was found among the cancer groups in terms of *D. folliculorum* detection. In the cancer group, mean age was significantly higher in *D. folliculorum* positive patients than negative ones.
Conclusion: It should be kept in mind that *D. folliculorum* incidence may increase in immunosuppressive states, such as cancer.
(Turkiye Parazitol Derg 2012; 36: 208-10)

Key Words: *Demodex folliculorum*, cancer, immunosuppression, Hatay

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GİRİŞ

Demodex sp. insanların genellikle yüz bölgesinde yerleşim gösteren kalıcı bir ektoparazittir. Patogenezi tam olarak bilinmeyen bu parazitin *Demodex folliculorum* ve *Demodex brevis* olmak üzere iki türü insanlarda enfestasyona yol açmaktadır. Bu parazitin bir bireyden diğerine yakın temas ile bulaştığı bildirilmektedir (1-4).

Patojenitesi tartışmalı olmakla beraber herhangi bir sebeple immün sistemin baskılandığı durumlarda, immunsupressif ilaç kullanımında ya da immünitenin zayıfladığı ileri yaşlarda ağır enfeksiyona sebep olabileceği bildirilmiştir (3, 4).

Vücutta yanak, çene, alın, boyun, dış kulak yolu, sırt, kalça, göğüs, meme ucu ve genital bölgelerdeki kıl foliküllerine ve derideki yağ bezlerine yerleştiği bildirilmektedir. Bu parazitin tanısında deri kazıntısı, selofan-bant yöntemi, punch biyopsi ve standart yüzeyel deri biyopsisi (SYDB) gibi yöntemler kullanılmaktadır (2-4). Forton ve Seys SYDB yönteminin *D. folliculorum'* un bulunduğu derinin korneum tabakasının yüzeyel kısmı ile birlikte folikül içeriği tamamen alınabildiğinden ve parazit yoğunluğunun ölçümü daha kolay olduğundan etkili bir yöntem olduğunu rapor etmişlerdir (5).

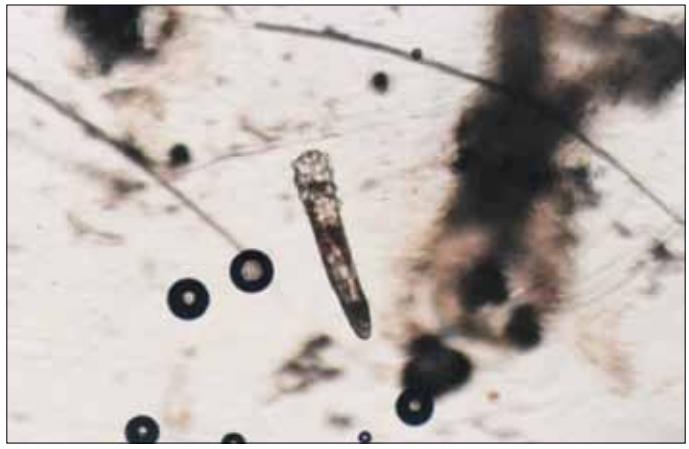
Bu çalışmada amacımız ürolojik kanser tanısı almış hastaların perine bölgesinde SYDB yöntemiyle *Demodex sp.* varlığını araştırmaktır.

YÖNTEMLER

Bu çalışmaya Mustafa Kemal Üniversitesi Tıp Fakültesi Üroloji Kliniği'ne başvuran ve herhangi bir ürolojik kanser tanısı almış 49 erkek hasta ile yaş ve cinsiyeti uyumlu 31 sağlıklı birey dahil edildi. Hastaların demografik özellikleri kaydedildi. Çalışma için Gaziantep Üniversitesi Tıp Fakültesi Yerel Etik Kurulundan onay alındı. Çalışma öncesi araştırılan parazit ve örnek alma yöntemi hakkında tüm bireyler bilgilendirildi. *Demodex sp.* aranması için perine bölgesinden cyanoacrilat içeren yapıştırıcı ile SYDB yöntemiyle örnekler alındı. Alınan örnekler Hoyer eriyiği ile tespit edilerek ışık mikroskobunda x40 ve x100 büyütmelerde Parazitoloji uzmanı tarafından incelenerek cm^{2}'deki *Demodex sp.* yoğunluğuna bakıldı. Tanıda cm^{2}'de 5 ve daha fazla *Demodex sp.* görülmesi pozitif olarak değerlendirildi (Şekil 1). Verilerin istatistiksel analizinde yaşlar için student t-test, diğer parametreler için ki-kare testi kullanıldı ve $p<0.05$ olan değerler anlamlı kabul edildi.

BULGULAR

Çalışmada kanser tanısı olan hastaların yaş ortalaması 60.2±18.6 (38-84 yıl) olarak bulundu. Hastaların 30'unun (%61.2) prostat, 16'sının (%32.7) mesane, 2'sinin (%4.1) böbrek ve 1'inin (%2.0) testis kanseri tanısı aldığı belirlendi. Alınan örneklerin mikroskobik incelemesinde kanserli hastaların 11'inde (%22.4), kontrol grubunun ise 1'inde (%3.2) *D. folliculorum* pozitif olarak değerlendirildi. *D. folliculorum* saptanma oranının kanserli grupta kontrol grubuna kıyasla anlamlı oranda yüksek olduğu bulundu ($p<0.019$). Hastaların tanıları ile *D. folliculorum* görülme sıklığı arasında istatistiksel olarak farklılık bulunamadı ($p\geq0.05$). Hastaların tanılarına göre *D. folliculorum* saptanma oranı Tablo 1'de gösterilmiştir. *D. folliculorum* pozitif olan hastaların yaş ortalaması 76.8±9.5, negatif olanların 55.4±17.8 olarak bulundu. İstatistiksel değerlendirmede pozitif olan grubun yaş ortalamasının negatif olan gruba kıyasla anlamlı oranda yüksek olduğu belirlendi ($p\leq0.001$).



Şekil 1. Erişkin *Demodex folliculorum* (orijinal büyütme x40)

Tablo 1. Hastaların tanılarına göre *Demodex folliculorum* görülme sıklığı

Kanserler	Demodex folliculorum pozitifliği n (%)
Prostat (n=30)	10 (33.3)
Mesane (n=16)	1 (6.3)
Böbrek (n=2)	0 (0)
Testis (n=1)	0 (0)
Toplam (n=49)	11 (22.4)

TARTIŞMA

Demodex cinsi parazit ilk olarak 1841 yılında Henle ve Berger tarafından bildirilmiş olup, 1982 yılında Simon pilosebase bezlere yerleştiğini saptayarak tür özelliklerini tanımlamıştır (1, 5). Ülkemizde ise Demodex konusundaki ilk olgu Saygı ve arkadaşları (6) tarafından perianal bölgeden selofan bant yöntemi ile alınan örneğin mikroskobik incelenmesi sonucu bildirilmiştir.

Normal şartlarda, foliküllerdeki akar popülasyonunun artışını kontrol eden mekanizmalar mevcuttur. Fakat bazı lokal ve sistemik faktörler onların proliferasyonlarını arttırabilir (7, 8). Literatürde Demodex görülme sıklığının yaşla birlikte arttığı belirtilmektedir (9, 10). Aycan ve ark. (11) çeşitli dermatolojik şikayeti olan hastalarda SYDB ile yüzden alınan örneklerin incelenmesinde 20 yaş üstündekilerde altındakilere kıyasla Demodex pozitifliği açısından anlamlı farklılık bulduklarını bildirmişlerdir. Bizim çalışmamızda ise bu bilgilerle uyumlu olarak *D. folliculorum* pozitif bulunan hastaların yaş ortalamasının negatif bulunanlara kıyasla anlamlı olarak daha yüksek olduğu bulunmuştur.

Demodex görülme sıklığını etkileyen bir başka faktör konağın immün durumudur. AIDS ve malignensiler gibi immün disfonksiyona yol açan durumların normalde kommensal yaşayan akarların proliferasyonuna sebep olabileceği bildirilmektedir (7, 8, 12). Bizim çalışmamızda da immünsüpresif hasta grubu olan kanser hastalarına odaklanılmıştır. Özçelik ve ark. (4), immün sistemi baskılanmış kronik böbrek yetmezliği olan kişilerin %12,76'sının

kirpik folikülünde, %25.53'ünün ise yüzünde *D. folliculorum* görüldüğünü ve bu oranların kontrol grubuna göre daha yüksek olduğunu bildimişlerdir. Karıncaoglu ve ark. (13) çalışmasında da benzer şekilde son dönem kronik böbrek yetmezliği olan hastalarda *D. folliculorum* insidansının kontrol grubuna göre arttığı belirtilmiştir. Yagdiran-Düzgün ve ark. (14) 87 hemodiyaliz hastasının %19.5'inde *D. folliculorum* saptadıklarını, bu oranın kontrol grubuna göre daha yüksek olmakla beraber aralarında anlamlı bir farklılık olmadığını saptamışlardır. Bizim çalışmamızda ise yukardaki çalışmalara benzer olarak kanserli hastalarda *D. folliculorum* görülme sıklığı kontrol grubuna göre anlamlı oranda yüksek bulunmuştur.

SONUÇ

İmmünsüpresyon derideki *D. folliculorum* yoğunluğunu etkileyebilir. Kanserli hastalarda *D. folliculorum* görülme sıklığının artabileceği hatırda tutulmalıdır.

Çıkar Çatışması

Yazarlar herhangi bir çıkar çatışması bildirmemişlerdir.

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Bağırsak Parazitlerinin Tanısında Direkt Mikroskobik İncelemedeki Bireysel Farklılıkların Karşılaştırılması

Comparison of Individual Differences in the Direct Microscopic Examination in the Diagnosis of Intestinal Parasites

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ÖZET

Amaç: Bağırsak parazitlerinin tanısında direkt mikroskobik incelemenin ve farklı mikroskobistlerden elde edilen sonuçlar arasındaki farklılığın öneminin vurgulanması amaçlanmıştır.
Yöntemler: Diyareli 225 çocuktan gaita örnekleri toplandı, makroskobik inceleme sonrası formalin-eter çöktürme yöntemiyle hazırlandı ve birbirinden bağımsız 3 farklı araştırmacı (parazitoloji uzmanı, mikrobiyoloji uzmanı ve ikinci yılını tamamlamış bir mikrobiyoloji araştırma görevlisi) tarafından mikroskobik incelemeye alındı. Ayrıca tüm örnekler modifiye Ehrlich Ziehl Neelsen yöntemiyle boyanarak *Cryptosporidium* sp. ve *Cyclospora* sp. açısından değerlendirildi.
Bulgular: Örneklerin 161'i her üç araştırmacı tarafından negatif olarak değerlendirildi. En az bir araştırmacı tarafından 64 (%28) örnekte parazit varlığı saptandı; 30 *Cryptosporidium parvum, 16 Blastocytis hominis, 5 Endolimax nana, 4 Giardia intestinalis, 3 Dientamoeba fragilis, 3 Ascaris lumbricoides, 2 Entamoeba histolytica/dispar, 1 Cyclospora cayetanensis.* Bunların 21'i (%33) için üç araştırmacı arasında uyum gözlenirken, lökosit ve/veya herhangi bir parazit varlığı açısından 64 örneğin 58'i (%91) için uyum saptandı. Etkenleri tür seviyesinde tanımlama açısından parazitoloji uzmanının sonuçlarında belirgin farklılıklar gözlendi.
Sonuç: Direkt mikroskobik inceleme ile değerlendiricinin deneyim ve eğitim düzeyine göre farklı sonuçlar elde edilebilir. Bu nedenle böyle testlerin yeterli eğitim ve tecrübeye sahip kişiler tarafından yapılması ve mümkünse farklı en az iki yöntemle kombine kullanılması gerektiğini düşünmekteyiz. *(Turkiye Parazitol Derg 2012; 36: 211-4)*

Anahtar Sözcükler: Direkt mikroskobik inceleme, bireysel farklılık, deneyim, bağırsak parazitleri

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ABSTRACT

Objective: The aim is to emphasise the importance for intestinal parasites' diagnosis by direct microscopic examination and the discrepancies among results from different microscopists.
Methods: Stool specimens were obtained from 225 children with diarrhoea after the macroscopic examination, prepared by formaline-ether sedimentation methods and included in microscopically examination by three different independent investigators (parasitologist, microbiologist, research assistant). Furthermore, specimens were stained with the modified Ehrlich Ziehl Neelsen method and evaluated for *Cryptosporidium* and *Cyclospora*.
Results: A total 161 specimens were evaluated as negative by all investigators. The number of specimens containing parasites detected by at least one investigator was *64; Cryptosporidium parvum 30, Blastocytis hominis 16, Endolimax nana 5, Giardia intestinalis 4, Dientamoeba fragilis 3, Ascaris lumbricoides 3, Entamoeba histolytica/dispar 2, Cyclospora cayetanensis 1*. The concordance among investigators was observed for 21 (33%) specimens; when specimens were evaluated for the presence of leukocytes and/or parasites, concordance was detected for 58 (91%) of the 64 specimens. In particular, significant differences were observed for the species level identification.

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Conclusion: Different results can be obtained by microscopic examination according to the experience and educational level of microscopists. Therefore, we think that these tests should be performed by persons who have sufficient education and experience, if possible, combined with at least two different methods. *(Turkiye Parazitol Derg 2012; 36: 211-4)*

Key Words: Direct microscopic examination, individual differences, experience, intestinal parasites

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GİRİŞ

Bağırsak parazitlerinin oluşturduğu enfeksiyonlar gelişmekte olan ülkelerde hala önemli bir sağlık sorunudur ve bu enfeksiyonların uygun tedavisi ancak doğru ve zamanında tanı ile mümkün olabilir. Özellikle endemik olmayan bölgelerdeki paraziter enfeksiyonların tanısı oldukça zordur. Enfeksiyonların nonspesifik seyri klinik tanı için genellikle yetersizdir, rutin laboratuvar testleri de nadiren yardımcı olabilir (1). Paraziter hastalıkların tanısı için tanımlanmış birçok testin duyarlılık ve özgüllükle ilgili sorunları bulunmaktadır. Bu nedenle en yaygın kullanılan tanı yöntemi hala direkt mikroskobik incelemedir.

Direkt mikroskobik inceleme yöntemi, hem kısa sürede sonuçlanması hem de uygulama kolaylığı nedeniyle hemen her parazitoloji laboratuvarında uygulanmaktadır. Bu yöntemle, protozoonların kist ve trofozoitlerinin tanımlanmasının yanı sıra, dışkıda bulunabilecek olan eritrosit ve lökosit varlığını da gözleme olanağı sağlamaktadır. Yumurta, trofozoit ya da kistlerin gaitada aralıklı olarak bulunması ya da sayının değişkenlik göstermesi nedeniyle, farklı günlerde alınmış üç ayrı örneğin değerlendirilmesi de direkt incelemenin başarısını artırmaktadır (2). Bununla birlikte şekilli dışkı örneklerinde parazitlerin trofozoit formlarının bulunması beklenmediğinden, konsantrasyon yöntemleri ile az sayıda bulunan protozoon kistleri ve helmint yumurtalarının yoğunlaştırılması tanımlama şansını arttırmaktadır (3).

Diğer klinik laboratuvarların aksine, klinik parazitoloji laboratuvarlarında bireysel değişiklikler ve kişisel değerlendirmeleri de içeren birçok manuel test kullanılmaktadır. Bu nedenle sonuçların doğruluğunun güvenilirliğini sağlamak için kullanılacak kalite kontrol sistemleri büyük önem taşımaktadır. Ancak böyle sistemleri oluşturmak son derece zor ve pahalı olabilir. Ayrıca test prosedürleri ve uygulayıcılar arasındaki farklılıklar nedeniyle bu tür sistemlerin değerlendirilmesinde de zorluklar yaşanmaktadır (4, 5). Modern tanı laboratuvarlarının tek sorunu bağırsak parazitlerinin rutin tanısında kullanılan yöntemin mikroskobik incelenmesi değil, aynı zamanda bu testin doğruluğunun da yeterince değerlendirilememesidir (6). Geleneksel tanı yöntemlerinde deneyimli personel ve zaman önemli bir faktördür. İyi bir değerlendirme için en az üç örneğe ihtiyaç duyulması, dışkıların zaman geçirmeden incelenmesi ve mutlak kalıcı dışkı boyama yöntemleri uygulanmasının gerekliliği de test performansı açısından önemlidir. Örneğin mukuslu dışkılar trofozoitin hareketlerini engelleyebildiğinden trofozoit formları rahatlıkla gözden kaçabilmektedir (7-10).

Çalışmamızda; bağırsak parazitlerinin rutin tanısında direkt mikroskobik incelemenin ve bu incelemeyi yapan mikroskobistin yeterliliğinin önemine değinilmeye çalışılmıştır.

YÖNTEMLER

Çalışmada, orta-düşük sosyoekonomik gelir seviyesine sahip semtlerde yaşayan, Eskişehir Kadın Doğum ve Çocuk Hastalıkları Hastanesi acil servisine; ateş, kusma ve diyare şikayetleri ile başvuran, 0-18 yaş çocuklara ait gaita örnekleri toplanmıştır. Temiz kapaklı kaplarda Eskişehir Osmangazi Üniversitesi Tıp Fakültesi Parazitoloji laboratuvarına getirilen örnekler, öncelikle şekilli, şekilsiz, kan ve mukus varlığı yönünden makroskobik olarak değerlendirilmiştir. Tamamı sulu ve/veya mukuslu yapıya sahip olan dışkı örnekleri ılık serum fizyolojikle seyreltilerek direkt olarak incelemeye alındıktan sonra, tüm örnekler formalin-eter çöktürme yöntemiyle hazırlanmıştır (11). Çöküntüden temiz bir lam üzerinde hem lugollü hem de serum fizyolojikli praparatları hazırlandıktan sonra, önemli bir diyare etkeni olan *Cryptosporidium spp*. ve *Cyclospora spp*. varlığı açısından değerlendirmek amacıyla da, modifiye Ehrlich Ziehl Neelsen yöntemiyle boyalı preparatlar hazırlanmıştır. Örnekler bu yöntemle boyanmadan önce bir kez serum fizyolojikle yıkanarak formalinden arındırılmıştır (11). Tüm örnekler; bir parazitoloji uzmanı, bir mikrobiyoloji uzmanı ve ikinci yılını tamamlamış bir tıbbi mikrobiyoloji araştırma görevlisinden oluşan, birbirinden bağımsız (kör) 3 farklı araştırmacı tarafından mikroskobik incelemeye alınmıştır.

BULGULAR

Toplam 225 gaita örneği çalışmaya alınmıştır. Örneklerin hiçbirinde kan varlığı tanımlanmazken, 123 örnekte mukus varlığı saptanmıştır. Hem lugollü direkt bakıda, hem de ARB boyalı preparatların mikroskobik incelenmesinde örneklerin 161'i her üç araştırmacı tarafından parazit varlığı açısından negatif olarak değerlendirilmiştir. Gaita örneklerinin direkt mikroskobik incelenmesinde; 28 örnekte araştırmacıların en az biri tarafından lökosit varlığı rapor edilmiştir. Lökosit varlığını tanımlama açısından üç araştırmacı arasındaki benzerlik, %67 (19 örnek) olarak değerlendirilirken, her alanda en az 8-10 lökosit bulunan 21 örnek dikkate alındığında uyum oranı %90 (19 örnek) bulunmuştur. Parazit varlığını saptayabilme açısından, mikrobiyoloji uzmanı ve araştırma görevlisi tarafından rapor edilen sonuçlar birbiriyle benzerlik gösterirken, özellikle etkenleri tür bazında tanımlama ve helmint yumurtalarını tanıma açısından parazitoloji uzmanının sonuçlarında belirgin farklılıklar saptanmıştır (Tablo 1). Herhangi bir araştırmacı tarafından parazit saptanan 64 örnekten sadece 21'i (%33) için üç araştırmacı arasında uyum tespit edilirken, lökosit ve/veya herhangi bir parazit varlığı açısından değerlendirildiğinde, 64 örneğin 58'i (%91) uyumlu bulunmuştur. Araştırmacılardan en az biri tarafından parazit saptanan 64 örneğin 50'si ve lökosit saptanan örneklerin tümünde mukus varlığı tespit edilmiştir. *A. lumbricoides*, *E.nana*, *D. fragilis* ve *C.cayetanensis* tanısı sadece parazitoloji uzmanı tarafından konulmuştur (Tablo1).

TARTIŞMA

Tanısal klinik mikrobiyoloji esas olarak etken patojenin kültürle izolasyonuna dayanmakla birlikte, paraziter hastalıkların tanısı hemen her zaman onların klinik örnekteki morfolojik görünümle-

Tablo 1. Parazit varlığı açısından sonuçlar

Araştırmacılar	*Blastocystis hominis*	*Cryptosporidium parvum*	*Cyclospora cayetanensis*	*Giardia intestinalis*	*Entamoeba spp.*	*Endolimax nana*	*Ascaris lumbricoides*	*Dientamoeba fragilis*	*Toplam*
1	10	24	0	2	1	0	0	0	37
2	11	23	0	2	1	0	0	0	37
3	12	20	1	4	1	5	3	3	49
Toplam[a]	16	30	1	4	2	5	3	3	64
Toplam[b]	6	13	0	1	0	0	0	0	21

1: tıbbi mikrobiyoloji araştırma görevlisi, 2: tıbbi mikrobiyoloji uzmanı, 3: parazitoloji uzmanı
[a]: en az bir araştırmacı tarafından pozitif bulunan örneklerin toplam sayısı
[b]: araştırmacıların tümü tarafından pozitif bulunan örneklerin toplam sayısı

rine göre yapılabilmektedir. İnsanlarda bulunan bağırsak parazitlerinin tanısı temel olarak dışkı, daha seyrek olarak da duodenal sıvı ve biyopsi örneklerinde parazitin çeşitli formlarının saptanmasına dayanmakta ve kullanılan direkt veya boyalı dışkı mikroskobisinin birçok avantajı bulunmaktadır (7, 9, 11). Dışkıda parazit incelemesi, özellikle örnekler uygun şekilde hazırlandığı ve yeterli sayıda örnek incelendiğinde oldukça duyarlı bir yöntem olup, tanıyı kesinleştirir ve altın standarttır. Işık mikroskobu ve bazı ucuz malzemelerin olduğu herhangi bir laboratuvarda yapılabilecek kadar basittir ve oldukça ekonomiktir. Ancak ucuz ve hızlı olan klasik dışkı bakısının bir takım dezavantajları da bulunmaktadır. Bunlar arasında en önemlisi yetişmiş insan gücüne duyulan gereksinimdir. Gerçekten bu yöntemler oldukça basit olarak görünmelerine ve kolay uygulanabilir olmalarına karşın, doğru tanı koyabilecek deneyimli mikroskopistleri yetiştirmek oldukça zor ve maliyetlidir. Bu yöntemlerin bir diğer dezavantajı da elde edilen sonuçların genellikle tekrarlanabilir, izlenebilir ve objektif olmamasıdır (9, 10). Bağırsak parazitlerinin aralıklı atılması nedeniyle tek bir dışkı örneği ile tanı koymak her zaman mümkün olamamaktadır. En az üç dışkı örneğinin incelenmesiyle bile *Giardia intestinalis* için %11.3, *Entamoeba histolytica* için %22.7'lik bir tanı düzeyine erişilebileceği bildirilmiştir (10). Bu durum ise hastaların ve/veya örneklerinin üç kez laboratuara ulaştırılmasını gerektirmektedir. Ayrıca son yıllarda giderek önem kazanan ve immün düşkün hastalarda ölümcül seyredebilen *Cryptosporidium* ve *Cyclospora* gibi etkenlerin laboratuvarda mikroskobi ile tanısını koymak tecrübe gerektiren bir işlemdir. Özellikle dışkıda az sayıda ookist bulunduğunda tanı koymak zordur. Rutin dışkı incelemelerinde *Cryptosporidium* sp. aranması önerilmemekle birlikte, diyareli hastalarda etkenin araştırılması pek çok araştırıcı tarafından tavsiye edilmektedir. Etkenin mikroskobik tanısında ARB boyalara gereksinim duyulmakta, bu yöntemle bile dışkıda bulunan bazı mayalar ve artefaktlar yanılmalara neden olabilmektedir (7-9). Son yıllarda klasik dışkı bakısı önemli ölçüde ihmal edilmektedir. Parija ve arkadaşları yaptıkları çalışmalarında bu ihmali üç önemli nedene bağlamışlardır. Bunlar; dışkının hazırlanması sırasında teknisyenin motivasyon eksikliği, eğitiminin yetersizliği ve dışkı hazırlamadaki zorluklar ile uzmanların yeterince eğitilememesinden kaynaklanmaktadır. Ayrıca bazı klinisyenlerin dışkı mikroskopisi sonuçlarına önem vermemesi de bu konunun diğer bir boyutudur (9). Son yıllarda bağırsak parazitlerinin tanısında; Enzim Immun Assay (EIA, ELISA), Polimeraz Zincir Reaksiyonu (PZR) gibi mikroskobiye ihtiyaç duyulmayan yöntemlerin kullanımının artması da dışkı mikroskopisinin ihmaline yol açan sebepler arasındadır (7-10).

Tanı amacıyla birden fazla yöntemin kullanılmasının sonuçların güvenilirliğini arttırdığı bilinmektedir, ancak intestinal sistemin paraziter enfeksiyonlarının tanısında yaygın olarak tek başına kullanılan yöntem, klinik örneğin direkt mikroskobik incelenmesidir. Bununla birlikte deneyimli bir mikrobiyolog bile ideal şartlarda toplanıp hazırlanmış dışkı örneklerinde trofozoid ya da kistlerin tanımlanmasında zorluklar yaşayabilmektedir. Biz de şimdiki çalışmamızda mikroskobik incelemenin sübjektifliğini ve tanıda birden fazla yöntemin gerekliliğini vurgulamaya çalıştık. Mikroskobik inceleme ile parazitlerin varlığını saptayabilme ve özellikle de helmintleri tanıma ve tanımlayabilme açısından üç araştırmacı arasında önemli farklılıklar saptanmıştır. Burada ilimizde helmint enfeksiyonlarına oldukça az rastlanmasının da önemi büyüktür. Bununla birlikte normal mikroskobik bulguların gözlenmediği örneklerin saptanması açısından araştırmacıların birbiriyle uyumu oldukça iyi bulunmuştur. Sonuç olarak her üç araştırmacı da normal mikroskobik özelliklere sahip olmayan örnekleri büyük oranda tespit edebilmiş olmakla birlikte, örnekteki gerçek sorunu tanımlayabilme konusunda belirgin farklılıklar ortaya çıkmıştır.

Gaitanın mikroskobik incelenmesi ile elde edilen sonuçların kullanılan yönteme, örneğin kıvamına, kan ve mukus içerip içermediğine, laboratuvar çalışanlarının niteliklerine göre farklılıklar gösterdiği bildirilmektedir (2, 4, 6). Gerçekten bağırsak parazitlerinin doğru tanı ve tedavisinde, gaita örneklerinin doğru şekilde toplanması, uygun şekilde ve sürede taşınması, saklanması, örneklerin yeterli miktarda olması gibi laboratuvar öncesi uygulamaların yanı sıra, laboratuvara ulaşan örneklerin doğru yöntemlerle işleme alınması, deneyimli uzmanlar tarafından değerlendirilmesi ve doğru şekilde raporlanması da son derece önemlidir (8, 9).

Çalışmamız, aynı yöntemlerle muamele edilmiş gaita örneklerinin 3 farklı eğitim ve deneyim düzeyine sahip araştırmacı tarafından değerlendirilmesi ile ortaya çıkabilecek raporlama farklılıklarını göstermesi bakımından önemlidir. Bu çalışma en az 2 yıl klinik mikrobiyoloji eğitimi almış tıbbi mikrobiyoloji araştırma görevlisi tarafından ve günlük en az 15 gaita mikroskobisi potansiyeline sahip bir parazitoloji laboratuvarında gerçekleştirilmiştir. Ülkemizde bu potansiyele ve/veya eğitim düzeyine sahip olmayan laboratuvarlarda da "gaitanın mikroskobik incelemesi" hizmetinin verildiği ve bu laboratuvarların sonuçlarına göre hastalık tanısı ve tedavilerinin yapıldığı unutulmamalıdır.

SONUÇ

Direkt mikroskobik inceleme sübjektif bir yöntemdir ve tek başına kullanıldığında değerlendiricinin deneyim ve eğitim düzeyine göre farklı sonuçlar gözlenebilmektedir. Bu nedenle böyle testlerin yeterli eğitim ve tecrübeye sahip kişiler tarafından yapılması, mümkünse diğer yöntemlerle kombine kullanılması ve farklı günlerde alınmış 3 farklı örneğin değerlendirilmesi gerektiğini düşünmekteyiz.

Çıkar Çatışması

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Larval Hook Length Measurement for Differentiating G1 and G6 Genotypes of *Echinococcus granulosus* Sensu Lato

Larval Çengellerinin Uzunluğunun Ölçülmesi ile *Echinococcus granulosus* Sensu Lato G1 ve G6 Genotiplerinin Ayrılması

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ABSTRACT

Objective: *Echinococcus granulosus* is a globally important cestode parasite causing remarkable medical and economical losses in the world. Ten genotypes (G1-G10) have been identified within this complex species. Protoscoleces rostellar hook characters e.g. total large and small hook lengths may be useful to differentiate genotypes. This study investigates the value of rostellar hook morphometry on genetically identified isolates of *E. granulosus* using mitochondrial *cox1* and *nad1* sequencing.
Methods: In total, 24 hydatid cyst samples of livestock and human origin were collected. The isolates were then sequenced for the mitochondrial *cox1* and *nad1* genes and total large and small rostellar hook lengths of protoscoleces were measured.
Results: Total large and small hook lengths could differentiate between G1 and G6 genotypes; however, G1 and G3 were not distinguishable by hook morphometry. Only large hook length was significantly different between the G3 and G6 isolates.
Conclusion: The G6 genotype is readily distinguishable from G1 by using both small and large hook lengths; however, only total large hook length was significantly different between the G3 and G6 genotypes. *(Turkiye Parazitol Derg 2012; 36: 215-8)*

Key Words: Hook morphometry, hydatid disease, Genotype, *cox1*, *nad1*

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ÖZET

Amaç: *Echinococcus granulosus* dünya çapında görülen, büyük ekonomik kayıplara neden olan ve halk sağlığı bakımından tıbbi önemi olan bir parazittir. Bu parazitin suş karakterizasyon yapılmış ve 10 tane genotipi (G1-G10) olduğu tespit edilmiştir. Protoscolexlerin rostellum etrafındaki büyük ve küçük çengellerin uzunluğu *Echinococcus granulosus* genotiplerini ayırt etmekte yararlı olabilir. Bu çalışma rostellum çengellerinin uzunluğunun ölçülmesinin değerini mitokondriyal *cox1* ve *nad1* sıralama kullanarak *Echinococcus granulosus* suşlarının ayrılmasını incelemektedir.
Yöntemler: Hayvan ve insan kaynaklı 24 hidatik kist örnekleri toplanıp ve daha sonra mitokondriyal *cox1* ve *nad1* genler sekanslandı. Protoskolekslerin büyük ve küçük rostellar çengellerinin uzunlukları ölçüldü.
Bulgular: Sonuçlar bunu gösteriyor ki büyük ve küçük çengellerinin uzunluğu G1 ve G6 genotiplerini arasında ayrım yapabilir, halbuki G1 ve G3 genotiplerini birbirinden ayıramamaktadır. Büyük çengellerin uzunluğu G3 ve G6 genotipleri arasında anlamlı olarak fark vardı.
Sonuç: G1 ve G6 genotipleri büyük ve küçük çengellerin uzunluğunu kullanarak birbirinden ayrıldığı; bununla birlikte G3 ve G6 genotiplerinin sadece büyük çengellerinin uzunluğunun birbirinden önemli derecede farklı olduğu belirlendi. *(Turkiye Parazitol Derg 2012; 36: 215-8)*

Anahtar Sözcükler: Çengellerinin morfometri, hidatik kist, genotip, *cox1*, *nad1*

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INTRODUCTION

Echinococcus granulosus, the aetiological agent of cystic echinococcosis (CE), is the smallest tapeworm of the family Taeniidae. CE is one of the most important parasitic zoonoses worldwide. The parasite is mainly transmitted among dogs as the definitive hosts and livestock animals as the intermediate host (1).

Several genetic studies have demonstrated the high intra-specific variability within this species that is collectively known as *Echinococcus granulosus* sensu lato. *E. granulosus* s.l. is comprised of a complex of ten genotypes (G1-G10) of which four genotypes are considered distinct species, i.e. *E. granulosus* sensu stricto (G1-G3), *E. equinus* (G4), *E. ortleppi* (G5), and *E. canadensis* (G6-G10). Different genotypes of the parasite have distinct morphological, epidemiological and transmission dynamics characterisations (2). It is believed that these characterisations have important implications in terms of epidemiology and the control of hydatid disease. However, solid evidence of these implications is still needed (3). Clearly, more large-scale molecular epidemiological studies in different geographical areas are necessary to elucidate the nature and significance of variability in *E. granulosus* s.l. and its implications for public health (2).

Regarding the relatively high cost of DNA sequencing on a large number of *E. granulosus* isolates, morphometric data could be potentially useful for screening large numbers of isolates for sequencing. Large-scale sequence-based studies are expensive and less accessible in many research laboratories in endemic areas, which constitutes a major obstacle for conducting high quality research projects. Larval rostellar hook characters, e.g. total large and small hook length, are potentially good tools for screening large numbers of *E. granulosus* isolates of different genotypes (4, 5). However, the value of morphometry for distinguishing genotypes of *E. granulosus* s.l. compared to DNA-based methods is not clearly shown. Very few studies have investigated the accuracy of hook morphometry on known genetically characterised isolates using DNA sequencing. The aim of this study is to determine the value of rostellar hook morphometry in the identification of different genotypes of *E. granulosus* s.l.

METHODS

Twenty-four hydatid cyst samples were collected from sheep (14), goat (2), cattle (1), camels (6) and humans (1) originating from locations within Kerman Province, south-eastern Iran. All animals were slaughtered in local abattoirs in Kerman and Rafsanjan cities. The human sample was from a female patient who was operated on at the Afzalipour Medical Centre in Kerman. Each individual cyst was considered an *E. granulosus* isolate. Protoscoleces were aspirated from cysts and washed three times with normal saline. After extracting DNA using the High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany), all of the isolates were genotyped by sequencing of two mitochondrial genes, *cox1* and *nad1*, using the primers JB3 and JB4.5 for *cox1* and JB11 and JB12 for *nad1* (6, 7). *E. granulosus* sequence data were deposited in the NCBI GenBank database (Figure 1).

For the morphometric study, protoscoleces were mounted in lactophenol on a glass slide and sufficient pressure was applied using a cover slip to cause the hooks to lie flat. Measurements were made of total hook length on two large and two small hooks per rostellum from each of five protoscoleces for each isolate using 100X magnification of a calibrated microscope (Figure 2, 3). One person (E.H.) carried out all of the measurements (8, 9).

Results of large and small hook data were analysed using SPSS ver. 15. Morphometric differences between the G1, G3 and G6 genotypes were analysed by a two-dimensional scatter plot and

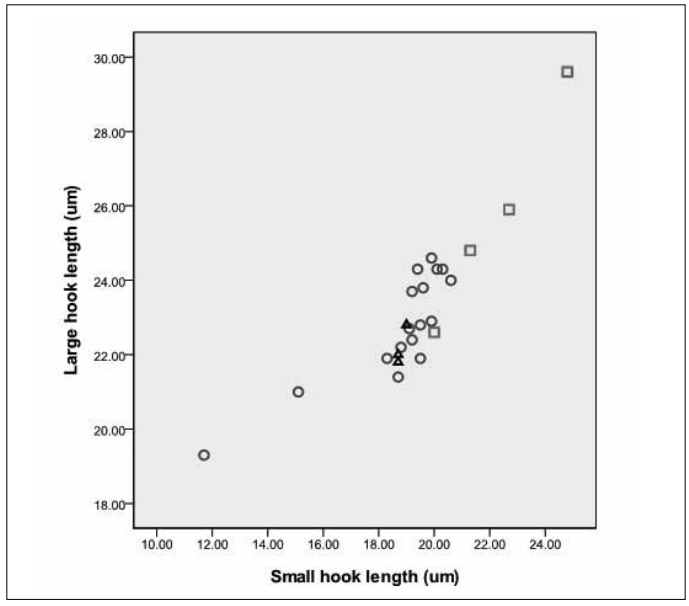

Figure 1. Scatter plot of the total large and small hook lengths according to genotypes for the 24 isolates of *E. granulosus* measured in this study. (○) G1; (Δ) G3; (□) G6. Sequence data of all of the isolates was submitted to NCBI GenBank with the following accession numbers: *cox1*: HM563002, HM563011-14, HM563016-20, and *nad1*: HM563023, HM563025-30, HM563032-33, HM563035-37

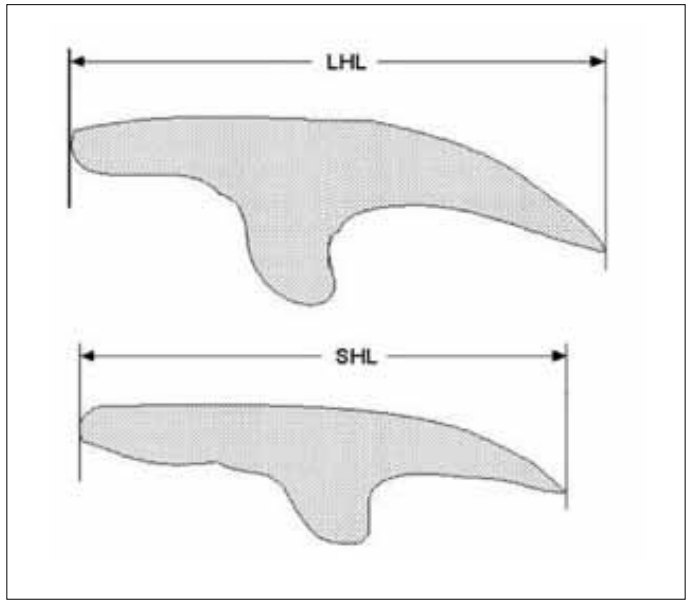

Figure 2. Diagram of rostellar hook measurements used in this study. Large Hook Length (LHL), Small Hook Length (SHL)

Table 1. Statistical analysis of mean differences of larval rostellar hook lengths in different genotypes of *E. granulosus* s.l. isolates

Dependent variable	Genotype (No.)	Mean (SD)	p value*	Group		p value**
Small Hook Length (µm)	G1 (17)	18.75 (2.18)	0.008	G1	G6	0.02
					G3	0.99
	G6 (4)	22.20 (2.05)		G6	G1	0.02
					G3	0.12
	G3 (3)	18.80 (0.17)		G3	G1	0.99
					G6	0.12
Large Hook Length (µm)	G1 (17)	22.79 (1.43)	0.055	G1	G6	0.02
					G3	0.85
	G6 (4)	25.72 (2.92)		G6	G1	0.02
					G3	0.04
	G3 (3)	22.20 (0.53)		G3	G1	0.85
					G6	0.04

*Kruskal-Wallis Test for mean difference of small and large hook length by genotypes
**Multiple comparison tests to determine where differences occur among group means

Kruskal-Wallis test followed by the Bonferroni multiple comparison test to determine where differences occur among group means. A statistical significance of $p<0.05$ was considered significant.

RESULTS

Hook length data of the present study showed that G6 isolates have significantly larger hook lengths than G1 isolates (Figure 3, Table 1); therefore, both genotypes could be differentiated using small and large hook length measurements. However, there was no statistically significant difference in hook dimensions between the G1 and G3 genotypes (Table 1). In hook morphometry, only the large hook length significantly differentiated the G3 and G6 isolates ($p<0.05$). According to our data, the sensitivity and specificity of total hook length for genotype identification was 100% and 75%, respectively.

DISCUSSION

The emergence of DNA-based molecular techniques provided a sensitive and reliable tool to understand the nature of variation within and between species and strains of helminth parasites (10). As a consequence, morphological tools have been undermined in recent years due to the loss of expertise and interest in traditional morphological studies (11). However, it is believed that molecular and morphological characters are complementary in epidemiological studies on cestode zoonoses, like cystic echinococcosis. Morphological and biological studies during the 1970s and 1980s had made major progress towards understanding intra-specific variation in *E. granulosus* leading to the identification of different strains of the parasite (12-15). Later, molecular DNA-based studies confirmed the presence of ten genotypes within this complex species (G1-G10). However, the taxonomic status of *E. granulosus* is not clear and large-scale molecular epidemiological studies in different endemic areas across the globe are obviously needed. Regarding high cost and availability considerations of this kind of studies in endemic areas, protoscoleces hook morphometry could be used as an alternative for strain identification of *E. granulosus* when screening large numbers of isolates. However, the value of rostellar hook characters for genotype identification of the parasite is not clearly shown (16). Among the different rostellar hook characters, the total lengths of small and large hooks were shown to be the most suitable characters for strain identification (4).

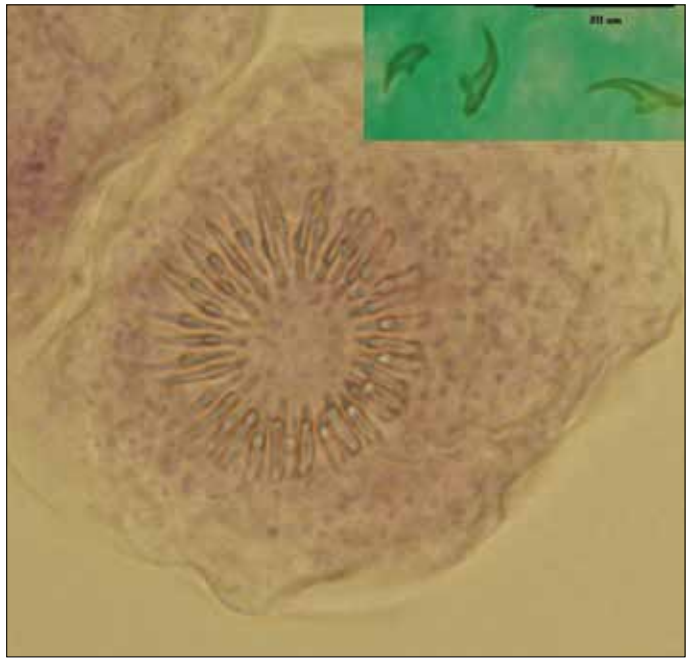

Figure 3. Rostellar hooks of a G6 camel isolate of *Echinococcus granulosus* protoscolex with one small and two large hooks shown in the inlet box. Bar = 50 µm

The results of the present study indicate that the G6 genotype is readily distinguishable from G1 by using both small and large hook lengths; however, only total large hook length was significantly different between G3 and G6 genotypes. Turceková et al. (17) showed that the shape and size of hooks were suitable for discriminating the G1 and G7 genotypes using *nad1* sequence

data as a reference. A study on Mexican pig isolates using PCR-RFLP and larval hook measurements showed that pig (G7), camel (G6) and horse (G4) isolates have significantly larger hooks than sheep (G1) strain isolates (18). The morphometry of rostellar hooks was investigated among human and livestock samples from Iran and the results showed that sheep and camel isolates could be differentiated by hook morphometry, although no molecular data was provided in the study for strain identification (5). Another study on ITS1 region, using PCR-RFLP, showed that sheep and camel strains are morphologically distinguishable by the measurements of the total and blade length of rostellar hooks of protoscoleces. This study showed that all human isolates of the G1 genotype had mean large hook length less than 25 µm, whereas all of the G6 isolates had hooks larger than 25 µm (8). In the present study, one G6 isolate of camel origin, confirmed by DNA sequencing, had a large hook length less than 25 µm in total, indicating that morphometry alone could not be relied on as the sole criterion for genotype identification; however, our human isolate was identified as G6 using both DNA-sequence and rostellar small/large hook measurements.

This study failed to differentiate between the G1 and G3 isolates, indicating remarkable phenotypic similarities between the two genotypes. This confirms recent categorisation of the G1 and G3 genotypes along with G2 (G1-G3 complex) as *E. granulosus* sensu stricto (19, 20). Similarly, morphometric analysis of larval rostellar hooks of *E. granulosus* from Tasmanian (G2) and Australian (G1) host origin showed that these two strains are indistinguishable by hook morphology (9).

Several studies have shown that at least a fraction of hook morphological variation is attributed to host-induced effects (4, 9, 21); however, this has not been analysed in the present study, because of the small number of isolates in each host category. Extensive studies on a larger number of isolates from different intermediate hosts using morphological and molecular tools are recommended.

CONCLUSION

We established the value of rostellar hook morphometry for differentiating genotypes of *E. granulosus* s.l. in Iran. The G6 genotype is readily distinguishable from G1 by using both small and large hook lengths; however, only total large hook length was significantly different between the G3 and G6 genotypes. Hook morphometry could not differentiate between the G1 and G3 isolates, indicating remarkable phenotypic similarities between the two genotypes.

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Conflict of Interest

No conflict of interest was declared by the authors.

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Kocaeli Derince Eğitim ve Araştırma Hastanesi Merkez Laboratuarına 2009-2011 Yılları Arasında Kistik Ekinokokkozis Şüphesiyle Başvuran Olguların Retrospektif Olarak Değerlendirilmesi

Retrospective Evaluation of Patients with Probable Cystic Echinococcosis to the Central Laboratory of the Kocaeli Derince Education and Research Hospital Between 2009 and 2011

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ÖZET

Amaç: *Echinococcus granulosus*'un etken olduğu Kistik ekinokokkozis (KE), dünyada ve ülkemizde yaygınlık gösteren zoonotik bir hastalık olup önemli bir halk sağlığı problemi olma özelliğini korumaktadır. Hastalığın ilimizdeki durumu ile ilgili ön bilgi edinmek için Kocaeli Derince Eğitim ve Araştırma Hastanesi Merkez Laboratuarı'na KE şüphesiyle gönderilen olguların retrospektif olarak değerlendirilmesi amaçlanmıştır.
Yöntemler: Aralık 2009-Mayıs 2011 tarihleri arasında laboratuarımıza başvuran toplam 225 olgunun *Echinococcus granulosus* indirekt hemaglutinasyon testi (IHA) sonuçları retrospektif olarak incelenmiştir. Pozitif olarak saptanan olgular geriye dönük olarak KE açısından klinik olarak değerlendirilmiştir.
Bulgular: Olguların 151'i (%67.11) kadın, 74'ü (%32.8) erkek olup IHA ile 18'inde (%8) pozitif, beş olgu (%2.2) borderline, geriye kalan 202 (%89.8) olguda ise antikor yanıtı belirlenememiştir. Seropozitif ve borderline olarak saptanan 23 olgunun 15'inde radyolojik olarak KE ile uyumlu lezyon belirlenirken, dört olguda tutulum gösterilememiş, dört olgunun radyolojik bilgilerine ise ulaşılamamıştır.
Sonuç: Hastane kayıtlarının KE hastalığında olguların sadece az bir kısmını temsil ettiği düşünüldüğünde ilimizde KE görüldüğü üzere varlığını sürdürdüğü ve önemini koruduğu anlaşılmaktadır. Bu nedenle gerek korunma gerekse hastalığın önlenmesinde gerekli önlemlerin alınması gerektiği görüşüne varılmıştır. *(Turkiye Parazitol Derg 2012; 36: 219-21)*

Anahtar Sözcükler: Kistik Ekinokokkozis, IHA, tanı

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ABSTRACT

Objective: Cystic echinococcosis (CE) is caused by metacestodes of *Echinococcus granulosus*, which is one of the most widespread zoonotic diseases in humans in both developing and developed countries, and also in Turkey. The aim of this retrospective study was to evaluate the situation of hydatid disease in Kocaeli.
Methods: The specific anti-*Echinococcus granulosus* indirect haemagglutination test results of 225 patients, who were referred with probable CE to the Centre Laboratory of the Kocaeli Derince Education and Research Hospital during December 2009-May 2011 was assessed retrospectively. Positive cases were also reassessed clinically.
Results: Of the total, 151 (67.1%) were female and 74 (32.8%) were male. The seropositivity ratio of IHA test was found to be 8% (18 patients), borderline ratio as 2.2% (5 patients), and seronegative ratio as 89.8% (202 patients). In 15 of the 23 seropositive and borderline patients, CE compatible radiological lesions were determined, while 4 of the remaining patients showed no lesion and the other 4 had no radiological data.
Conclusion: Considering that hospital records can represent only a small part of the CE cases, it can be said that CE still subsists and retains its importance in our city. Essential precautions should be taken for the prevention and protection for this disease.
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Key Words: Cystic Echinococcosis, IHA, diagnosis

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GİRİŞ

Kistik ekinokokkozis (KE) yurdumuzda büyük bir kesimin hayvancılıkla uğraşması, korunmada gerekli önlemlerin uygun şekilde alınamaması nedenleriyle gerek insanlarda gerekse sığır koyun gibi hayvanlarda yaygın görülen bir parazit hastalığıdır (1). İnsan kist hidatik hastalığı, insanda görülme oranı, ameliyatın ve tedavinin pahalı olması sebebiyle önemli bir sağlık sorunu oluşturmaktadır (1, 2). KE'in Eski Dünya'nın büyük bir kısmında, özellikle Yunanistan, Kıbrıs, Bulgaristan, Lübnan ve Türkiye'de; diğer bazı Avrupa ülkelerinde ve Afrika'da halen endemik olarak görüldüğü bildirilmektedir (3). Ülkemizde KE yaygın olarak görülmekle birlikte İç Anadolu, Doğu ve Güneydoğu Anadolu bölgelerinde daha sık görüldüğü belirtilmektedir (4, 5). KE'de organ lokalizasyonunun çoğunlukla karaciğer ve akciğer olduğu ve daha az sıklıkla dalak, kalp, beyin kemik ve diğer organlarda yerleşimin görüldüğü bilinmektedir (6).

Bu çalışmada ilimizdeki KE durumunu belirlemek amacıyla Aralık 2009-Mayıs 2011 tarihleri arasında Kocaeli Derince Eğitim ve Araştırma Hastanesi Merkez Laboratuarı'na KE şüphesiyle çeşitli kliniklerden gönderilen olguların İndirekt Hemaglütinasyon (IHA) testi sonuçları retrospektif olarak değerlendirilmiştir.

YÖNTEMLER

Çalışmamızda, Aralık 2009-Mayıs 2011 tarihleri arasında Kocaeli Derince Eğitim ve Araştırma Hastanesi Merkez Laboratuarı'na farklı kliniklerden KE şüphesiyle gönderilen 151'i kadın, 74'ü erkek, 225 hastaya ait serum örnekleri incelenmiştir. IHA testi ticari kit (Hydatidose, Fumouze Laboratoires, France) test prosedürüne uygun olarak çalışılmıştır. Serum sulandırımları U tabanlı mikroplaklarda 1/80, 1/160, 1/320 olmak üzere üç dilüsyon çalışılmış (pozitiflik saptanması halinde 1/80, 1/160, 1/320, 1/640, 1/1280, 1/2560 olmak üzere altı sulandırım yapılmıştır) ve antijenli eritrosit süspansiyonu eklenerek, 2 saatlik inkübasyon sonrası düğme iliği şeklinde çökelti negatif, kenarı tırtıklı, dantela gibi görülmesi pozitif olarak değerlendirilmiştir. Test prosedürüne göre 1/320 ve üzerindeki değerler seropozitif, 1/160 sınır değer (borderline), 1/160'dan küçük değerler ise negatif olarak değerlendirilmiştir. Olguların yaş ve cinsiyete göre dağılımları retrospektif olarak değerlendirilmiştir.

BULGULAR

Yaşları 2 ile 84 yıl arasında değişen, yaş ortalaması: 32,5 olan toplam 225 olgunun IHA ile 18'i (%8) pozitif, beş (%2.2) olgu borderline, 202 (%89.8) olgu ise negatif olarak saptanmıştır. Seropozitif olarak saptanan yaşları 11 ile 60 arasında değişen yaş ortalaması 36.6 olan olguların yedisini kadınlar 11'ini ise erkekler oluşturmuştur. Borderline saptanan yaşları 22 ile 55 arasında değişen yaş ortalaması 36 olan olguların ikisini erkekler oluştururken, üçünü kadınlar oluşturmuştur. Seropozitif 13 olgu ve borderline 2 olgu olmak üzere yaşları 11 ile 60 arasında değişen yaş ortalaması 38.7 olan toplam 15 olguda radyolojik olarak (Batın US, Batın BT, Akciğer Direkt Grafi) KE ile uyumlu lezyon belirlenmiştir. İkiyüz yirmi beş olgudan 108'i Kocaeli, dört olgu Sakarya, bir olgu Bolu, bir olgu Bursa, bir olgu Kars, bir olgunun Van'da ikamet ettiği belirlenirken dokuz olgunun adres bilgilerine ise ulaşılamamıştır.

Seropozitif olarak saptanan 18 olgunun 13'ünde radyolojik olarak KE ile uyumlu lezyon belirlenirken, iki olguda tutulum gösterilememiş, üç olgunun radyolojik bilgilerine ise ulaşılamamıştır. Olguların 11'inde karaciğer tutulumu saptanırken, bir olguda böbrek tutulumu, bir olguda da akciğer tutulumu belirlenmiştir. Borderline olarak belirlenen 5 olgunun ikisinde radyolojik tutulum (karaciğer, dalak) gösterilirken, iki olguda tutulum gösterilememiş, bir olgunun ise bilgilerine ulaşılamamıştır.

Radyolojik tanı alan ve seropozitif ve borderline saptanan 15 olgunun yaş grubu ve cinsiyete göre dağılımları Tablo 1'de verilmiştir.

TARTIŞMA

Ülkemizde verilerin düzenli olarak toplanamaması veya eksik bildirilmesi sebebi ile Sağlık Bakanlığı verilerinin KE hakkında gerçeği tam olarak yansıtmadığı düşünülmektedir. Bu verilere göre 1975-1994 yılları arasında 40.242 olgu bildirilmiştir (1). Yazar ve ark. (7) 2001-2005 yılları arasında Türkiye genelinde yaptıkları çalışmada KE görülme oranını 6.3/100.000, Kocaeli›nde ise 0.84/100.000 olarak belirtmişlerdir. Karaman ve ark.'nın (8) Kars'ta yaptıkları çalışmada %34.6 seropozitiflik oranı saptanırken cinsiyetler arasında anlamlı bir farka rastlanmadığı belirtilmiştir.

Todorov ve ark.'nın (9) 1999 yılında yayınlanan çalışmalarında Bulgaristan'da KE görülme oranı 3,3/100.000 olarak bildirmişlerdir. Türkiye genelinde yapılan çalışmalarda hastalığın kadınlarda daha fazla görüldüğünü bildiren çalışmalar bulunmaktadır. Akar ve ark.'nın (10) KE olgularını değerlendirdikleri çalışmalarında; saptanan 165 olgunun %58'inin kadın, %42'sinin erkek olduğunu; Özekinci ve ark.'nın (11) yaptıkları çalışmada saptanan 234 olgunun %60.25'inin kadın, %39.74'ünün erkek olduğunu bildirmişlerdir. Bununla birlikte enfeksiyonun her iki cinsiyette eşit oranlarda görüldüğünü bildiren çalışmalar da vardır (9). Çalışmamızda saptadığımız 15 olguda kadın erkek oranlarının birbirlerine yakın olduğu görülmüştür, buna göre KE olarak belirlenen olguların yedisini erkekler oluştururken, sekizini kadınların oluşturduğu saptanmıştır.

Değişik çalışmalarda KE olgularına her yaşta rastlanabildiği bildirilmişse de orta yaşlarda yüksek görülme oranı gösterdiği belirtilmiştir (12-14). Çalışmamızda saptanan olguların yaş dağılımı 11 ile 60 arasında değişmekle birlikte olguların %33.33'ünü 20-40 yaş arasındaki olguların oluşturduğu saptanmıştır.

Ertabaklar ve ark.'ı (13), araştırmalarında KE olgularının %66.4'ünde kistin karaciğerde, %21.66'sında akciğerde ve %0.83'ünde dalakta yerleşim gösterdiğini tespit etmişlerdir.

Tablo 1. KE tanısı kesinleşen olguların yaş grubu ve cinsiyetine göre dağılımı

Yaş Grupları	Erkek (%)	Kadın (%)	Toplam (%)
10-20	1 (%6.67)	1 (%6.67)	2 (%13.33)
20-30	2 (%13.33)	2 (%13.33)	4 (%26.66)
31-40	-	1 (%6.67)	1 (%6.67)
41-60	4 (%26.66)	3 (%20)	7 (%47.67)
60-	-	1 (% 6.67)	1 (% 6.67)
	7 (%47.67)	8 (%53.33)	15 (%100)

Ertuğ ve ark.'ı (14), Aydın ve çevresinde yaptıkları çalışmalarında karaciğer lokalizasyonunun %89.3 ile birinci sırada, akciğer lokalizasyonunun ise %7.1 ile ikinci sırada yer aldığını bildirmişlerdir. Delibaş ve ark.'nın (12), yaptıkları çalışmada KE ön tanısıyla operasyon geçiren olgular arasında en sık tutulan organlar sırasıyla karaciğer %70 ve akciğer %11 olarak belirtilmiştir. Bu çalışmalarda olduğu gibi bizim çalışmamızda da, radyolojik tanı alan ve seropozitif ve borderline saptanan olguların %73.33'ünde karaciğer tutulumu en sık olarak belirlenirken, %6.66'sında akciğer, %6.66'sında böbrek, %6.66'sında dalak tutulumu gösterilmiştir.

Kist hidatik tanısı karaciğer enfeksiyonu için ultrason veya bilgisayarlı tomografi (BT) taraması ile yada akciğer enfeksiyonları için göğüs filmi veya BT ile yapılmaktadır. Görüntüleme yöntemleri ile birlikte serolojik yöntemlerin de kullanılmasının tanıda hassasiyeti arttırdığı bilinmektedir (1).

Klinik tanıda KE'in diğer kitleler ile karıştırılması sebebiyle hastanın hikayesinin tanıda öneminin büyük olduğu bildirilmektedir (15). IHA testinin duyarlılık ve özgüllüğünün diğer serolojik testlerle karşılaştırıldığında daha yüksek olduğu belirtilmektedir (16). Sarı ve ark.'nın (17) yaptıkları çalışmada KE olduğu kanıtlanmış olgularda IHA testinin %97.5 özgül, %90 duyarlı, Bilge ve ark.'nın (18) yaptıkları çalışmada IHA testinin %100 özgül, %74.6 duyarlı olarak bildirilmiştir. Serolojik tanıda kullanılan testlerin duyarlılık ve özgüllüğünün kullanılan kitin özelliklerine, kistin yerleşim yeri ve sayısı, antijenin özelliklerine, antijenin elde edildiği konağa, hastanın antikor yanıtına, seçilen yönteme göre değiştiği bildirilmiştir (18-20). Serolojik testlerin duyarlılık ve özgüllüğünü arttırmak için aynı serum örneğinin farklı yöntemlerle çalışılması önerilmektedir (19, 20).

Ayrıca KE serolojik tanısında çeşitli yöntemler kullanılmaktadır. IHA ve ELISA yöntemlerinin kolay uygulanabilmesi, pahalı laboratuvar gereçleri gerektirmemesi, yüksek özgüllük ve duyarlılıkları nedenleri ile sıklıkla IHA ve ELISA yöntemleri tercih edilmektedir (12, 17, 21). Biz de laboratuarımızda bu nedenle IHA yöntemini KE serolojisinde kullanmaktayız.

İlimizde görüldüğü üzere kısa bir zaman diliminde sadece hastanemiz Merkez Laboratuvarına başvuran olgular değerlendirildiğinde KE olgularının azımsanamayacak sayıda olduğu görülmektedir. Saptadığımız olguların ildeki genel durumu yansıtması mümkün olmamakla birlikte fikir vermesi açısından anlamlı olduğu düşünülmektedir. Saptadığımız olguların gerçek olgu sayısının sadece küçük bir bölümünü oluşturduğu düşünüldüğünde gerçek olgu sayısının çok daha fazla olduğu yadsınamayacak bir gerçek olup gerçek durumu saptamak amacı ile yapılacak epidemiyolojik çalışmalara ihtiyaç duyulmaktadır. Enfeksiyon Doğu ve Güney Doğu Anadolu bölgelerine göre düşük oranda saptanmakla birlikte bölgemizde önemli bir halk sağlığı sorunu oluşturan ve ekonomik kayba neden olan bu hastalığın eradikasyonu için korunma ve kontrol programlarının planlı bir şekilde yapılması ve toplumun bilinçlendirilmesi gerektiği kanaatine varılmıştır.

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Samsun'da Sülünlerde (*Phasianus colchicus*) Nekropsi ve Dışkı Bakısında Saptanan Helmintler

Helminths of Pheasant (*Phasianus colchicus*) Detected by Necropsy and Faecal Examination in Samsun, Turkey

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ÖZET

Amaç: Bu çalışma, sülünlerde bulunan helmintleri tespit etmek amacıyla Orman ve Su İşleri Bakanlığı Doğa Koruma ve Milli Parklar Genel Müdürlüğü'ne bağlı Gelemen Sülün Üretme Çiftliği'nde gerçekleştirilmiştir.
Yöntemler: Araştırma kapsamında 203 dışkı bakısı ve 17 nekropsi yapılmıştır.
Bulgular: Dışkı bakısında *Capillaria* spp. (%28.6) ve *Heterakis* spp. (%17.2) yumurtalarına, nekropside ise *Capillaria annulata* (%17.6), *C. bursata* (%35.3), *C. caudinflata* (%23.5), *C. contorta* (%64.7), *C. obsignata* (%5.9) ve *Heterakis gallinarum*'a (%58.8) rastlanmıştır.
Tartışma: Sülünlerde sıklıkla rastlanan helmintlerden *Syngamus trachea*'ne dışkı bakısında ne de nekropside rastlanmamıştır.
Sonuç: Bu çalışma Türkiye'de sülünlerde bulunan helmintlerin tespitine yönelik kapsamlı ilk araştırma niteliğinde olup, *Capillaria bursata, C. caudinflata, C. contorta, C. obsignata ve H. gallinarum* Türkiye'de sülünlerde ilk bildirimdir. *(Turkiye Parazitol Derg 2012; 36: 222-7)*

Anahtar Sözcükler: Kanatlı, sülün, helmint, Samsun, Türkiye

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ABSTRACT

Objective: This study was performed at the Gelemen Pheasant Procreation Farm affiliated to the General Directorate of Nature Conservation and National Parks, Ministry of Forest and Water Affairs.
Methods: A total of 203 faecal samples and 17 necropsies were examined.
Results: In the faecal examination, *Capillaria* spp. accounted for 28.6% and *Heterakis* spp. for 17.2% of eggs; in necropsy, *Capillaria annulata* (17.6%), *C. bursata* (35.3%), *C. caudinflata* (23.5%), *C. contorta* (64.7%), *C. obsignata* (5.9%) and *Heterakis gallinarum* (58.8%) were detected.
Discussion: Syngamus trachea, often reported in helminths in pheasant, could be found neither upon faecal examination nor on necropsy.
Conclusion: This study is the first extensive research to identify helminths of pheasants in Turkey. *Capillaria bursata, C. caudinflata, C. contorta, C. obsignata and H. gallinarum* are the first reports from pheasants in Turkey. *(Turkiye Parazitol Derg 2012; 36: 222-7)*

Key Words: Bird, pheasant, helminth, Samsun, Turkey

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GİRİŞ

Sülünler Galliformes takımında Phasianidae ailesinde yer almakta, 13 cins altında 29 türü bulunmaktadır. Çoğu tür Doğu Asya'da kendine özgü habitatlarda bulunurken, adi ya da bayağı sülün olarak bilinen *Phasianus colchicus*'a ise kuzey yarım kürede ılıman iklim kuşağında yaygın olarak rastlanır. Dünyada büyük çoğunluğu bayağı sülün olmak üzere yetiştiricilik yapılmaktadır. Sülün üretimi ile doğal-özel av alanlarına stok takviyesi ya da yeni av alanları oluşturmak amaçlandığı gibi hobi-zevk amaçlı da sülün yetiştirilmektedir. Damızlık üretimi ve eti için ise başta Fransa olmak üzere İtalya ve Belçika'da sülün çiftlikleri bulunmaktadır (1). Türkiye'de doğal ortamı Marmara ve Karadeniz'de kıyıya yakın ormanlık, çalılık alanlar olan sülünler, ülkemizde daha çok av turizmine yönelik olarak üretilmektedir. Bunun yanı sıra az miktarda da olsa özel çiftliklerde sülün türlerine rastlanmaktadır (2, 3). Türkiye'de en büyük çiftlik olma özelliğinde olan Gelemen Sülün Üretme Çiftliği Samsun'da 1958 yılında faaliyete geçmiştir. Her yıl 10000'in üzerinde sülün burada yetiştirilip türün doğal alanlarında devamını sağlamak ve av alanlarına stok takviyesi yapmak amacıyla salınmaktadır.

Sülünler birçok helminte konaklık yapar. Özefagus-kursak mukozası, incebağırsak ve sekuma yerleşen *Capillaria* spp., sekumda bulunan *Heterakis* spp. ve trakede yerleşen *Syngamus trachea* sülünlerde en sık rastlanan türlerdir. Sülünlerde bulunan helmintler, yerleşim yerleri ve yayılış oranları Tablo 1'de verilmiştir.

Bu çalışma Türkiye'de sülünlerde bulunan helmintlerin tespitine yönelik olarak yapılan kapsamlı ilk çalışma niteliğinde olup, bölgemizde sülünlerde bulunan helmintler ve yayılışları hakkında bilgi vermek amacıyla yapılmıştır.

YÖNTEMLER

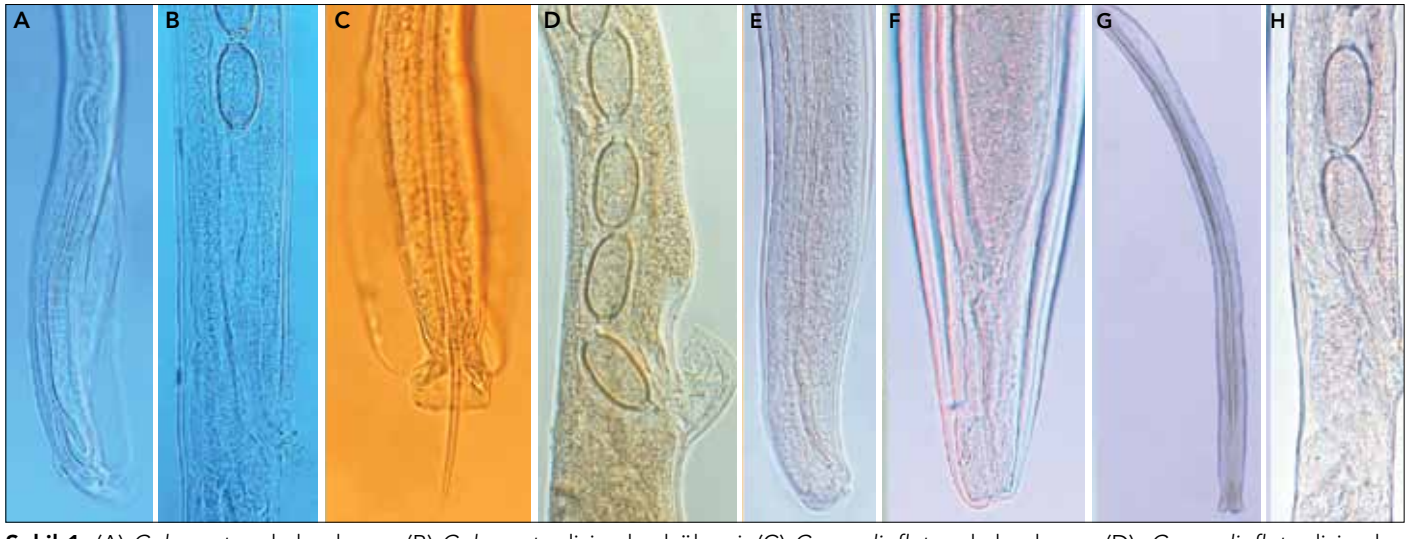
Araştırma Mart-Ağustos 2012 tarihleri arasında Orman ve Su İşleri Bakanlığı Doğa Koruma ve Milli Parklar Genel Müdürlüğü'ne bağlı Gelemen Sülün Üretme Çiftliği'nde gerçekleştirilmiştir. Çalışma dışkı incelemesi ve nekropsi muayenesi olmak üzere iki kısımdan oluşmaktadır.

Dışkı muayenesi amacıyla kafeslerden 101'i dişi, 102'si erkek sülünlere ait olmak üzere toplam 203 dışkı örneği incelenmiştir. Gelemen Sülün Üretme Çiftliğinde erkeklerin ve dişilerin ayrı olarak bulunduğu iki büyük kafes ile çiftleşme döneminde kullanılan damızlık grupların bulunduğu çok sayıda küçük kafes bulunmaktadır. Dışkı örnekleri sülün bulunan tüm kafeslerde yerden toplanmıştır. Kontaminasyon riskini azaltmak amacıyla dışkılar toplanırken tazeleri seçilmiş, ayrıca tüm kafesi temsil etmesi amacıyla örnekler homojen olarak toplanmıştır. Kafeslerin içerisine serçe gibi küçük kuşlar girebileceğinden toplanan dışkıların sülünlere ait olmasına dikkat edilmiştir. Toplanan dışkılar ayrı torbalara konmuş, numaralandırılmış ve incelenmek üzere parazitoloji laboratuvarına getirilmiştir. Örnekler flotasyon yöntemi ile (çinko sülfat sol.) kullanılarak incelenmiş, inceleme sonunda enfekte örnekler kaydedilmiş, helmint yumurtaları ilgili ölçümleri yapılarak literatürler eşliğinde teşhis edilmiştir (8, 16, 24, 25).

Nekropsi materyalini çiftleşme kafeslerinde araştırma süresince ölen 17 sülün oluşturmuştur. Ölen sülünler kısa sürede parazitoloji laboratuvarına getirilmiş, göz ve vücut yüzeyi makroskobik olarak muayene edildikten sonra karın ve göğüs boşluğu açılmış, makroskobik incelemeden sonra sindirim sistemi organları ayrım yerlerinden ligatüre edilerek ayrı kaplara alınmıştır. Trake akciğere giriş yaptığı bifurkasyo bölgesi ve larenks hizasından bir makas yardımıyla kesilmiş, içi açılarak makroskobik ve stereo mikroskop altında incelenmiştir. Böbrekler bir petri içinde diseke edildikten sonra stereo mikroskop altında incelenmiştir. Sindirim sistemi içerikleri delik çapı 150 µm olan elekten geçirildikten sonra stereo mikroskopta incelenmiş, toplanan parazitler tür teşhisi yapılana kadar %70'lik alkolde saklanmıştır. Toplanan helmintler laktofenolde saydamlaştırıldıktan sonra önemli morfolojik ölçümleri kaydedilmiş ve ilgili literatürler yardımıyla tür teşhisleri yapılmıştır (13, 24-26). Bulunan türler OMUPAR.824.12.01-06 numaralarıyla kayıt altına alınarak saklanmıştır.

BULGULAR

Araştırma sonunda incelenen 203 dışkı örneğinden 93'ü (%45.8), nekropsi yapılan 17 sülünden 16'sı (%94.1) çeşitli helmint türleri ile enfekte bulunmuştur. Dışkı bakısında *Heterakis* spp. ve *Capillaria* spp. yumurtalarına, nekropside *Heterakis gallinarum*, *Capillaria annulata*, *C. bursata*, *C. caudinflata*, *C. contorta* ve *C. obsignata*'ya rastlanmıştır (Şekil 1, 2). Dışkı bakısı ve nekropside



Şekil 1. (A) *C. bursata* erkek arka uç, (B) *C. bursata* dişi vulva bölgesi, (C) *C. caudinflata* erkek arka uç, (D) *C. caudinflata* dişi vulva bölgesi, (E) *C. contorta* erkek arka uç, (F) *C. contorta* dişi arka uç, (G) *C. obsignata* erkek arka uç, (H) *C. obsignata* dişi vulva bölgesi

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Tablo 1. Sülünlerde bulunan helmintler, yerleştiği organ ve yayılış oranları

Helmint türü	YO	Yayılış oranı (Literatür)
Echinostoma revolutum	İB	3 (4)
Paratanaisia bragai	B	22 (5)
Choanotaenia infundibulum	İB	5.7 (6)
Raillietina echinobothrida	İB	13 (4); + (7)
Ascaridia spp.		10.5* (8); 12.5* (9)
Ascaridia galli	İB	0.4 (6); 12.9-23.1 (10)
A. columbae	İB	10.3-18.2 (10)
Capillaria spp.		38.4* (8); 38.1-43.9 (11)
Capillaria annulata (Sin: *Eucoleus annulatus*)	Ö, K	+ (7); + (11); + (12); 1.9 (13); 2 (14)
Capillaria bursata	İB	+ (11); 20.5 (13)
Capillaria caudinflata (Sin: *C. gallinae; C. longicollis; Aonchotheca caudinflata*)	İB	23-28 (4); + (10); + (11); + (12); 0.1-1.9 (15); + (16)
Capillaria contorta (Sin: *E. contortus*)	İB	73 (4); + (11); + (12); 20.5 (13); + (16)
Capillaria obsignata (Sin: *C. columbae; Baruscapillaria obsignata*)	İB, S	+ (10); + (11); 2 (14); 0.3 (15)
Capillaria perforans (Sin: *E. perforans*)	Ö, K	12.6 (13); 72 (14)
Capillaria phasianina	İB, S	+ (10); + (11); 59.6 (13); 12 (14); + (15) + (16)
Capillaria retusa	İB, S	6-75 (4); 0.7 (15)
Cheilospirura hamulosa (Sin: *Acuaria hamulosa*)	T	14.3 (17)
Dispharynx nasuta (Sin: *D. spiralis*)	Ö, M	3 (4); 1 (18); 10 (19)
Gongylonema ingluvicola	Ö	2 (19)
Heterakis gallinarum	S	74-81 (4); 35.1 (6); 4.6-11,1 (10); + (12); 84.1 (13); 5.4 (15); 90 (20)
Heterakis isolonche	S	31.7* (8); 7.4-27.9 (10); + (21)
Oxyspirura mansoni	G	24 (19)
Strongyloides sp.		12.5* (22)
Subulura brumpti	S	8 (19)
Syngamus trachea	T	1-10 (4); 45.8* (8); 34.5-37.2 (10); + (12) 51.5 (13); 5.1-6.8 (15)
Tetrameres fissispina	MB	4 (19)
Thominx cyanopicae (Sin: *Echinocoelus cyanopicae*)	S	2.4 (11)
Trichostrongylus tenuis	İB	6-22 (4); 2.1* (8); 0.1 (15); + (23)
Prostorhynchus transversus	İB	1 (4)

+ : Olgu bildirimi ya da oran verilmemiş; *Dışkı bakısı, Sin: Sinonim
YO: Yerleştiği organ; B: Böbrek; G: Göz; İB: İncebağırsak; K: Kursak; M: Mide; MB: Mide bezleri; Ö: Özefagus; S: Sekum; T: Taşlık

bulunan helmintlerin enfeksiyon oranları ve tespit edilen parazit yükü Tablo 2 ve Tablo 3'de verilmiştir.

Dışkı incelemesi ve nekropsi sonuçlarına göre enfekte hayvanlarda miks enfeksiyonlara rastlanmıştır. Üçü dişilerden, altısı erkeklerden alınan örneklerden olmak üzere dışkı bakısında 9 enfekte örnekte *Capillaria* spp. ve *Heterakis* spp. birlikte bulunmuştur. Nekropsi sonuçlarında ise 16 enfekte sülünün 10'unda miks enfeksiyon görülmüş, sonuçlar Tablo 4'de sunulmuştur.

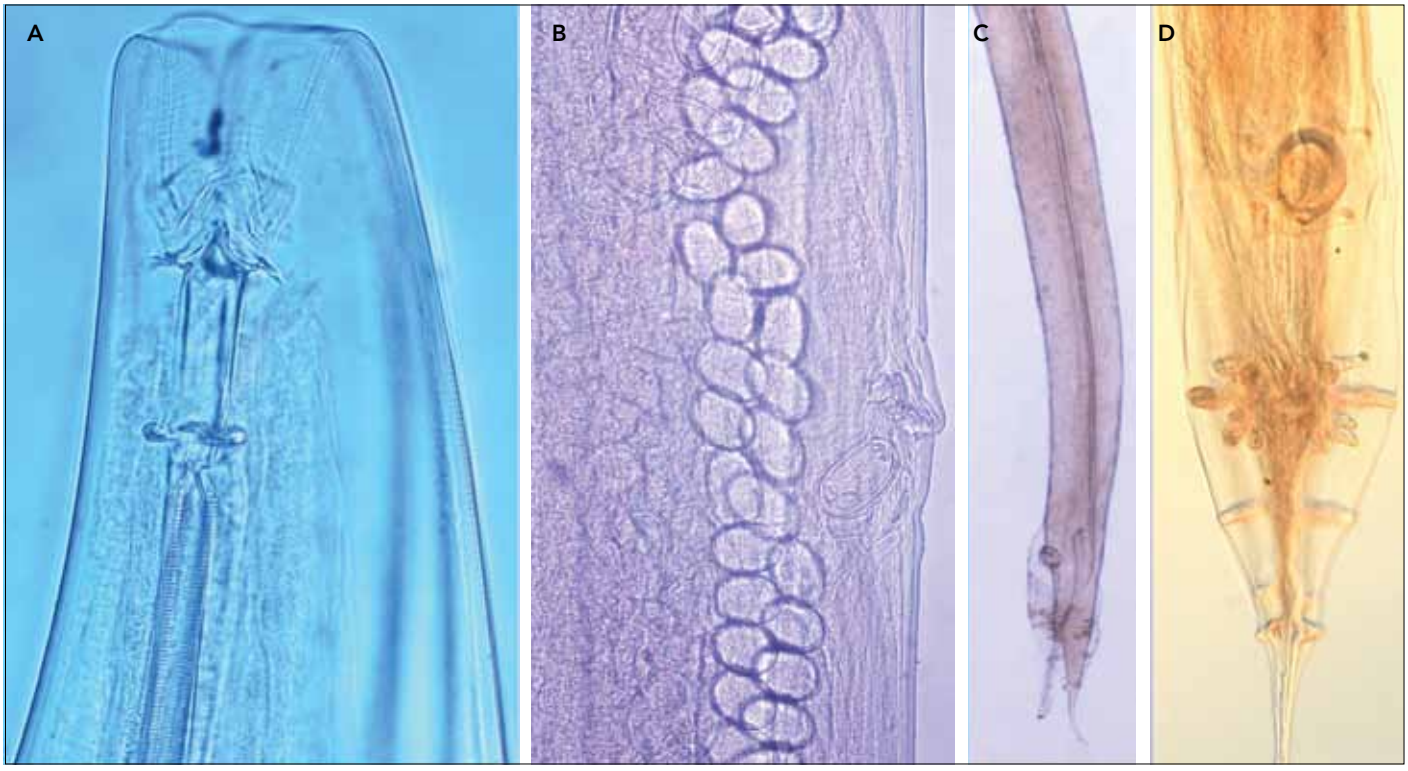
Türkiye'de sülünlerde ilk bildirim olan türlerin morfolojik ölçümleri yapılmış, ortalama değerleri Tablo 5'de verilmiştir. Ölçümler her parazitten 10 örnek üzerinde, sayısı az ise bulunduğu kadarında yapılmıştır.

Tablo 2. Dışkı bakısında bulunan helmintler ve sülünlerde cinsiyete göre dağılımları

Helmint	Dişi (n=101)	Erkek (n=102)	Toplam (n=203)
Capillaria spp.	22 (%21.8)	36 (%35.3)	58 (%28.6)
Heterakis spp.	15 (%14.8)	20 (%19.6)	35 (%17.2)
Toplam	37 (%36.6)	56 (%54.9)	93 (%45.8)

TARTIŞMA

Yapılan çalışmalara bakıldığında sülünlerde çok sayıda nekropsi çalışması yapıldığı halde dışkı bakısına dayalı fazla araştırma



Şekil 2. *H. gallinarum* (A) ağız bölgesi, (B) vulva bölgesi, (C) spikülümler, (D) erkek arka uç

Tablo 3. Nekropside bulunan helmintler, yayılışları ve parazit yükü

Helmint türü	Enfekte hayvan/ nekropsi sayısı (%)	Parazit yükü		
		♂	♀	Toplam
Capillaria annulata	3/17 (17.6)	4-9 (7)	2-16 (7.7)	6-25 (14.7)
C. bursata	6/17 (35.3)	0-187 (37)	0-305 (60.2)	1-492 (92.2)
C. caudinflata	4/17 (23.5)	1-7 (3.3)	1-2 (1.8)	2-9 (5)
C. contorta	11/17 (64.7)	2-32 (14.2)	1-31 (12.3)	4-62 (12.3)
C. obsignata	1/17 (5.9)	5	16	21
Heterakis gallinarum	10/17 (58.8)	4-118 (38.8)	6-114 (40.8)	10-232 (79.6)
Toplam	16/17 (94.1)			1-589 (101.9)

olmadığı görülmektedir (8, 9). Türkiye'de yapılan çalışmalar ise hayvanat bahçesi verilerine dayanmaktadır. Tiğin ve ark. (22) Ankara Hayvanat Bahçesi'nde yaptıkları çalışmada sülünlerde *Strongyloides* sp. yumurtalarına rastladıklarını, Gürler ve ark. (27) Samsun Hayvanat Bahçesi'nde sülünlerde helmint yumurtalarına rastlamadıklarını bildirmiştir. Slovakya'da Goldova ve ark. (8) 1030 sülün dışkından 497'sini (%48.2) çeşitli helmint yumurtaları ile enfekte bulmuşlar: *Capillaria* spp. (%38.4), *Syngamus trachea* (%45.8), *Heterakis isolonche* (%31.7), *Ascaridia* spp. (%10.5) ve *Trichostrongylus tenuis* (%2.1) türlerinin bulunduğunu belirtmişlerdir. Başka bir çalışmada, Hindistan'da Patel ve ark. (9) dışkı incelemesi yaptıkları 8 sülünden 1'inde (%12.5) *Ascaris* spp. yumurtasına rastlamışlardır. Bu çalışmada dışkı bakısında *Capillaria* spp. (%28.6) ve *Heterakis* spp. (%17.2) yumurtaları bulunmuş, ancak sülünlerde sık rastlandığı bilinen *S. trachea*'ya ne dışkı bakısında ne de nekropside rastlanmamıştır. Bu farklılı-

Tablo 4. Nekropsi bakısında görülen miks enfeksiyonlar

Miks enfeksiyon	Enfekte hayvan sayısı
H. gallinarum + *C. bursata*	1
H. gallinarum + *C. contorta*	3
H. gallinarum + *C. bursata* + *C. contorta*	1
H. gallinarum + *C. caudinflata* + *C. contorta*	2
C. annulata + *C. bursata* + *C. contorta*	1
C. annulata + *C. bursata* + *C. caudinflata* + *C. contorta*	1
H. gallinarum + *C. annulata* + *C. bursata* + *C. caudinflata* + *C. contorta*	1

Tablo 5. Türkiye'de sülünlerde ilk bildirim olan türlerin morfolojik ölçümleri (mm)

mm	*C. bursata*		*C. caudinflata*		*C. contorta*		*C. obsignata*		*H. gallinarum*	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Uzunluk	17.4	35.9	15.3	19.1	21.7	35.2	14.3	18.3	9.4	10.1
Genişlik (µm)	59.1	86.4	54.3	60.5	68.2	134.4	71.6	98.1	346.4	406.6
Özefagus	6.49	8.15	6.2	6.4	5.54	8.97	6.3	6.4	1.06	1.13
Bulbus	-		-		-		-		0.18	0.21
Vulva-ön uç	-		-		-		-		-	4.9
Kuyruk	-		-		-		-		-	1.13
Spikülüm	1.75	-	1.7	-	-		0.75	-	0.69/2.2	-
AÇ* çapı	-				-		-		0.07	-
AÇ-arka uç	-				-		-		0.71	-
Yumurta (µm)	-	58.8x24.4	-	60.7x24.5	-	45.2x22.7	-	56.7x23.4	-	71.1x41.7

*AÇ: Anal çekmen

ğın nedeni Türkiye'de bu parazitin düşük oranlarda (%0.9-2) ve nadiren görülmesi olarak yorumlanmıştır (26, 28).

Heterakis gallinarum ve *H. isolonche* enfeksiyonlarına sülünlerde sıklıkla rastlanmaktadır (8, 10, 13). *Heterakis gallinarum* düşük patojeniteye sahip olarak görülürken *H.isolonche*, özellikle sülünlerde nodüler tifilitise neden olan patojen bir tür olarak kabul edilmektedir (21). Menezes ve ark. (20) *H.gallinarum* ile enfekte buldukları sülünlerden bazılarında nodüler tifilitis olgularına rastladıklarını bildirmişlerdir. Yapılan bu çalışmada, nekropside *H. isolonche*'ye rastlanmazken, *H. gallinarum* ile enfekte bulunan örneklerin bazılarında, yaygın olmamakla birlikte makroskobik olarak sekumda birkaç nodül gözlenmiştir.

Capillariidae ailesinde bulunan türler diğer kanatlılarda olduğu gibi sülünlerde de en sık rastlanan helmintlerdendir (4, 13, 14). Çalışmamızda gerek dışkı bakısında, gerek nekropside en fazla capillarid nematoda rastlanmıştır. Teşhis edilen 5 farklı türden en yaygın tür literatür bilgi (Tablo 1) ile aynı şekilde *C. contorta* olmuştur. Son yıllarda yapılan çalışmalar ile birlikte Capillariidae ailesinde bulunan helmintler farklı cinsler altına sokulmuştur. Yayında capillarid helmintlerin yeni cins isimleri yerine daha yaygın olarak bilinen "*Capillaria*" cins ismi kullanılmış, yeni adlandırmaları ise Tablo 1'de sinonimi olarak verilmiştir. Farklı olarak Tablo 1'de yalnızca *Echinocoelus cyanopicae* yerine literatürde bildirdiği şekliyle *Thominx cyanopicae* kullanılmıştır. Morfolojik ölçümler yapılırken *C. contorta*'nın spikülümü tam belirgin olmadığı için uzunluk ölçümü yapılamamıştır.

Güralp ve ark. (23) Gelemen Sülün Üretme Çiftliği'nde *Trichostrongylus tenuis* kaynaklı sülün ölümleri görüldüğünü bildirmiş olmasına rağmen, bu çalışmada hem dışkı hem nekropsi incelemesinde aynı parazite rastlanmamıştır. İki çalışma arasında uzun zaman olması, ayrıca parazitin sülünlerdeki yaygınlığının düşük olması parazite rastlanmama nedenleri olarak değerlendirilebilir (4, 13, 15).

Dışkı bakısı sonuçları ve nekropsi incelemesi sonuçları karşılaştırıldığında dışkı bakısında genel helmint enfeksiyon oranı %45.8 iken, nekropsi incelemesinde bu oran %94.1'e çıkmıştır. Nekropsi sonuçlarının dışkı bakısına göre daha güvenilir olduğu bilinmektedir. Bunun yanı sıra arada oluşan bu farkın nedenleri olarak enfekte hayvanların bazılarında parazit yükünün az olması, teşhis edilen dişi helmintlerin bir kısmında uterusta yumurta olmaması da sayılabilir.

SONUÇ

Türkiye'de konu ile ilgili yapılan çalışmalarda nekropsi incelemesi sonucunda sülünlerde *T. tenuis*, *C. annulata* ve *Raillietina echinobothrida*, dışkı bakısı sonucunda *Strongyloides* spp.'ye rastlanmıştır (7, 22, 23). Bu çalışma sonunda teşhis edilen helmintlerden *Capillaria bursata*, *C. caudinflata*, *C. contorta*, *C. obsignata* ve *H. gallinarum* Türkiye'de sülünlerde ilk bildirim olarak kaydedilmiştir.

Teşekkür

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Çıkar Çatışması

Yazarlar herhangi bir çıkar çatışması bildirmemişlerdir.

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Kastamonu Civarındaki Evcil Sığırların (*Bos taurus taurus*) İşkembesinde Tespit Edilen İki Siliyat Türü *Entodinium palmare* ve *E. okoppensis* (Protista: Ciliophora: Ophryoscolecidae) Hakkında

About Ciliates *Entodinium palmare* and *E. okoppensis* (Ciliophora: Ophryoscolecidae) in the Rumen of Domestic Cattles (*Bos taurus taurus*) in the vicinity of Kastamonu

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ÖZET

Amaç: Bu çalışmanın amacı Kastamonu civarında yaşayan evcil sığırların işkembesinde bulunan siliyat türleri *Entodinium palmare* ve *E. okoppensis*'in sitolojik özelliklerini tespit etmek, türlerin morfolojik karakterlerini orijinal tanımlarıyla karşılaştırarak benzerlik ve farklılıkları tartışmaktır.
Yöntemler: Yirmi dört sığırdan elde edilen örnekler %10'luk formalinle tespit edildikten sonra metil formalin salin (MFS) ile boyanmıştır.
Bulgular: Ülkemizdeki sığırlardan elde edilen bireylere ait ölçümler, morfolojik karakterler ve biyometrik veriler orijinal tanımlarla benzer bulunmuştur.
Sonuç: *E. palmare ve E. okoppensis* ülkemizdeki sığırlardan ilk kez dünyadaki sığırlardan ise ikinci kez bu çalışmayla rapor edilmiştir. *E. palmare* ve *E. okoppensis* ülkemiz sığırlarında oldukça düşük bir görülme sıklığı ve bulunma oranına sahiptirler. Bu durumun nedenleri olarak sığırların beslenme alışkanlıkları, beslenme sıklıkları, işkembenin fizyolojik durumu veya türler arasında görülen rekabetin önemli olabileceği sonucuna varılmıştır. *(Turkiye Parazitol Derg 2012; 36: 228-31)*

Anahtar Sözcükler: *Entodinium palmare*, *E. okoppensis*, işkembe, siliyat, sığır

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ABSTRACT

Objective: The aim of this study was to determine the cytological features of the rumen ciliates *Entodinium palmare* and *E. okoppensis* living in the rumen of domestic cattle in the vicinity of Kastamonu, compare the morphological characters of species with their original descriptions and discuss the similarities and differences.
Methods: The specimens were obtained from 24 cattle, fixed with 10% formalin and stained with methylgreen formalin saline (MFS) solution.
Results: Specimens were measured from cattle in our country, and were found to be similar to the original description on the basis of morphological characteristics and biometric data.
Conclusion: With this investigation, *E. palmare* and *E. okoppensis* were firstly detected from our cattle and were secondly detected from cattle throughout the world. *E. palmare* and *E. okoppensis* have low frequency appearance and percentage composition in our cattle. This study concluded that the feeding habits and feeding frequencies of cattle, the physiological conditions of rumen or competition between species can be important reasons for this situation. *(Turkiye Parazitol Derg 2012; 36: 228-31)*

Key Words: *Entodinium palmare*, *E. okoppensis*, rumen, ciliate, cattle

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GİRİŞ

Dünyanın farklı bölgelerinde yaşayan herbivor konakların işkembesindeki siliyat türlerinin belirlenmesi siliyatların coğrafi varyasyonları, konakların beslenme alışkanlıkları ve siliyatların filogenileri hakkında bilgi sağlamaktadır (1-5). İşkembe siliyat tür kompozisyonu farklı konaklar arasında, hatta aynı konak türün farklı coğrafik alanlarında yaşayan bireylerinde farklı olabilir (6-8).

Entodinium palmare ve *E. okoppensis* Ophryoscolecidae familyasına ve Entodiniomorphida ordosuna dahil endo-

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kommensal siliyatlardır (9). *E. palmare* ilk kez 2009 yılında Tanzanya'daki kısa boynuzlu Zebu sığırlarından, *E. okoppensis* ise ilk kez 1990 yılında Japonya'daki Holstein sığırlardan tanımlanmıştır (10, 11).

Bu çalışmanın amacı Kastamonu civarında yaşayan evcil sığırların (*Bos taurus taurus* L.) işkembesinde bulunan siliyat türleri *E. palmare* ve *E. okoppensis*'in sitolojik özelliklerini tespit etmek, türlerin morfolojik karakterlerini orijinal tanımlarıyla karşılaştırarak benzerlik ve farklılıkları tartışmaktır.

YÖNTEMLER

İşkembe örnekleri Kastamonu ve civarındaki çeşitli mezbahalardaki 24 sığırdan Mart 2012 ve Haziran 2012 tarihleri arasında alınmıştır. Örnekler siliyatların bozulmasını engellemek için hemen %10'luk formalinle tespit edilmiştir. Laboratuara getirildikten sonra ağ gözü sayısı 50 ve açıklığı 582.5 µm olan hücre ayrıştırma eleğinden (Sigma) geçirilerek süzülmüştür. Daha sonra elde edilen depo örneklerden küçük cam tüplere ölçekli pipet yardımıyla bir miktar alınarak, MFS ile geçici preparatlar hazırlanmışıtr (12-14).

Siliyatların orientasyonu için Dogiel'den yararlanılmıştır (15). Bu orientasyon sisteminde, öncelikle hücrenin anteriyör-posteriyör yönelimi saptanır. Sitoproktun bulunduğu taraf daima posteriyör, karşı taraf ise anteriyördür. Nukleus apareyine en yakın vücut kısmı dorsal karşı tarafı hücrenin ventralidir. Sağ ve sol taraflar ise organizmanın dorsal tarafının gözlemcinin sırt tarafıyla aynı doğrultuda olduğu düşünülerek saptanır.

Işık mikroskobunda inceleme, fotoğraf çekimi ve örneklere ait ölçümler Leica DM 3000 görüntüleme sistemiyle gerçekleştirilmiştir. Sınıflandırma ve tür tayini Lynn (9), Mishima ve ark. (10) ve Ito ve Imai (11) dayandırılarak verilmiştir. Morfolojik karakterlerle ilgili istatistiksel verilerin elde edilmesinde SPSS (Vers. 10.0) istatistik programı kullanılmıştır.

BULGULAR

***E. palmare* Mishima et al. (10)**

Vücut ovoittir, yanlardan basıktır, ön uca doğru hafifçe daralır. Vücudun ön ucunda ileri-geri çekilebilen (retraktil) oral sil zonunun belirgin bir dudağı bulunur. Vücudun posteriyör dorsal parçası sağ tarafta konkavdır ve bu parça dorsal taraftan bakıldığında sağ uca doğru eğimlenir. Yüzeysel ve geniş oluk vücudun sol yüzeyinde boyuna uzanır (sol tarafta hücrenin uzun eksenine paralel uzanır). Vestibulum huni şekillidir ve dorsale yönelir. Sitoprokt konkavlığın ventral kenarında ve arka uçtadır. Makronukleus çubuk şekillidir ve vücudun dorsal tarafındadır. Küçük ovoid mikronukleus makronukleusun ventral sol tarafına yerleşmiştir. Tek kontraktil vakuol mikronukleusun ön tarafında, makronukleusun ön ucunun sol ventral tarafında bulunur (Şekil 1).

E. palmare incelenmiş olan 24 sığırın 1'inde gözlemlenmiştir. Görülme sıklığı %4.2'dir. Sığır 10'da bulunma oranı %2.4'tür.

E. palmare'ye ait sığırlarımızdan saptanan morfometrik değerler Tablo 1'de verilmiştir. Tablo 2'de ise *E. palmare*'nin vücut ölçümleri orijinal tanımlamadaki örnekle karşılaştırılmıştır.

***Entodinium okoppensis* Ito ve Imai (11)**

Vücut dikdörtgenle kare arası değişir. Vücudun arka ucunda çeşitli büyüklükte 1-3 kaudal ışın veya lob bulunur. Vücudun ön ucu düz veya konkavdır. Ön dudak, oral siller geri çekildiğinde hemen hemen hiç görülmez. Vestibulum geniş ve huni şekillidir. Dikey olarak uzanır fakat hafifçe sola yönelir. Sitoprokt vücudun posteriyör ucunda orta sol taraftadır. Makronukleus düz ve ince çubuk şekillidir, vücut uzunluğunun 4/5'i kadar uzunluktadır. Vücudun sağ-dorsal tarafına yerleşmiştir. Makronukleusun ön ucu düz, arka ucu ise yuvarlaktır. Oval mikronukleus makronukleusun orta sol kenarında bulunur. Kontraktil vakuol makronukleusun tam önünde ve biraz üzerindedir.

Rumen sıvısı örneklerinde bu türe ait 2 morfotipi tespit edilmiştir.

***E. okoppensis* m. okoppensis (Şekil 2a)**

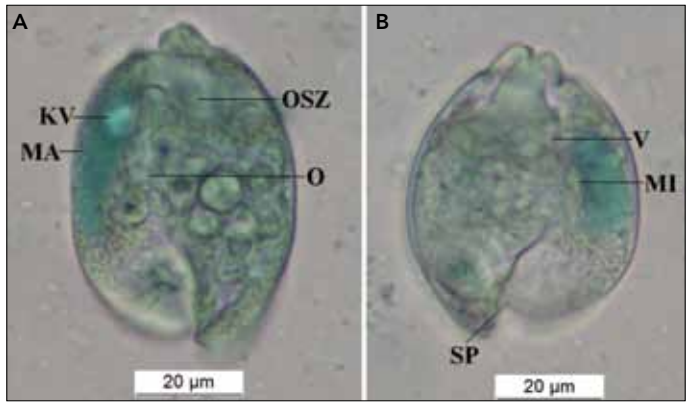
Üç kaudal ışın bulunur. Sağ olan sivri veya küt ışın şeklindedir, bazen dış tarafa doğru bükülür, sol alt ve sol üstteki kaudal çıkıntılar sivri ışınlar şeklindedir.

***E. okoppensis* m. bifidum (Şekil 2b)**

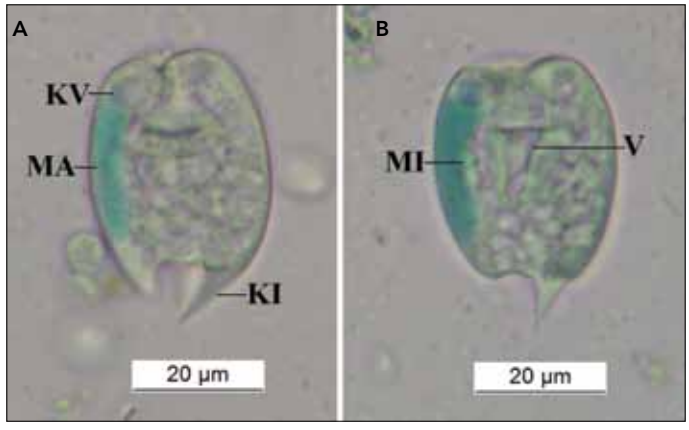
Sadece sol tarafta aynı uzunlukta 2 kaudal ışın mevcuttur.

E. okoppensis incelenmiş olan 24 sığırın 1'inde gözlemlenmiştir. Görülme sıklığı %4.2'dir. Sığır 15'de bulunma oranı %6.3'tür.

E. okoppensis'e ait sığırlarımızdan saptanan morfometrik değerler Tablo 3'te verilmiştir. Tablo 4'te ise *E. okoppensis*'in vücut ölçümleri orijinal tanımlamadaki örnekle karşılaştırılmıştır.



Şekil 1. *E. palmare* (A) sağdan, (B) soldan



Şekil 2. *E. okoppensis* m. okoppensis (a) sağdan, *E. okoppensis* m. bifidum (b) sağdan

Tablo 1. *E. palmare*'ye ait ölçüm değerleri ve bu karakterlere ilişkin biyometrik veriler

	***Entodinium palmare* (n=25)**			
Karakterler	**Ekstr.**	**Ort.**	**SD**	**SE**
[U]	42.2-68.3	60.0	7.2	1.4
[G]	37.1-52.4	42.9	4.4	0.9
[MaU]	17.5-35.5	27.6	5.7	1.1
[MaG]	6.6-10.4	7.9	1.0	0.2
[U/G]	1.1-1.6	1.4	0.1	‹0.1
[MaU/MaG]	2.0-5.1	3.6	0.9	0.2
[U/MaU]	1.7-3.0	2.2	0.3	0.1

n: Örnek sayısı, U: Hücre uzunluğu, G: Hücre genişliği, MaU: Makronukleus uzunluğu, MaG: Makronukleus genişliği, Ekstr.: Ekstrem değerler, Ort.: Aritmetik ortalama, SD: Standart sapma, SE: Standart hata

Tablo 2. *E. palmare*'nin vücut ölçümleri ile ilgili bulguların orijinal tanımlamadaki örnekle karşılaştırılması

Kaynak	**Ülke**	**Konak**	**[U]**	**[G]**	**[U/G]**
10	Tanzanya	Sığır	44.4±4.7	31.9±4.9	1.4±0.3
Bu çalışma	Türkiye	Sığır	60.0±7.2	42.9±4.4	1.4±0.1

10: Mishima ve ark.

Tablo 3. *E. palmare*'e ait ölçüm değerleri ve bu karakterlere ilişkin biyometrik veriler

	***Entodinium palmare* (n=25)**			
Karakterler	**Ekstr.**	**Ort.**	**SD**	**SE**
[U]	22.5-39.6	33.2	4.7	0.9
[G]	21.4-32.2	27.5	3.0	0.6
[KIU]	3.2-10.9	5.5	1.5	0.3
[MaU]	17.6-34.7	23.9	4.1	0.8
[MaG]	3.9-7.1	5.1	0.9	0.2
[U/G]	0.9-1.4	1.2	0.1	‹0.1
[MaU/MaG]	3.3-6.3	4.8	0.8	0.2
[U/MaU]	1.1-1.7	1.4	0.2	‹0.1

n: Örnek sayısı, U: Hücre uzunluğu, G: Hücre genişliği, KIU: Kaudal ışın uzunluğu, MaU: Makronukleus uzunluğu, MaG: Makronukleus genişliği, Ekstr.: Ekstrem değerler, Ort.: Aritmetik ortalama, SD: Standart sapma, SE: Standart hata

Tablo 4. *E. okoppensis*'in vücut ölçümleri ile ilgili bulguların orijinal tanımlamadaki örnekle karşılaştırılması

Kaynak	**Ülke**	**Konak**	**[U]**	**[G]**	**[U/G]**
11	Japonya	Sığır	35.7±6.3	26.2±3.0	1.4±0.2
Bu çalışma	Türkiye	Sığır	33.2±4.7	27.5±3.0	1.2±‹0.1

11: Ito ve ark.

TARTIŞMA

E. palmare ve *E. okoppensis* bu çalışmayla ülkemiz sığırlarından ilk kez, dünyadan ise ikinci kez rapor edilmiştir. Araştırmada sığırlarımızdan ölçülen örnekler, morfolojik karakterler ve biyometrik veriler bakımından orijinal tanımlarıyla benzer bulunmuştur.

Ülkemiz sığırlarında belirlenen *E. palmare*'nin vücut uzunluğu ve genişliği Tanzanya'daki Zebu sığırlarından belirlenen değerlere göre büyük olmasına rağmen, uzunluğun genişliğe oranı aynıdır (Tablo 2). Ülkemiz sığırlarından belirlenen *E. okoppensis*'in *vücut uzunluğu ve genişliği* Japonya'daki örneklerle hemen hemen aynıdır (Tablo 4).

Sığırlar üzerinde daha önce ülkemiz ve dünyada yapılan çeşitli çalışmalarda bu türlere rastlanmaması ve sadece *E. palmare*'nin Tanzanya'daki ve *E. okoppensis*'in Japonya'daki sığırlardan tespit edilmesi ve ülkemizde Kastamonu civarındaki sığırlardan rastlanması oldukça şaşırtıcıdır (2, 4-6, 16-28). Japonya'daki sığırlardan *E. okoppensis*'in 4 morfotipi rapor edilmesine rağmen, ülkemiz sığırlarında sadece 2 morfotipi tespit edilmiştir. Ayrıca *E. okoppensis*'in bir morfitipi *E. okoppensis* m. cameli Çin'deki develerden kaydedilmiştir (11). Bununla birlikte, sığırlardan tespit edilen 4 morfotip develerde gözlenmemiştir (29).

SONUÇ

E. palmare ve *E. okoppensis* oldukça düşük bir görülme sıklığı ve bulunma oranına sahiptir. Bu durumun nedenleri olarak sığırların beslenme alışkanlıkları, beslenme sıklıkları, işkembenin fizyolojik durumu veya türler arasında görülen rekabetin önemli olabileceği sonucuna varılmıştır.

E. palmare'nin Tanzanya ve Türkiye gibi farklı kıtalarda bulunan ülkelerin işkembe siliyat faunasında yer alması, *E. okoppensis*'in ise aynı kıtada fakat birbirine oldukça uzak coğrafi bölgelerden kaydedilmesi herbivorların işkembe siliyat faunası üzerine daha fazla çalışmanın yapılması gerektiğinin göstergesidir. Böylece farklı bölgelerde yaşayan farklı konakların incelenmesiyle bu türün kıtalararası nasıl geçiş gösterdiğinin anlaşılması açısından önemlidir.

Teşekkür
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Çıkar Çatışması
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The Parasitic Communities of the Rock Pigeon *Columba livia* from Iraq: Component and Importance

Irak'ta Kaya Güvercini (*Columba livia*) Parazitleri: Türler ve Önemi

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ABSTRACT

Objective: The main objectives of the present study were to investigate the rock pigeon parasitic communities from Iraq as well as reporting on the prevalence and intensity of various infections from both sexes.
Methods: An examination of 128 specimens of the live rock pigeon *Columba livia* from Iraq was undertaken. The samples were obtained from several localities of Iraq. Blood samples were examined for haemoprotozoa, carcasses were investigated for the ectoparasites throughout their body skins and feathers, and the alimentary canal was examined for protozoans and helminths.
Results: Twenty-seven species of parasites were identified. They comprised 1 Fungi, *Candida* sp.; 4 Protozoa, *Eimeria labbeana*, *Trichomonas gallinae*, *Haemoproteus columbae* and *Plasmodium* sp.; 8 Cestoda, 4 of each of the genera *Cotugnia* and *Raillietina*; 4 Nematoda, *Ascaridia columbae*, *A. galli*, *Capillaria obsignata* and *Synhimantus spiralis*; and 10 Arthropoda, the commonest of which were the wing and tail feather louse *Columbicola* sp. and the pigeon louse fly *Pseudolinchia canariensis*. Infection indices are provided for each species and in respect to both sexes of the host.
Conclusion: The issue of zoonosis is raised, so is the role of the rock pigeons in acting as a reservoir and spreading some of the disease agents associated with other avian populations including poultry. Seven of the species are newly introduced to the parasitological list of Iraq and for this country the rock pigeon is a new host record for another 9 of the endoparasites that were diagnosed.
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Key Words: Rock pigeon, parasitic communities, endoparasite, ectoparasite, Iraq

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ÖZET

Amaç: Bu çalışmanın temel amacı, Irakta'ki kaya güvercini topluluklarında parazit türleri, enfeksiyon sıklığı, şiddeti ve cinsiyete bağlı dağılımını araştırmaktır.
Yöntemler: Irak'ın farklı yerlerinden 128 canlı kaya güvercini (*Columba livia*) temin edildi. Kan örnekleri haemoprotozoa yönünden, deri ve tüyler ektoparazit yönünden ve sindirim kanalı ise protozoa ve helmint yönünden muayene edildi.
Bulgular: Yirmi yedi tür parazit tespit edilmiştir. Bunlar 1 mantar; *Candida* sp., 4 protozoa türü; *Eimeria labbeana, Trichomonas gallinae, Haemoproteus columbae* ve *Plasmodium* sp., 8 sestod türü; 4 *Cotugnia* ve 4 *Raillietina* türü; 4 nematod; *Ascaridia columbae, A. galli, Capillaria obsignata* ve *Synhimantus spiralis* ve 10 arthropod türü (en yaygın türler *Columbicola* sp. ve *Pseudolinchia canariensis*) olarak belirlenmiştir. Enfeksiyon yaygınlığı, şiddeti her tür için ve her iki cinsiyet açısından irdelenmiştir.
Sonuç: Irak parazit faunasına eklenen yedi tür tanımlanmış ve Irak kaya güvercinlerinde ilk kez rastlanan 9 endoparazit hakkında bilgi verilmiştir. *(Turkiye Parazitol Derg 2012; 36: 232-9)*

Anahtar Sözcükler: Kaya güvercini, parazit, endoparazit, ektoparazit, Irak

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INTRODUCTION

The rock pigeon, *Columba livia,* is essentially a free-living and cliff-dwelling granivorous species, but which is also a direct predecessor of the domestic subspecies *C. l. domestica.* It is readily recognisable that these two populations have kept many properties in common and actually even retained a certain degree of synanthropy. This suggests that their relation is much closer than some links of consanguinity.

Pigeons are amongst the most prevalent and readily observable birds in all provinces of Iraq. Besides constituting a small part of human food, they are used for ornamental or appearance purposes (fancy pigeons) as well as pets, human sport (racers and performers), for biological and medical experimentations, and for teaching the art of taxidermy. Pigeons in this part of the world also have a rich historical and anecdotal background.

Due to the fact that flocks of rock pigeons have adapted so well to life in association with human habitation, they become pests in domestic, commercial and public premises. More importantly, pigeons pose a serious health threat to millions of people and some of their economic animals. In Iraq, Al-Jumaily et al. (1) presented evidence that the population of the rock pigeon carry such dangerous disease agents as the bacterium *Salmonella typhimurium* and Newcastle virus. Besides, according to Soulsby (2), Ritchie et al. (3), Kaufmann (4), and Barnek et al. (5), wherever found across the globe, pigeons might be potential carriers of numerous other harmful pathogens and therefore a habitual tool to contaminate food and water and circulate illnesses such as psittacosis (ornithosis), histoplasmosis, aspergillosis, cryptococcosis, toxoplasmosis, tuberculosis, vibriosis, encephalitis, Q fever, pox, staphylococcosis, and listeriosis. Furthermore, pigeons themselves are subject to contracting not only the Newcastle virus but many other serious diseases, produced by other viruses, rickettsiae, bacteria, fungi, protozoa, helminths and arthropods (6-10). The reassessment of the bulk of the data produced some invaluable information that these birds would might appear to be of relevance to both wild and domestic avifauna as well as to host-parasite relationships, antigenic cross connections, zoonoses, sanitary surveillances, and control measures.

Judging from the available data directly relevant to the parasitism in the Iraqi populations of rock and domestic pigeons, the information regarding the identity, prevalence, intensity and endoparasite-ectoparasite assemblage of the parasitic forms likely to be associated with these birds is still far from adequate (11-24). Therefore, the main objectives of the present study were to investigate the above themes in a relatively larger sample of the rock pigeon in the hope of providing new data and clarifying some other pertinent issues.

METHODS

A sample of 128 specimens of the eastern rock pigeon *Columba livia* (Gmelin, 1789), subspecies *gaddi* Zarudny and Loudon, 1906, was randomly selected and used in this study. It comprised 58 males and 70 females, all of which were at the adult stage, albeit of different ages. These pigeons were captured alive between February 1990 and July 1991 from different localities within the country.

A general examination of each pigeon was performed and the majority of them were found to be lively and seemingly healthy looking. The average figures of some physical parameters of the specimens were: body weight 221.3 gm, wing cord 20.7 cm, tail length 10.3 cm, tarsus length 3.3 cm and bill length 1.88 cm.

A small amount of blood was obtained from the brachial vein of each bird and used for the preparation of thin smears. These were air-dried, fixed in absolute methanol and stained with 5% Giemsa solution (pH 7.2) before examining them using an oil immersion lens for the presence of haemoprotozoans. *Haemoproteus* and *Plasmodium* parasites were differentiated following their respective descriptions and illustrations in the literature, for example, presence in the host's peripheral red blood corpuscles (RBCs) of gametocytes for the former and schizonts for the latter (3, 5, 25). In both cases, a considerable number of the infected cells were not of normocytic size range but rather revealed some enlargement and structural distortion, as may also happen to the seriously damaged and dying cells of other tissues as a result of radiation and various other types of cyto-injurious agents (26). The parasitisation levels for either of these blood organisms were estimated on the basis of randomly examining 1,000 RBCs from each bird and then determining the percentage of infected cells. The data were averaged and so presented.

Within an hour after a pigeon was euthanised, any ectoparasites were collected from the skin and/or plumage. Lots obtained from different host specimens were separately kept to avoid any confusion. In many cases, it was possible to identify these species depending on their distinctive characteristics, and hence also determine their prevalence and intensities, by directly examining them under low-power objectives (2, 12, 27). Otherwise, they were preserved in 70% isopropyl alcohol (lice and mites) or 70% ethanol (other species) to be prepared and definitely identified later. To facilitate this process, the ectoparasites were subjected to an overnight clearance in 10% potassium hydroxide, washed, stained in 0.7% solution of Congo red, washed to remove excess stain, dehydrated in alcohol, cleared in xylene and permanently mounted in Canada balsam.

The cadaver of each pigeon was eviscerated. This process involved complete separation of the digestive tract from other organs by laparotomy. The tract, from gullet to rectum, including the gizzard and paired caeca, was then cut open. The content and scrapings from the mucosa of each anatomical part waere examined. When present, helminths from each bird were collected, cleaned in saline, their numbers counted, initially identified with the help of a dissecting microscope, and then preserved in 70% ethanol for subsequent processing. Attempts to identify the species of unicellular organisms (fungi and protozoans) were also made from fresh samples in the form of impermanent preparations examined with high-power objectives.

Permanent preparations of the gastrointestinal parasites were frequently made. This was done following the standard procedures for unicellular organisms, cestodes and nematodes. Relevant descriptions, found in Levine (28), Schmidt (29), Kaufmann (4), and Barnek et al. (5), aid the process of analysing the diagnostic features of the parasites and ultimately in achiev-

ing species-rank identification for most of them. Furthermore, definite identification of some of the species was decided by the experts' opinions previously received from the British Museum (Natural History) and CAB International.

Infection by the flagellate protozoan *Trichomonas gallinae* was infrequently predicted by the presence of pale cream-coloured lesions inside the host's mouth. However, it was always confirmed by actually finding the motile trophozoites of this organism in the microscopic preparations of fresh scrapings taken from the oral mucosa. The identification of other gastrointestinal unicellular parasites (fungi and coccidians), and sometimes helminths' ova, was achieved by microscopic examination of fresh faecal samples prepared, as needed either by the sedimentation or zinc sulfate floatation techniques. The level of parasitisation, in terms of parasite burdenfor the unicellular organisms other than for haemoprotozoans, was arbitrarily determined by designating 1+ for very mild asymptomatic form of infection and where only a small population of the parasite was detectable, and 2+ for moderate degree of infection and symptoms such as slight diarrhoea. No case of massive infection and acute symptoms, including emaciation, listless, dull and ruffled plumage and severe diarrhoea, was observed for any of the sampled pigeons.

Thus, whenever possible, both of the principal parasitisation indices, namely, "infection rate" and "parasite burden", were determined. The first index represents the prevalence of a parasite species among the examined specimens of its host, whereas the second is the average load of the parasite number actually present and hence may be taken as an indicator for the intensity of infection.

RESULTS

Twenty-seven forms of parasites were identified in and on the rock pigeon specimens examined in this study (Table 1). The global infection prevalence was 100%; that is, every bird was positive for at least one parasite. In fact, it was a parasitic association, which was distinguished as a common feature, since the majority of pigeons harboured 6-9 species concurrently, a few of them hosted up to 12 , and one specimen even had 15 species. These parasites belong to 5 main taxonomic categories, namely, Fungi, Protozoa, Cestodes , Nematodes and Arthropodes .

With regard to the unicellular organisms, a specific identification of *Eimeria labbeana*, *Trichomonas gallinae* and *Haemoproteus columbae* was accomplished. However, such definite identification was not possible for the yeast-like fungi of the genus *Candida* and apicomplexan protozoan of the genus *Plasmodium*. Therefore, these latter 2 forms were retained at the generic rank.

In respect to the gastrointestinal unicellular organisms, the dominant species was *T. gallinae*. As to haemoprotozoans, the infection rate of *H. columbae* was higher than for *Plasmodium* sp. Only in *H. columbae* was the infection rate significantly higher in males than females. In no case was a pigeon solely infected with one of these unicellular parasites and almost always the presence of *Plasmodium* was associated with that of *Haemoproteus*.

Eight species of cyclophyllidean cestodes were found. They are *Cotugnia polyacantha*, *C. satpuliensis*, *C. columbae*, *C. digonopora*, *Raillietina* (=*Skrjabinia*) *tetragona*, *R. carpohagi*, *R. bonini* (=*Hymenolepis columbae*) and *R. fuhrmanni* . All these tapeworms belong to the family Davaineidae. They were mostly recovered from the ileal part of the small intestine. The vast majority of their specimens were attached to the mucosa through their scolices and probably enforced their anchoring by a cushion-shaped rostellar apparatus. The differential diagnosis of some of them was only possible upon close examination of their scolices and comparison with the published descriptions and illustrations. In other words, when scolices were missing, examination and morphometrics of the strobilae, proglottids and ova of these tapeworms were not always sufficient for a definite identification.

Among these tapeworms, the representative degree of *R. fuhrmanni* and *R. bonini* seemed to be less than those of the rest. Variations in the individual relationships between sexes of pigeons and indices of their parasitisation with the tapeworm species appeared to be significant in the majority of cases. Nonetheless, figures for such variations seem less suggestive of a genuine difference due to certain intrinsic factors, whether in terms of prevalence or intensity, upon averaging the data for the 2 tapeworm genera or for all their species involved in that matter.

Four species of nematode parasites were found in this study. They comprise the 2 allied large roundworms *Ascaridia columbae* and *A. galli* the hairworm *Capillaria obsignata*, *C. columbae* and the spiralworm *Synhimantus* (=*Dispharynx*) *spiralis*. All these parasites inhabit the small intestine and the incidence data indicate that they were dominated by the pigeon ascarid species. Then, with the exception of the fowl ascarid, male pigeons were found to be more prone to infection by these nematodes than their female counterparts, whereas the reverse was the case in respect to the index of parasite burden.

As shown in Table 1, as many as 10 species of ectoparasites were collected and differentiated from the pigeons that were sampled. Eight of them were insects, 6 belonging to Mallophaga, namely, the wing and tail feather chewing lice *Columbicola* sp., the down feather or fluff louse *Goniocotes gallinae*, and the small pigeon louse *Campanulotes bidentatus*; the small body louse *Menacanthus cornutus*, the head and neck louse *Cuclotogaster heterographus* and the pigeon whole body louse *Hohorstiella lata*; 1 belongs to Diptera, namely, the pigeon flatfly or louse fly *Pseudolynchia canariensis*, and 1 belongs to Siphenoptera, namely, the cat flea *Ctenocephalides felis*. The other 2 ectoparasites found belong to Arachnida, comprising 1 Acarina, namely, the fowl red mite *Dermanyssus gallinae* and 1 Parasitiformes, namely, the Persian poultry soft tick *Argas* (*Persicargas*) *persicus*.

The infestation rates of the above external parasites were variable, but only the *Columbicola* sp. and *P. canariensis* were noted infesting more than half of the sampled pigeons. The level of infestation in terms of parasite burden also showed variation and in such a pattern that *Columbicola* sp was the highest level and *D. gallinae* the lowest. Differences in the infestation of the 2 sexes were often manifested. However, the average data for all

species involved tends to diminish them, where p-values fall under the threshold for significance level.

DISCUSSION

A randomised sample of the rock pigeons probably provided a helpful set of morphometric measurements, employed before in connection with the infection prospective study by Dranzoa et al. (8). Such data should be provided in time to serve as an arbitrary indicator of the development and maintenance of body condition and viability fitness by the adults, as well as to hygiene practice. Data from different countries and various health conditions for these birds are needed to substantiate the impact of such a relationship. On the other hand, the parasitological findings seem to validate a fairly good health status of the pigeons. Such a situation may be explained by a number of interrelated factors. For example, suitable climatic conditions from the parasite aspect, and from the host aspect poor sanitation, crowding, low-energy food, interspecific and intraspecific competitions for resources.

Candida sp. was encountered in about a quarter of the pigeons. -However this was always in a non-invasive form and without a single case of massive infection and associated clinical signs, which often cause the appearance of whitish plaque in the upper digestive tract. The prediction of Tsai et al. (30), Ritchie et al. (3), and Kaufmann (4) is that this worldwide parasite may occur naturally in the alimentary canal of pigeons and doves as well as a wide range of other avian species, including the commercially important ones like chickens, turkeys, ducks, geese, pheasants, partridges and quails.

The oocysts of *Eimeria* are also commonly transmitted to and found in otherwise intact pigeons without gross pathological manifestations of acute gastroenteritis (31). Indeed, low infection by these parasites may even be protective by boosting the

Table 1. The parasite component community of the eastern rock pigeon (*Columba livia gaddi*) from Iraq together with infection sites and indices

Parasite name	Infection site	Infection rate (%)		Parasite burden	
		♂	♀	♂	♀
Candida sp.	Upper digestive tract	22.0	28.3	1+	1+
E. labbeana	Intestine (oocysts in faeces)	17.1	24.5	1+	1-2+
T. gallinae	Upper digestive tract	58.5	71.7	2+	2+
H. columbae	Gametocytes in RBCs	73.2	49.1	1.5	0.9
Plasmodium sp.	Schizonts in RBCs	31.7	41.5	1.1	1.6
C. polyacantha	Small intestine	19.5	18.9	4.5	5.0
C. satpuliensis	= =	17.6	11.8	5.3	4.7
C. columbae	= =	11.8	8.8	2.0	3.3
C. digonopora	= =	7.3	5.7	1.7	1.3
R. tetragona	= =	9.8	20.8	2.5	1.8
R. (R.) carpohagi	= =	8.8	2.9	4.3	3.0
R. bonini	= =	4.9	1.9	2.5	2.0
R. fuhrmanni	= =	-	3.8	-	1.5
A. columbae	= =	24.4	13.2	2.6	4.0
A. galli	= =	5.9	11.8	2.0	3.3
C. obsignata	= =	9.8	3.8	2.8	2.3
S. spiralis	= =	4.9	1.9	3.5	3.0
Columbicola sp.	Wing and tail feather	64.4	57.6	~ 60	~ 45
G. gallinae	Down feather	17.1	15.1	~ 30	~ 25
C. bidentatus	Body skin	7.3	1.9	~30	~15
M. cornutus	= =	12.2	13.2	~ 30	~ 30
C. heterographus	Head and neck	4.9	17.0	~ 35	~ 25
H. lata	= =	7.3	11.3	~ 20	~ 40
P. canariensis	= =	61.0	43.4	3.4	5.7
C. felis	= =	4.9	9.4	2.0	2.7
D. gallinae	= =	7.3	18.9	3.0	2.8
A. persicus	= =	14.6	17.0	4.2	11.5

immune responses against further infections, as is the case with poultry (4, 5). However, when large numbers of mature and sporulated oocysts are ingested, especially by the susceptible and debilitated birds, inevitably profound immunosuppression will have the upper hand which leads to the development of a true coccidiosis (2, 3). *E. labbeana* was first recorded from Iraq in domestic pigeons by Al-Janabi et al. (15) and in rock pigeons by Shamsuddin et al. (22). In comparison with the present infection rate of 21.3%, the data from other countries cover a full range. Dranzoa et al. (8) detected no oocysts among the intestinal material of the rock pigeons from Uganda; whereas Kulisic (32) from Yugoslavia, Martinez-Moreno et al. (33) from Spain, Boado et al. (6) from Cuba, Catelli et al. (7) from Italy and Koroglu et al. (31) from Turkey obtained infection rates in specimens of the same host of 24.2%, 63.3%, 68.8%, 25.0% and 15.1%, respectively. The two remarkably similar species of coccidians more often found by these workers, whether or not confirming their identification through cultural sporulation, were *E. labbeana* and *E. columbarum.*

The infection rate of *T. gallinae* was the highest among all the parasites isolated in this study. This is a finding that one would expect, for example, since this flagellate protozoan is of cosmopolitan distribution and generally looked upon as a normal digestive tract inhabitant. Then, as also mentioned by Levine (28) and Soulsby (2), the vast majority of the older pigeons may be carriers of this organism. This means that they easily can introduce it to their young squads while feeding them the regurgitated holocrine substance or "cropmilk". The levels of parasite burden in male and female pigeons, as noted in this study, would prove that the presence of light infections by *T. gallinae* is by no means indicative of a true and cankerous trichomoniasis. Martinez-Moreno et al. (33) and Toulah (34) identified trichomonads in the upper digestive tract and associated organs of the normal wild and domestic pigeons from Spain and Saudi Arabia, respectively. The respective incidence rates they presented were 79.2% and 63.0%, which are comparable to those of the present study. Noticeably lower figures for *T. gallinae* infections in rock pigeons were presented by other, for example, 11.0% from Chile (9), 26.5% from Brazil (35) and 32.0% from Italy (7). I It would be possible to say that a considerable proportion of the world's populations of the rock pigeon at present harbour this protozoan.

The haemoprotozoan *H. columbae* has been recorded previously from Iraq, in both rock pigeons and domestic pigeons (15, 22, 24). The latter researcher obtained an approximateinfection incidence and intensity of 39% and 23% in RBCs respectively; the first figure is somewhat lower and the second one significantly higher in comparison with the present data. This is the first mention of a *Plasmodium* sp. occurrence in the rock pigeons from Iraq. Parasitaemia due to these parasites has been repeatedly detected in pigeons and many other species of wild and domestic birds worldwide, for example, for the haemoproteid species, Martinez-Mareno et al. (33) from Spain, Earle and Little (36) from South Africa, and Dranzoa et al. (8) from Uganda found in the rock pigeon samples infection rates of 26.7%, 73.0% and 76.5%, respectively. Gulanber et al. (37) and Senlik et al. (38) obtained average infection rates for *H. columbae* in adult domestic pigeons from Turkey of 43.2 and 18.8%, respectively.

In contrast to the findings of Earle and Little (36), male pigeons in this study were found more prone than females to infection by *H. columbae*. On the other hand, Senlik et al. (38) were unable to detect a significant difference in the infection rate of this parasite in terms of host sexuality, but they found that such an infection was a subject of marked seasonal variation. Several endogenous and exogenous factors may have an accumulative influence on the parasitisation of both sexes of the pigeons by these parasites, such as host's hormones and humoral compounds, age and nutritional state, behaviour and habits, as well as the season of the year and ecological and physical features of the regions.

Concurrent with the infection by *H. columbae*, Dranzoa et al. (8) provided a figure of 29.4% for infection of the rock pigeons by the *Plasmodium* parasites. This figure is close to that obtained for male but lower than that for female pigeons in the present study. The general opinion is that this and the preceding blood parasite are related in many characters and considered by many to be either benign or of mild effect (36, 39). They are continually circulated around the world and any variations in their prevalence, intensity and health impact, whether sex-related, seasonal-related or spatial-related, might depend on the susceptibility of the host species involved, their ages, habitats, as well as congruence, transmission, density and feeding habits of their vectors. As also referred to by the forenamed workers, the respective incriminated vectors of these parasites have been identified. They include a number of mosquitoes of the genera *Culex* and *Aedes* for *Plasmodium* and flies of the genera *Hippoboscid* and *Phlebotomus* and midges of the genus *Culicoides* for *Haemoproteus* spp., respectively. Many species of these haematophagous biting insects are present throughout Iraq and its neighbours.

With regard to the tapeworms, the present findings suggest the existence of many interspecific similarities for the members of both of the genera *Cotugnia and Raillietina*. This is in agreement with Schmidt (29), Sawada et al. (21) and Dranzoa et al. (8), the information gathered from the study of ova and gravid proglottids from the host's excrement may be useful but often not sufficient for their definite diagnosis. The identification of a species level among these cestodes is best made by detecting the parasites' strobilae and scolices among the scrapings of the intestinal mucosa and carefully analysing the characteristics revealed by the microscopic inspection.

For *R. bonini*, this is the first mention in Iraq. However, it has been reported in samples of the rock pigeons from several other counties; frequently but not always with comparably low rates, for example, 0.9% from Italy (7), 1.0% from Spain (33), 7.5% from Yugoslavia (32), and 45.7% from Brazil (40). The fowl tapeworm *C. digonopora* was previously reported from Iraq only in the wood pigeon (41). It is known to infect chickens and other poultry in many parts of the world. With respect to the rock pigeon, Chahota et al. (42) found the infection rate of this species in a sample from India to be 6.25%. The other species of tapeworms encountered were all recorded previously in pigeons, doves or chickens from Iraq, viz., *C. polyacantha* in 18.0% of rock pigeons from the Baghdad area (14); *C. satpuliensis* in 29.4% of domestic

pigeons from Arbil (21); *C. columbae* in 0.22% of domestic pigeons from northern Iraq (24); *R. tetragona* in 18.5% of domestic fowls from the Mosul district (43), and in 0.22% of domestic pigeons from northern Iraq (24); *R. carpohagi* in 15.4 % of domestic pigeons from Arbil (21); and *R. fuhrmanni* in 17.0% of palm doves from the Baghdad area (14). It is worth mentioning that, among the new davaineids described from the rock pigeons of Saudi Arabia by Magzoub et al. (44), there was a species having morphometrics close to those of *R. tetragona* and another one to *C. digonopora*.

In conformity with the situation in the chukar partridge, when the global data for the cestode infection of male and female pigeons were considered, no significant sex differences were apparent (45). Such observations seem to contradict those of Srivastava and Srivastava (46), where significantly higher annual mean figures for infection by these parasites were obtained in the female rock pigeons from Allahabad, India. These researchers attributed such differences to enhanced susceptibility in female birds produced by greater stress due to frequent fluctuation in their hormonal and metabolic activities during the active reproductive stages. On the other hand, the significant seasonal variations noted by Al-Aloosi (41) in the infection rates of some intestinal tapeworms in the wood pigeon (*C. palumbus*) from Iraq might be attributed to the changeable behaviour of this host species, particularly in relation to annual fluctuations in the foraging habitat.

No doubt availability of information relevant to the ecology and life histories of the avian tapeworms in Iraq will be very useful in order to investigate systematically their epidemiology and control. Elsewhere, these parasites were shown to have evolved indirect cycles whereby the ova develop into infective larvae (cysticercoids) in the tissues of some invertebrates (4, 5, 29, 47). The identity of these intermediaries may vary with the tapeworm species but it often comprises one or other of such insects as houseflies and allied dipterans, some hymenopterans such as ants, and some beetle species.

Only one of the species of the presently found nematodes in the rock pigeons was previously identified from Iraq which is *A. galli* in domestic fowls (43, 48) and domestic pigeons (24). The approximate figures for infection rate and parasite burden presented by the latter researcher were 56.4% and 30%. Higher infection figures with nematode parasites were found by Senlik et al. (49) in the domestic pigeons from the Bursa province of Turkey. Similar to many other pigeon parasites, infection by nematodes that involve low incidences and/or low intensities are expected to be often endurable. Only those infections that become too severe are dangerously harmful, which are frequently found in disabled, weakened or senile hosts.

The individual and global infection indices obtained for the nematode species are in general agreement with the range of data obtained by other workers also for apparently healthy-looking and free-living pigeons (6, 7, 9, 32, 33, 40). Nevertheless, such hosts must be treated as a potential source of infection to domestic pigeons and other susceptible birds. In this connection, each of the 4 nematode parasites that were identified in this study has a direct life cycle (2-5, 50). This means that the ova are passed in faeces and under favourable environmental factors (especially high temperature and adequate humidity) they develop into infective stage.

Ectoparasites are prevalent on wild and domestic pigeons throughout the world. Both their host specificity and deleterious impact vary greatly. In Iraq, the works of Khalaf (18, 19) and Abul-Hab (12, 13) reveal that all species of these parasites presently found were previously encountered on poultry and sometimes on pigeons and doves. However, these earlier studies were largely faunistic in nature. On the other hand, the infestation rates presented by Zangana (24) and Abdul-Karim et al. (10), for some of these parasites fit within the respective ranges of the present data. The infestation situation of pigeons is probably quite similar to that of chickens (51, 52); that is, that the patterns of relationship between ectoparasites and their surrounding environment are important in this respect. Specifically, it is likely that the atmospheric temperature constitutes the dominant factor in controlling their occurrence, abundance and diversity throughout the year.

As stated in results, the *Columbicola* lice collected in this study were reserved at the generic level. These interesting insects were first noticed in Iraq on pigeons and collared doves in Baghdad and identified as *C. columbae* and *Columbicola* sp., respectively (18). Pigeons from the Na'maniyah district about 120 km south of Baghdad were also found to be infested by *C. columbae* (19). This largely pigeon and dove specific louse was recently thought to be the same as that found in north Iraq on turkeys and chukar partridges (45, 53). However, earlier examination by Eichler and Abul-Hab (17) of the *Columbicola* forms of feather lice occurring on domestic and rock pigeons at Baghdad concluded that they all belong to *C. montschadskyi*. Hitherto, only a third species of this group of closely allied lice was reported from the Iraqi pigeons, and that is *C. tschulyschman* (11, 12, 24). The comparable forms of lice infesting pigeons in Turkey were invariably classified as *C. columbae* (54, 55). Furthermore, this species is of common occurrence on pigeons across Europe (27, 56, 57).

The pigeon flatfly *P. canariensis* was previously observed on pigeons in the Mosul province of north Iraq (16). It was also found infesting Istanbul pigeons (54). This louse-like dipteran fly feeds on blood and develops especially in juvenile birds. It also contributes in spreading some obligate blood parasites including *Haemoproteus* spp. and *Leucocytozoon* spp. (25, 39). Furthermore, a number of lice and mites species are believed to have evolved a phoretic association with this particular species of hippoboscid fly (8). They normally do not feed on these flies but rather may use them for transport from one bird individual to another.

The poultry red mite *D. gallinae* is another blood-sucking parasite. According to Ritchie et al. (3) and Kaufmann (4), *D. gallinae* may even be fatal especially to young birds, as well as transmitting viral, rickettsial and protozoan diseases including equine encephalitis and borreliosis. Inhabitants of the pigeon-infested buildings in Iraq often complain of bites. Indeed, some coincident events involving the red mites on the pigeons sampled for the present study confirmed that this species is prone to attack and bite human beings.

The Persian soft tick *A. persicus* is also a bloodsucker. It is prevalent among poultry in north Iraq, especially on traditionally-raised free-range chickens that have been denied adequate health care (Al-Barwari and Saeed - unpublished observation) (58). It is well documented that this tick is an important agent in spreading viruses, rickettsiae, bacteria and protozoa among pigeons and many other wild and domestic animals (2, 4, 59).

CONCLUSION

A number of final conclusions can be drawn from the findings of this study and reflections upon their comparison with the data of other workers. They are:

1. The rock pigeons constitute a continuum subject of infection by a diversified community of endoparasites as well as infestation by ectoparasites.
2. Adult rock pigeons can tolerate certain thresholds of naturally occurring disease agents without displaying gross pathological changes and clinical signs of sickness. Therefore, these birds should better be looked upon as a potential reservoir for spreading many parasitic and microbial species to other types of pigeons and doves, poultry, game birds, prey birds and some members of other avian orders.
3. Hyper-parasitisation is generally rare in the population of free-living rock pigeons,but, when occurring, may result in developmental instability. This may be attributed to a disturbance in the natural equilibrium between the hosts and parasites leading to a disease outbreak accompanied by acute symptoms and increased mortality rate (8, 20).
4. To man, pigeons of today seem to have completely outlived all their past usefulness and instead classified as vermin. An increased number of them in the intimate human environment may constitute sanitary, nuisance and other troubles.
5. Any comprehensive strategy aiming at the effective control of pigeon parasites, should adopt the policy of simultaneously controlling a number of invertebrate species, which serve either as vectors or intermediaries for many forms of these parasites.
6. The quality of eradication of pigeons from around the poultry breeding and layer farms as well as from food and water handling facilities is an essential factor forcounteracting disease dissemination. The same of course applies to other bird pests like house sparrows and starlings.

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Conflict of Interest

No conflict of interest was declared by the authors.

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Parasitosis of the Chukar Partridge, *Alectoris chukar* in North Iraq

Kuzey Irak'ın Kınalı Keklik (*Alectoris chukar*) Parazitleri

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ABSTRACT

Objective: The aim of this study was to establish the identity of the parasitic communities of the chukar partridge from Iraq as well as reporting on the prevalence and intensity of various infections from several localities and comprising both sexes.
Methods: Twenty-nine live adult chukar partridge (*Alectoris chukar*) were obtained from several localities across the northern provinces of Iraq. Blood samples were examined for haemoprotozoa, carcasses were assessed for the ectoparasites throughout the body skins and feathers, and the alimentary canal was examined for protozoans and helminths.
Results: All of the examined animals were infected with the 18 different endo- and ectoparasite species identified. These include 2 protozoans, 3 cestodes, 2 nematodes, and 11 arthropods. The overall figures suggest no significant differences in infection indices in terms of the host's sexuality. Pathogenicity of the parasites involved is briefly emphasised. Furthermore, the role of the chukar in the dissemination of these disease agents among populations of other avian species is discussed.
Conclusion: With the exception of *C. latiproglottina*, all of the species differentiated represent new records for chukar from Iraq, and the 2 coccidians are new addenda to the country parasitological list. *(Turkiye Parazitol Derg 2012; 36: 240-6)*

Key Words: Chukar partridge, parasite, endoparasite, ectoparasite, Irak

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ÖZET

Amaç: Irak'tan toplanan kınalı kekliklerde bulunan parazitlerin yaygınlığı ve yoğunluğunu belirlemek amacıyla yapılmıştır.
Yöntemler: Irak'ın kuzey eyaletlerinden 29 yetişkin kınalı keklik (*Alectoris chukar*) canlı olarak toplanmış, deri ve tüyler ektoparazit, sindirim kanalı protozoon ve helmintler, kan ise Haemoprotozoa yönünden incelenmiştir.
Bulgular: İncelenen örneklerin tümü parazitlerle enfekte bulunmuş ve hayvanlardan 2 protozoan, 3 sestod, 2 nematod ve 11 artropod olmak üzere toplam 18 farklı tür parazit saptanmıştır. Sonuçlar, konak cinsiyetine göre enfeksiyon sıklığında önemli bir farklılık olmadığını göstermiştir. Parazitlerin patojenitesi de kısaca incelenmiş, bu hastalık ajanlarının diğer kuş türleri arasındaki dağılımında kınalı kekliklerin oynadığı rol de tartışılmıştır.
Sonuç: *Cotugnia latiproglottina* dışında diğer türlerin tamamı Irakta kınalı keklikte ilk kez bildirilmekte, *Eimeria kofoidi* ve *E. caucasica* ise Irak için ilk kayıttır. *(Turkiye Parazitol Derg 2012; 36: 240-6)*

Anahtar Sözcükler: Kınalı keklik, parazit, endoparazit, ektoparazit, Irak

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INTRODUCTION

The chukar partridge (*Alectoris chukar*) is a common Eurasian species. It is the most important native game bird of the northern region of Iraq (1). Two of the 14 suggested subspecies are seen in the wild in this country, namely, *A. c. kurdestanica* and *A. c. werae* (2, 3). They are undoubtedly very similar to each other in size and other morphometric traits, general appearance and habits, to the extent that

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cross-breeding may occur between them. However, it is generally believed that the populations of these subspecies have little overlap in their geographical distributions; for example, the range of *kurdestanica* is much wider extending from Armenia across southeast Turkey and north Iraq to Iran, while that of *werae* is merely confined to certain habitats in northeast Iraq and west Iran.

The chukar partridge is known to be capable of producing hybrids with some other members of the genus *Alectoris* (2, 4). Indeed, its hybrids are often more tamed and therefore much easier to be bred and raised on a large-scale. Several nations of the Mediterranean and Middle East regions have taken the male of *Alectoris* partridge as a symbol of bravery, intelligence and wickedness, and its female as a symbol of passion and motherliness.

Parasitological studies have been conducted on a number of *Alectoris* species and subspecies in several parts of the world. A wide variety of endo- and ectoparasite species were recovered and identified from them. The first category is mainly composed of gastrointestinal protozoans and helminths, with average infection figures roughly clustering around 50%. Relevant data were made available upon many investigations, such as those of Ruff and Wilkins (5) on *A. chukar* from the USA; Vasilev (6) on *A. chukar kleini* from Bulgaria; Reck and McQuistion (7), Koroglu and Tasan (8, 9) and Perrucci et al. (10) on *A. graeca* from the USA, Turkey and Italy, respectively; Belleau and Leonard (11) and Rizzoli et al. (12) on *A. graeca saxatilis* from France and Italy, respectively; Moretti et al. (13) and Carvalho-Varela and Ferradeira (14) on *A. rufa* from Italy, Spain and Portugal, respectively, and Calvete et al. (15) also on *A. rufa* from Spain. With respect to the ectoparasites, less systematic research seems to have been done (14, 16, 17), but nevertheless reveals infestation of these birds by many species, most commonly lice, fleas and ticks.

On the other hand, the situation in Iraq, as in other Middle East countries, still lags behind. That is to say, the knowledge on *Alectoris* partridges is mostly narrative and zoogeographical. Indeed, the literature survey shows that these birds were examined with a parasitological intention on only two occasions. Arthur (18) described a few specimens carrying a new species of ixodid ticks (*Ixodes tatei*), while Sawada et al. (19) found a single specimen of a new species of davaneid tapeworms. Upon the description of the latter, the name *Cotugnia latiproglottina* was assigned to it (typewritten in the original abstract as *C. paucitesticulata* by mistake).

The present study, therefore, aims to establish the identity of the parasitic communities of the chukar partridge from Iraq as well as reporting on the prevalence and intensity of various infections. The sample size employed was much larger than the preliminary ones referred to above, as well as being more representative by virtue of obtaining it from several localities and comprising both sexes.

METHODS

The study depended on examining 29 (11 male and 18 female) chukar partridge, *Alectoris chukar* (Gray, 1830) (Galliformes, Phasianidae). All of the specimens were adult and older than one year, as judged from the observation of the plumage pattern and weight. Sex differentiation was always confirmed by gonadal checking. The birds were obtained alive from hunters or dealers on different occasions from several localities across the northern provinces of Iraq. The vast majority of them were recognised as *A. c. kurdestanica* Meinertzhagen, 1923, and only a couple (probably 3) as *A. c. werae* (Zarudny and Loudon, 1904). Accordingly, no attempt was made to discriminate the parasitological data on the basis of the host subspecies.

Each bird was given a thorough examination with the results showing that almost all of them were vigorously active. None of them showed injuries, marked skin lesions (such as hyperdermatosis, epidermitis and acanthosis) or excessive feather loss or damage. However, some of them discharged moistened faeces and a pair had severe diarrhoea. The latter exhibited poor feeding and drinking and their bodies were markedly dehydrated.

The respective average figures of 6 morphometric parameters that were determined for 10 male and 10 female chukar specimens were as follows: body length (bill to tail) 36.4 and 31.7 cm, body mass 703.8 and 448.5 g, wing cord 47.9 and 45.9 cm, tail length 83.3 and 80.5 cm, tarsus length 47.5 and 43.9 cm, and bill length 15.0 and 14.4 cm.

Blood samples were employed following a procedure specified by Al-Barwari and Saeed (20). From each partridge, a small amount of blood was drawn from the wing vein. This was prepared into thin smears that were air-dried, fixed in absolute methyl alcohol and stained following the Giemsa method (5%, pH 7.2). The slides were microscopically examined for the presence or absence of haemoprotozoa.

Partridge carcasses were assessed for the ectoparasites throughout their body skins and feathers. Wherever found, these organisms were mostly removed by fingers and sometimes by small forceps. Preservation, clearance, staining and species differentiation of these parasites were also performed as described previously (20). The same was applied to the determination of the prevalence rates and intensities of infection for each species and for both sexes of the host.

Evisceration of each bird was then performed and the alimentary canal was opened by a longitudinal incision. Fresh samples of the contents of and scrapings from the mucous membranes of each part (mouth, oesophagus, crop, stomach, gizzard, small intestine, rectum and caeca) were transferred to glass slides, mixed with drops of saline, covered with coverslips and examined microscopically for protozoans, helminth segments and ova. Livers and gallbladders were examined for trematodes, and tracheas for nematodes. Upon finding the adult helminths, they were collected separately from each bird, cleared in saline, counted, identified as far as possible, fixed in 70% ethanol, and then stored in 70% ethanol + 5% glycerine for subsequent use.

Standard procedures were frequently employed for the purpose of permanent preparations of any gastrointestinal protozoan, cestode and nematode parasites that were present. Definite identification to the species level was achieved by studying the characteristic features of the parasite forms. A floatation technique was often employed to confirm the identification of *Eimeria* species; it was also used to arbitrarily determine the

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infection density for these parasites, for example, by designating 1+ for asymptomatic infection and where relatively only small numbers of oocysts were detectable; 2+ for moderate infection and symptoms, such as slight diarrhoea; and 3+ for acute symptoms, including severe diarrhoea, emaciation and in appetence.

RESULTS

The total number of parasite species encountered in and on the chukars was 18. Thirteen (44.8%) of the specimens examined were found to harbour at least one helminthic species. However, when combined with the results on ectoparasites, all of the host specimens were found to be parasitised with one or more species. Indeed, about 50% of the partridges harboured 7 or more species of parasites simultaneously.

Table 1 Lists the scientific names of the parasites that were found together with their infection sites in or on both sexes of the host, as well as the respective infection rates and parasite burdens. Internal infections were only encountered in the small intestine and caeca. The upper digestive tracts of all of the sampled chukars were found to be negative for the infection by the flagellate protozoan *Trichomonas gallinae*, and no haemoprotozoans, like *Haemoproteus* and *Plasmodium,* were detected in blood smears or trematode species in livers or gallbladders. The mouth, oesophagus and crop of all of the sampled chukars revealed no infection with *Capillaria* nematodes. Also, the tracheas of all specimens were found to be free of infection by helminths like the gapeworm *Syngamus trachea.* However, two species of intestinal protozoans, three species of intestinal tapeworms, one species of intestinal and another of caecal nematodes, and 11 species of ectoparasites were identified.

The two eimeriid protozoans recovered were identified as *Eimeria kofoidi* Yakimoff and Malikaschwili, 1936 and *E. caucasica* Yakimoff and Buewisch, 1932. The host site preference for either of the above species is the small intestine. Their prevalence was moderately higher in female than male hosts. The index of parasite burden, although only arbitrarily measured for infection by these species, tends to suggest the existence of an association between the intensity of infection and severity of symptoms in the diseased specimens.

The three forms of tapeworms differentiated were all found to be davaineid species, namely *Raillietina* (*Raillietina*) *tetragona* (Molin, 1858), *Cotugnia latiproglottina* Sawada et al. (19) 1990 and *Choanotaenia infundibulum* (Bloch, 1779). The vast majority of the specimens were bound through their scolices to the mucosa of small intestine, but some were present free in the lumen of this organ. Male chukars seemed to be more prone to parasitisation by anyone of these tapeworms than the female counterparts, but it is unclear whether that is in terms of prevalence or intensity.

One of the two nematode species that were identified, namely *Heterakis gallinarum* (Schrank, 1788) was encountered in the

Table 1. Parasites harboured by the chukar partridge from north Iraq together with the infection indices in male and female sample

Parasite name	Infection site	Infection rate (%)*		Parasite burden**	
		♂	♀	♂	♀
*E. kofodi***	Intestine (oocyctc in faeces)	36.4	50.0	1+-2+	1+-3+
*E caucasica***	= = = =	27.3	44.4	1+-3+	1+-3+
R. (R.) tetragona	Small intestine	27.3	16.7	2.3	2.7
C. latiproglottina	= =	18.2	11.1	6.0	3.0
C. infundibulum	= =	9.1	0	2.0	0
H. gallinarum	Intestinal caecum	18.2	27.8	4.0	2.4
A. galli	Small intestine	9.1	16.7	1.7	1.3
M. cornutus	Body skin	45.5	38.9	54.2	27.6
C. columbea	Wing feather and skin	9.1	22.2	2.0	3.8
G. dispar	Feather and skin	9.1	5.6	7.0	28.0
C. heterographus	Head and neck	0	11.1	0	10.5
L. caponis	Wing feather and skin	0	5.6	0	7.0
C. lectularis	Body skin	9.1	11.1	2.0	1.3
C. felis	= =	18.2	0	1.5	0
D. gallinae	= =	9.1	16.7	1.7	2.3
I. frontalis	= =	9.1	5.6	2.0	1.0
H. asiaticum	= =	0	11.1	0	1.0
A. persicus	= =	27.3	16.3	3.3	2.7

*The average percentage of prevalence of the parasite species among all the host's specimens sampled
**The infection intensity or the average number of the parasite individuals in the infected cases
***Intensity of infection is arbitrarily determined (see Materials and Methods)

caeca, whereas the second species, *Ascaridia galli* (Schrank, 1788), was found in the small intestine. The posterior extremity of an adult male of the latter species revealed some minor morphological variations in comparison with the specimens commonly found in Iraqi poultry (chicken, turkey and duck). For both of these nematode parasites, it was the female chukar that showed the higher susceptibility for infection, while the opposite was noted in respect to the index of parasite burden.

The study encountered 11 species of ectoparasites. Seven of them were insects, comprising five Mallophaga, namely, the small body louse *Menacanthus cornutus* Schrommer, 1913, the feather louse *Columbicola columbae* (Linnaeus, 1758), the large louse *Goniodes dispar* Burmeister, 1838, the head louse *Cuclotogaster heterographus* Nitzsch, 1866, and the wing louse *Lipeurus caponis* (Linnaeus, 1758); one Heteroptera, the common bed bug *Cimex letularius* Linnaeus, 1758, and one Siphonaptera, the common cat flea *Ctenocephalides felis* (Bouche, 1835). The other four species were Arachnida, the first an Acarina, the poultry red mite *Dermanyssus gallinae* (De Geer, 1778), and the second, third and fourth Acari, the front hard-bodied ticks *Ixodes frontalis* (Panzer, 1798), Asiatic hard-bodied tick *Hyalomma asiaticum* Schulze and Schlottke, 1929, and Persian poultry soft-bodied tick *Argas persicus* (Oken, 1818).

Harbouring of chukar to ectoparasite species revealed a wide range of variability in terms of prevalence (approximately 0-46% in males and 0-39% in females). The highest sex-combined infestation rate recorded was for *M. cornutus*, followed by *C. columbae* and then *D. gallinae*; whereas the lowest such rate was for *L. caponis* and *H. asiaticum,* where in either of them only a single case of infection was found (Table 1). Differences were also detected with respect to parasite burden (approximately 0-54 in males and 0-28 in females), again with higher average figures for *M. cornutus* but followed by *G. dispar*; whereas the lowest burden was in *H. asiaticum* followed by *C. felis.*

DISCUSSION

A couple of the chukar specimens suffering from acute diarrhoea were constantly lighter in weight than the rest. They often harboured more parasite species, with coccidians always being present in their intestines. This finding supports a previous one made on the rock pigeon, which indicated that some of the biometric measurements might turn out to serve as an arbitrary indicator of the general health status of the adult bird (20).

Examination of the chukar for endo-parasitosis revealed its infection with cestodes and nematodes besides coccidians, but not with any trichomonal, blood protozoan, trematode or acanthocephalan species. This, in a general sense, is in agreement with what one would expect in the structure of the parasitic communities of this species; partly because of being a seedeater and preferring dry and rocky habitats, and partly in light of the results obtained by several other researchers in comparable ecosystems. However, Millan et al. (21) were able to record for the first time a *Haemoproteus* sp. in *A. rufa* from Spain. Also, few digenetic trematodes, such as *Brachylaemus fuscatus*, *Dicrocoelium petrovi* and *Dicrocoelium* sp., have been recovered from adults of other *Alectoris* partridges (6, 11, 12, 15, 22). The absence of *Trichomonas* infection in partridges may be explained through the mode of their feeding that does not involve "crop milk", even at a very early age after hatching, without totally eliminating the possibility of some inherited resistance to such infection. The scarcity of blood parasites in these birds is likely to be due to the scarcity of vector agents, but again without eliminating the possibility of some immunological resistance and probably the density of the definitive hosts in that matter. As to the relevance to trematodes, they may well be related to the availability of ill-suited intermediary hosts, which are essential to the survival of their infective stages as well as their free-living stages surviving the outside physical and environmental factors.

The present study showed that at least two species of eimeriid coccidians *(E. kofoidi* and *E. caucasica*) are capable of parasitising the Iraqi population of chukar. Besides Russia, which is the country in which they were first discovered, both were also found to be widely distributed among the partridge species in some other countries, for example, 76% and 38% of *A. graeca* from Turkey, respectively, and *E. kofoidi* in 59% of *A. graeca* in the USA (7, 8, 23, 24). It is worth mentioning here that the total number of *Eimeria* spp. described from *Alectoris* partridges may have reached 13 (10, 25, 26). However, several of them are widely looked upon as being synonyms of only two or three genuine ones, that is, those which were also recovered in this study and *E. alectorae* (27, 28). The latter species was first found in the chukar from India.

The varied pathogenicity produced by coccidian parasites in the sampled partridges was comparable to that observed elsewhere by others (5, 11, 13, 14). That is to say, such infections may or may not be associated with clinical symptoms of acute disease. This suggests that mild infection of these birds by the gastrointestinal coccidians, similar to the situation in poultry and many other species, might be of little effect if not of some advantage to them due to the development of an immunological mechanism (27, 29). Several anti-coccidial drugs have been tried in chukar partridges, such as ormetoprim, sulphamethazine, amprolium, dinitolmide, monensin, lasalocid, and sulphonamides. However, it is a medication through feed with sodium sulphamethazine, or a 5:3 mixture of sulphadimethoxine and ormetoprim (Rofenaid), which is apparently much more effective in the treatment and prevention of this type of parasitism in these birds (5, 7).

The tapeworm *C. latiproglottina* was first described by Sawada et al. (19) in chukar from north Iraq. They encountered it as a single mature, non-gravid worm in the small intestine of one out of three *kurdestanica* specimens obtained from Hareer County, east of Erbil province. The original description of its specific characteristics were found to be justifiable, for example, it is similar to *C. transvaalensis* Ortlepp, 1963, which was first recorded from the Guinea fowl, but differ from it by having no less than 3-fold longer strobila, a longer vitelline gland and a more massive ovary, with the outline of the latter organ being finally and not coarsely lobated. The present study also extends the epidemiological knowledge pertinent to the infection of chukars by this parasite.

With regard to the two other species of tapeworms found (*R. (R.) tetragona* and *C. infundibulum*), this is the first record of their

presence in partridges from Iraq. However, both of them have been reported in these birds from some other places, for example, the former with a prevalence of 6.0% in *A. graeca* from Turkey (9), and 8.6% (intensity of 1-5) in *A. Rufa* from Spain (15); and the latter with a prevalence of 4.0% in *A. graeca* from Turkey (9), and 8.6% (intensity of 1-10) in *A. rufa* from Spain (15).

The fact that the tapeworm species mentioned above are capable of producing diseases in poultry reveals the importance of encountering them in this study (29). Furthermore, they are also capable of infecting several other avian species. In as far as Iraq is concerned, *R.* (*R.*) *tetragona* has been found in 18.5% (intensity of 4.7) of chickens from Mosul (30), 0.2% of domestic pigeons from north of Iraq, 16.0% (intensity of 2.2) of rock pigeons from various localities, and 16.0% (intensity of 2.5) of the white-cheeked bulbuls from around Baghdad City (20, 30-32). This species was also found to infect the Iraqi populations of the domestic pigeon and wood-pigeon (*C. palumbus*), which in the latter host exhibited discernible seasonal variations (33, 34). As for *C. infundibulum*, it has been identified in 18.5% (intensity of 6.7) of chickens from Mosul district (30).

The findings of two species of nematodes (*H. gallinarum* and *A. galli*) represent new records in chukar from Iraq. However, both of them have been identified in some other partridge species from abroad. For example, the prevalence of the former was about 1.0% of *A. rufa* from the United Kingdom, 12.0% of *A. graeca* from Turkey, and about 1.2% (intensity of 1.0) of *A. g. saxatilis* from Italy; the latter totalled 5.0% (intensity range of 2-27) of *A. rufa* from Spain (9, 12, 15, 17).

The above nematode species have also been found in other avian species from Iraq, for example, *H. gallinarum* in 45.8% (intensity of 39.4) of chickens from Mosul, and *A. galli* in 56.4% (intensity of 29.6) of chickens from Mosul, 0.5% (intensity of 25) of domestic pigeons from the north of Iraq, and in 6.4% (intensity of 2.6) of rock pigeon from various localities (20, 30, 31). As is well documented, the deleterious effect of *H. gallinarum* may stretch beyond its own parasitisation, for this caecal worm is an important vector of the virulent flagellate protozoan *Histomonas meleagridis*. According to Potts (17) and Calnek (29), histomoniasis (blackhead disease) may be a cause of a considerable number of deaths in the partridge population, as is the case in many other species of gallinaceous birds in the wild where the food comprises insects and other invertebrates, like earthworms.

In agreement with the findings of Rizzoli et al. (12) on the rock partridge from Italy and Al-Barwari and Saeed (20) on the rock pigeon from Iraq, the data of the present study revealed that the association between chukar sex and the overall prevalence rate or infection intensity of any main category of gastrointestinal parasites was not significant.

Parallel to their parasitisation with endoparasites, the infestation of the chukars with ectoparasites was found to be a common phenomenon in north Iraq. A comparable situation was found regarding infestation of the red-legged partridge *A. rufa* in Spain and Portugal (14, 16). The latter researchers identified and analysed the data relevant to 13 helminthic (one trematode, four cestodes and eight nematodes) and nine ectoparasite species (six chewing lice and three ticks). Generally speaking, the observations made by them, like those of Al-Barwari and Saeed (20) on the rock pigeon, would suggest that the overall intensity of infestation by lice is primarily controlled by environmental factors. However, the data of the present study as a whole do not collate with that of Calvete et al. (16) regarding a greater association between louse species infestation and male but not female partridges. This is true, whether investigating intensity of infestation or species richness.

The 11 species of ectoparasites identified from the chukar collectively represent a new record for this host from Iraq. However, the lists presented in previous studies in this country, such as those of Al-Barwari and Saeed (20), Al-Hubity (30), Khalaf (35), Abul-Hab (36), and Abdul-Karim et al. (37), would reveal that all of the species have been fairly well recorded. Which of them is also an actual parasite of chukar and which is incidentally encountered due to contamination from other more suitable sources is difficult to say with certainty until further samples are analysed. What can be inferred from observations already made in Iraq is that *M. cornutus*, *G. dispar*, *C. heterographus*, *L. caponis*, and *D. gallinae* are commonly found on chickens and other poultry; *A. persicus* is also prevalent among chickens in north Iraq, with high infection rates and heavy parasite burdens in some localities that are difficult to cope with (38); *C. columbae* is a specific parasite of pigeons and doves; while the rest of species encountered parasitise a range of mammals, including humans, as well as many birds. The ixodid tick *I. tatei* was not encountered in this study. This parasite was first described in Iraq by Arthur (18) from the common red fox *Vulpes vulpes,* as well as from the chukar, upon which it preys. The prevalence rates of ectoparasites and the intensity of their infection reported by the above sources from Iraq varied considerably due to variation of such factors like the host species, seasonality, geographical location within the country and nutritional status. As far as the overall differences in the parasitisation of the two sexes is concerned, the averages of the global data for all species involved in the present study tend to suggest that the individual findings may not be as significant as they appear. In other words, the observed differences are perhaps due to such factors like small sample size, the source, and the ecological circumstances and duration of confinement before examination, more than to the impact of some host-specific intrinsic factors like hormones.

The infection indices for any one of the ectoparasites encountered was generally found to be low and probably not of sufficient severity to be solely responsible for the production of clinical symptoms of illness or alterations in the physiological conditions of the host. However, as discussed by Calnek (29), Calvete et al. (16), and Al-Barwari and Saeed (20), they should be looked upon not only as obligate external parasites but also under certain circumstances where the defence mechanisms are weakened (such as in sick, deformed or senile birds), food deprivation, and heavy and prolonged infection with blood and intestinal parasites, as being capable to populate rapidly and causing serious health deterioration, sexual selection, reduction in fecundity and even mortality of the host by predation or otherwise. What should also be emphasised is the vectorial and reservoir roles of these arthropodan parasites, and the fact that some of

them act as intermediary hosts to a number of helminthic parasites. Of special interest in this regard was the recovery in this study of 2 specimens of the Asiatic hard tick *H. asiaticum*. This ixodide species is among a number of suspected vectors that may play a role in the transmission of the causative agents of some deadly diseases in Iraq from animals to humans, such as CCHF and RVF viruses (39, 40). The researchers were informed that the source of both partridges carrying these ticks was a north-eastern area not too far from the border with Iran.

CONCLUSION

The researchers recommend carrying out periodic investigations on both endo- and ecto-parasites of the chukar partridge, as well as any other Iraqi wild species wherever possible. This approach will be useful to evaluate the wildlife situation and perhaps also sense the dangers of dissemination of some of the parasitic species to the economically important animals, pets and humans themselves. A policy involving the strict prevention of infection should be employed to prevent the possibility of introduction of an extinct as well as a new species to a particular territory of the country. As emphasised by Tompking et al. (41), parasite-mediated competition between different species need to be defined and dealt with wherever possible. The likelihood of any potential interaction between autochthonous and allopatric species is threatening and should be avoided as much as possible.

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Conflict of Interest

No conflict of interest was declared by the authors.

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Isospora belli in a Patient with Liver Transplantation

Karaciğer Transplantasyonlu Bir Hastada *Isospora belli*

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ABSTRACT

Isospora belli is an opportunistic protozoon which should be monitored in patients with gastrointestinal complaints such as abdominal pain, nausea and diarrhoea, in both immune-compromised and immune-competent patients. Our case was a 35 year-old male patient who had received a liver transplant because of cirrhosis and hepatic fibrosis. A diarrhoeic stool sample of the patient was sent to the laboratory for microbiological and parasitological analyses. Faecal occult blood was positive and bacteriological analysis was negative. *Isospora belli* infection was diagnosed by detection of the oocysts in stool samples. Per oral trimethoprim-sulphamethoxazole treatment was given in 500 mg bid dose for 10 days. At the end of the treatment, no oocyst of *Isospora belli* was seen but non-pathogenic cysts of *Entamoeba coli* and vacuolar forms of *Blastocystis hominis* were observed. Two months later the patient had abdominal pain, fatigue and diarrhoea again and parasitological re-evaluation showed oocysts of *Isospora belli*. *(Turkiye Parazitol Derg 2012; 36: 247-50)*

Key Words: *Isospora belli*, post-transplant infections, liver transplantation

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ÖZET

Isospora belli, immün yetmezlikli ve/ veya immun sistemi baskılanmış olgularda, karın ağrısı, ishal gibi gastrointestinal şikayetlerle başvuran hastalarda akla getirilmesi gereken fırsatçı bir protozoondur. Olgumuz; 2008 yılında Hepatit B'ye bağlı karaciğer sirozu ve fibroz tanısıyla karaciğer transplantasyonu uygulanmış 35 yaşında erkek hastadır. Hasta parazitolojik inceleme için başvurduğundan bu yana immmun-supresif tedavi almaya devam etmektedir. Karın ağrısı, halsizlik, ishal yakınmaları nedeniyle dışkının mikrobiyolojik ve parazitolojik açıdan değerlendirilmesi istenmiştir. Hastanın yapılan dışkı incelemesinde gizli kan pozitif, bakteriyolojik inceleme negatif olarak saptanmıştır. Parazitolojik incelemede nativ-lugol, parakon dışkı konsantrasyon tüpü ile çöktürme yöntemi ve modifiye asit-fast boyama yöntemleri ile Isospora belli ookistleri görülmüştür. Hastaya 500 mg bid dozda 10 günlük 2x1 trimethoprim-sulfamethoxazole tedavisi uygulanmıştır. Tedavi sonrası dışkı incelemesinde I. belli ookistleri görülmemiş, ancak apatojen amip olan *Entamoeba coli (E. coli)* kistleri görülmüştür. Hastanın 4 ay sonra yine karın ağrısı, halsizlik, ishal yakınmaları nedeniyle parazitolojik incelemeler yapılmıştır. Dışkı örneği incelendiğinde *I. belli* ookistleri yeniden görülmüş ve tedavi önerilmiştir. Sonuç olarak; klinisyen hekimlerin, özellikle immün yetmezlikli ve/veya immun sistemi baskılanmış olgularda karın ağrısı ve ishal gibi gastrointestinal şikayetleri olan hastalarda parazitolojik inceleme yaptırmaları faydalı olacağı kanaatindeyiz. *(Turkiye Parazitol Derg 2012; 36: 247-50)*

Anahtar Sözcükler: *Isospora belli*, transplantasyon sonrası infeksiyonlar, karaciğer transplantasyonu

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INTRODUCTION

Isospora belli (*I. belli*) is a coccidian parasite found in epithelium of the intestines, only causing infection in humans (1-3). Although widely seen all over the world, isosporiasis, which is caused by *I. belli*, is more frequent, especially in tropical and subtropical regions (2, 4-6). In developing countries, the incidence is particularly high in patients with chronic diarrhoea and those affected with-AIDS (1, 2, 7). It accounts for 10-20% of chronic diarrhoea cases of AIDS patients in Haiti and Africa (2).

Infected oocysts of *I. belli* measured about 22-33x12-15 µm in size, with round granular centres (2, 7, 8). In the oocyst wall, each one of the two sporoblasts develops into a sporocyst, which contains four sporozoites that are released from the cysts. Oocysts can survive for months in normal environmental conditions (2, 7). Fluidic and bloodless diarrhoea develops one week after the intake of water and foods contaminated with mature sporule oocysts via digestion. Colic, anorexia, weight loss and abdominal cramps may accompany diarrhoea problems, which sometimes last for 2 or 3 weeks. Fever is generally not common or rarely seen. Oocyst excretion may last for a few weeks after recovery. Cases with extra-intestinal isosporiasis where liver, spleen and lymphatic nodules were affected have also been reported (2, 5, 9).

Isosporiasis leads to acute and self-limiting diarrhoea in people with healthy immune systems, whereas it causes life-threatening persistent enteritis in immune-deficient individuals, particularly in those with AIDS (4, 6, 7, 10, 11).

Isospora oocysts are diagnosed by microscopic detection in stool by an ethyl alcohol-formaldehyde concentration technique, once with and once without iodine for staining; this can reveal suspicious oocyst-like features, so modified acid-fast stains are performed on a fresh smear.

CASE REPORT

This study was approved by the Ethics Committee of Dokuz Eylul University. A 35 year-old male patient who had been living in Balikesir underwent a liver transplantation 4 years ago due to liver cirrhosis and fibrosis. Since the right hepatectomy transplanted from his brother was rejected, he was operated on for a second time (a cadaveric transplantation). A Takrolimus, Mikofenolik asit Prednisolone and immunosuppressive treatment was conducted, and continued. The patient had colic and debilitating diarrhoea when he came to our clinic. Microbiological and parasitological analyses of the stool were performed. The results showed that the occult blood test was positive, and the bacteriological analysis was negative. *I. belli* oocysts were seen in the parasitological analysis conducted with native, lugol and modified acid-fast dye techniques (Figure 1). The biochemical results are as follows: creatinine: 1.30 mg/dL), Uric acid: 7.7 mg/dL, AST: 48 U/L, ALT: 67 U/L, ALP: 269 U/L, GGT: 188 U/L, Total protein: 9.1 g/dL, K: 5.2 mmol/L, Cl: 111 mmol/L. Other parameters were normal. The patient received a 10-day Trimethoprim/sulphamethoxazole (TMP-SMX) bid treatment. No *I. belli* oocysts were found in the stools in the post-treatment analyses. However, there were *Entamoeba coli* (*E. coli*) cysts, which are apathogenic amoebae, and *Blastocystis hominis* (*B. hominis*) vacuolar forms (less than 5 of each in X40 microscopic area). *I. belli* oocysts were found again in the stools examined 2 months later when the patient had recurrent diarrhoea.

DISCUSSION

Isosporiasis is a parasitic infection which can be seen worldwide and can cause long-lasting and persistent diarrhoea in people whose immune system has been suppressed. In our country, it is seen in patients who have undergone organ transplantations, those affected with AIDS, cancer patients, patients with tuberculosis, those with malignant haematological diseases, in cases with other haematological diseases and in people whose immune systems are healthy (2-4, 7, 12-15). Our case was a patient whose immune system had been suppressed due to liver transplantation. In a study by Yazar et al. (7), *I. belli* oocysts were found in a kidney-transplant patient with abdominal colic, fatigue, nausea, vomiting, and bloodless, fluidic, temporarily-recovering and recurrent diarrhoea for 15 days. He was seen after a one-week TMP-SMX 60/240 mg, BID treatment; following this the clinical symptoms disappeared and microscopic analyses revealed that there were no *I. belli* oocysts. Koru et al. (3) reported *Entamoeba histolytica* in the stool analysis of a 32 year-old male, kidney-transplanted patient who had been complaining about abdominal cramps, fluidic, bloodless and mucousless diarrhoea, mild fever and nausea. Metronidasole treatment was given. In the stool analysis, *Salmonella typhi* C was found and native-lugol and acid-fast dye techniques showed that there were also *I. belli* oocysts. A ciprofloxacin treatment was prescribed. Analysis after 1 week demonstrated that oocysts were still there and, thus, a 10-day TMP-SMX treatment was started. Aksoy et al. (12) examined 554 diarrhoea patients' stools using Kinyoun acid-fast dye technique and sporulated and unsporulated *I. belli* oocysts were found in two patients, one of whom was HIV-positive and the other who had undergone a liver transplantation. In Büyükbaba-Boral's study, a 33 year-old female patient from Elazig suffering from acute diarrhoea, nausea, vomiting, fatigue and sweating accompanied with significant weight loss was diagnosed with AIDS, and lamivudine + zidovudine + nevirapin treatment was started. She was reported to have no complaints other than bloodless, mucousless and yellow diarrhoea 8-10 times a day. The stool samples were diagnosed by microscopic detection three times and no pathogenic bacteria or parasites were found. A fourth analysis revealed many *I. belli* oocysts. The TMP-SMX treatment was given 4x1 for the first 16 days and then 2x1 for the following 16 days, after which the diarrhoea ceased (2). Balcioglu et al. (4) investigated the incidence of parasitic infections in village children and the case of a 12 year-old boy diagnosed with isosporiasis. Although the patient had no complaints at first, the detailed history revealed that he had been having recurrent colic and diarrhoea with nausea, vomiting, and joint pain for some time and had lost 3 kg during the last month. After diagnosis, all immunoglobulin, IgG subgroups, complement levels and CD4+/CD8+ levels of the patient were normal. He was given 5-25 mg/kg TMP-SMX four times a day during the first ten days and TMP-SMX 5-25 mg/kg twice a day for the following three weeks. Three different stool samples were analysed 30, 60 and 90 days after the treatment

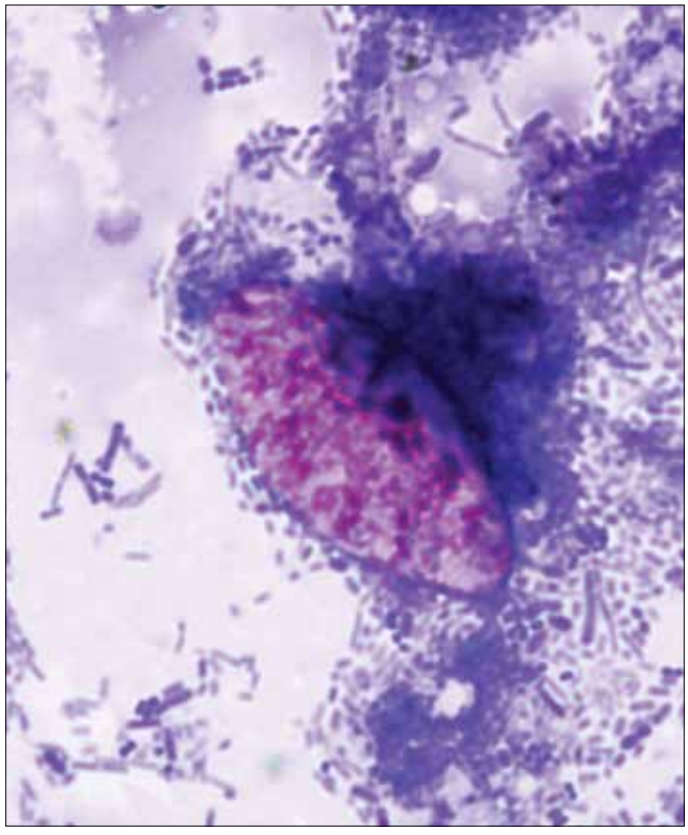

Figure 1. *I. belli* unsporulated oocyst in modified acid-fast stain technique

was completed to test the efficiency of the treatment; no parasites were found in the analyses. Atambay et al. (11) found many oocysts in a stool analysis of a female patient (aged 25) who had been receiving immunosuppressive treatment for 8 months after having a liver transplant and had colic, nausea and diarrhoea. TMP-SMX was given twice daily for ten days. Her diarrhoea disappeared on the second day of the antibiotic treatment and no oocysts were found in the subsequent stool analyses.

In our report, a male patient who had received a liver transplant four years ago and who had been suffering from colic, fatigue, and diarrhoea came to our clinic. Parasitological analysis of the stools using native, lugol and acid-fast dye techniques showed *I. belli* oocysts. In our case, after TMP-SMX 60/240 mg, BID, P.O. treatment, no *I. belli* oocysts were found in the stool analysis, but there were less than five *E. coli* cysts and there were *B. hominis* vacuolar forms. The stool was analysed 2 months later due to the recurrent diarrhoea and *I. belli* oocysts were found.

Bialek et al. (16) reported chronic bilier isosporiasis in a 60 year-old male patient whose immune system was healthy. Oral cotrimoxazole, oral nitazoxanide and 5-nitrothiazole benzamide treatment was given; however, due to the patient's malabsorption problem, the treatment did not produce a good result. Thus, a 5-day intravenous cotrimoxazole treatment was given which eradicated the oocysts in the stool sample. It was noted that extra-intestinal isosporiasis was seen not only in cases with immune suppression, but also in those with a healthy immune system. In the study by Mudholkar et al. (8), which investigated a 35 year-old HIV-affected male patient suffering from weight loss, vomiting, bloody and mucous diarrhoea continuing for two years and fever for eight days, *I. belli* oocysts were found and TMP-SMX treatment was given. However, the patient died one week after the treatment. Gruz et al. (10) reported a 23 year-old male patient with short bowel syndrome and found inflammation in biopsy following the transplantation, although there was no rejection or clinical problems after the operation. Diarrhoea occurred three months later and *I. belli* oocysts were found in the stool analysis. TMP-SMX treatment was started as a four times a day regime for ten days, then decreased to 2 times a day for three weeks and further decreased to prophylactic treatment once a day for one month, and led to complete recovery. *I. belli* oocysts were found in duodenum and colon biopsy in the study by Meamar et al. (6) which investigated a male patient (aged 43) who had intermittent fever, severe dehydration, vomiting, colic, diarrhoea at times over 8 months, weight loss, debility, gastrointestinal problems apart from coughs, phlegm and chest pain. It was reported in this study that there was a large number of *I. belli* oocysts in the patient's stools. Diarrhoea ceased two days after oral TMP-SMX treatment started. Thymectomy was performed, but it was found that the patient had diarrhoea three times following discharge from hospital due to isosporiasis. Prophylactic treatment continued after the antibiotic treatment which lasted for three weeks. No recurrent diarrhoea was reported in the subsequent examinations for 6 months.

CONCLUSION

We believe that coccidian parasites should always be taken into account when investigating the aetiologies of long-term and intermittent diarrhoea which appears in cases where the immune system has been suppressed. Equally important is the application of an effective treatment.

Conflict of Interest

No conflict of interest was declared by the authors.

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Hidatik Akut Pankreatit

Hydatid Acute Pancreatitis

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ÖZET

Karaciğer hidatik kistlerinin safra ağacı içine rüptürü bilinmektedir, fakat akut pankreatit nadir bir komplikasyonudur. İntrabiliyer rüptür ilk kez Dew tarafından 1928'de bildirilmiştir. İntrabiliyer rüptür hepatik hidatik kistin ciddi bir komplikasyonudur. Erişkin hastalarda insidansı %1-25 arasındadır. Bizim olgumuzda hepatik kist materyalinin ana safra kanalına göç edebileceğini ve papiller orifise impakte olup akut pankreatiti provake edebileceğini göstermeyi amaçladık. Akut pankreatitle gelen hastada ayrıcı tanıda biliyer nedenler arasında parazitik enfeksiyonların da düşünülmesi gerekmektedir. *(Turkiye Parazitol Derg 2012; 36: 251-3)*

Anahtar Sözcükler: Hidatik kist, akut pankreatit, intrabiliyer rüptür

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ABSTRACT

Liver hydatid cysts are known to rupture into the biliary tree, but acute pancreatitis is a rare complication of intra-biliary rupture. Intra-biliary rupture was first reported in 1928 by Dew. Intra-biliary rupture is a serious complication of hepatic hydatid cysts. The incidence varies from 1% to 25% in adult patients. In our case, we aimed to show the migration of the material of a hepatic hydatid cyst to the common bile duct and the impaction of hydatid membrane in the papillary orifice, which may cause acute pancreatitis. Parasitic infections should be considered as a differential diagnosis in patients with acute biliary pancreatitis. *(Turkiye Parazitol Derg 2012; 36: 251-3)*

Key Words: Hydatid cyst, acute pancreatitis, intra-biliary rupture

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GİRİŞ

Kistik ekinokokkozis (KE), *Echinococcus granulosus*'un neden olduğu zoonotik bir hastalıktır. Bu hastalık Akdeniz, Asya, Uzak Doğu ve Latin Amerika'da birçok ülkede endemiktir. Hidatik kistlerin %75'i karaciğerde bulunur. Hastalar yıllarca asemptomatik olabilirler ve genellikle non-spesifik şikayetlere sahiptirler. Fakat herhangi bir zamanda ortaya çıkan bazı komplikasyonlar erken tedavi edilmezse hayatı tehdit edebilirler (1).

Karaciğer, hidatik kistlerin en sık yerleştiği yerdir, daha az sıklıkla akciğerler, nadiren de dalak, böbrekler, beyin, kaslar, kemik ve pankreasta bulunabilir. Kistin biliyer kanala rüptürü nadirdir ve obstruktif sarılık ya da kolanjit ile kendini gösterebilir (2, 3). Biliyer kanalda hidatik membranların görüldüğü birkaç olgu bildirilmiştir (4, 5). Karaciğer kistlerinin safra ağacı içine rüptürü bilinmektedir, fakat akut pankreatit nadir bir komplikasyonudur. İntrabiliyer rüptür ilk kez Dew tarafından 1928'de bildirilmiştir (6). İntrabiliyer rüptür hepatik hidatik kistlerin ciddi bir komplikasyonudur. Erişkin hastalarda insidansı %1-25 arasındadır (7).

Bizim olgumuzda hepatik kist hidatik materyalinin ana safra kanalına göç edebileceğini, papiller orifise impakte olup,

Bu olgu 27. Ulusal Gastroenteroloji Haftası 2010'da poster olarak sunulmuştur.

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bunun da akut pankreatiti provoke edebileceğini göstermeyi amaçladık. Akut pankreatitle gelen hastada ayrıcı tanıda biliyer nedenler arasında parazitik enfeksiyonların da düşünülmesi gerekmektedir.

OLGU SUNUMU

Altı gün önce ishalle başlayan karın ağrısı, bulantı ve kusma yakınması olan 60 yaşında erkek hasta, karın ağrısının giderek artması ve sağ üst kadrandan başlayıp göbek çevresine yayılması üzerine acil servise başvurmuştur. Hastanın özgeçmişinde; iki yıldır Tip 2 diyabetes mellitus hastası olduğu ve metformin 1000 mg 2x1 kullandığı öğrenildi. Hastada safra taşı, alkol kullanımı, geçirilmiş operasyon öyküsü yoktu.

Fizik muayenede; kan basıncı 110/70 mmHg, nabız 72/dk, ateş 37°C idi. Batın muayenesinde sağ üst kadranda hassasiyet mevcuttu, defans ve rebound yoktu. Laboratuar incelemesinde; Hgb: 12.6 g/dL, Htc: %37.9, Plt: 264 000/mm^3, Sedimantasyon: 109 mm/h idi. AST: 88 U/L, ALT: 156 U/L, GGT: 921 U/L, ALP: 379 U/L, t.bil: 6.9 mg/dL, d.bil: 4.16 mg/dL idi. Amilaz: 655 u/L, Lipaz: 1113 u/L idi. Hastanın serum elektrolitleri normaldi. Üst batın USG de; karaciğer konum ve konturu tabii idi. Parankim ekosu yağlanmaya sekonder artmıştı. Kaudot lob düzeyinde yaklaşık 36x50 mm çapta periferi kalsifiye içerisinde kistik alanlar ve hiperekoik görünümler olan hidatik kist lehine lezyon izlendi. İntrahepatik safra yollarında hava imajları vardı. Safra kesesi duvarı belirgin olup lümende belirgin posterior gölgelenmesi olmayan ekojeniteler (kolesterol kristalleri) izlendi.

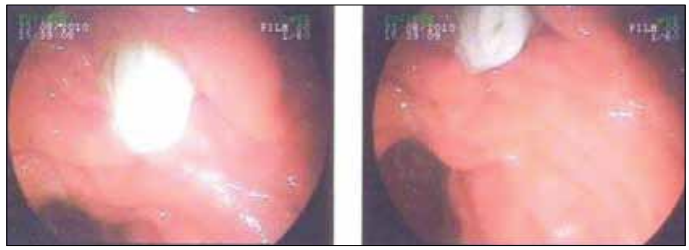
Biliyer pankreatit düşünülen hastaya Endoskopik Retrograd Kolanjio Pankreatografi (ERCP) planlandı. ERCP'de; Ampulla waterii'ye oturmuş beyaz renkli hidatik kist membranı izlendi. Bu membran snare ile çıkarıldı. Koledok kanülize edildi. Opak madde verildiğinde koledoğun hafif dilate olduğu görüldü. Endoskopik sfinkterotomi yapıldı, koledok balonla sıvazlanarak işleme son verildi (Resim 1).

ERCP sonrası karın ağrısı olmayan hastanın amilaz, lipaz değerleri düştü, kolestatik enzimleri ve bilirubinleri geriledi. ERCP sırasında çıkarılan materyal patolojiye gönderildi. Histopatolojide hidatik kist membranı ve protoskoleksler izlendi (Resim 2). İndirekt hemaglutinasyon (IHA) ile yapılan analizde anti-*Echinococcus granulosus* antikorları 1/640 dilusyonda pozitif idi. Albendazol 200 mg 2x2 başlanan ve genel durumu ile şikayetleri gerileyen hastaya medikal tedavi sonrası karaciğer KE cerrahi tedavi planlandı.

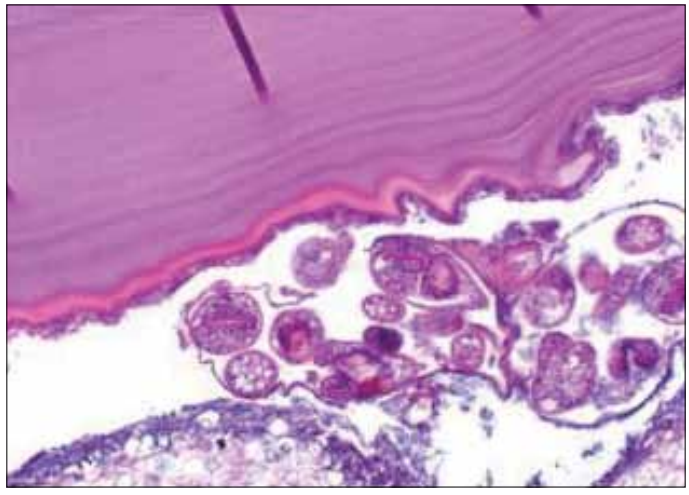
TARTIŞMA

Biliyer ağacın parazitik enfeksiyonları tropikal ve subtropikal ülkelerde yüksek prevelans ve insidansa sahiptir. Biliyer ağaca rüptür karaciğer KE'nin bilinen bir komplikasyonudur. Bu komplikasyon neticesinde kist kavitesinde enfeksiyona ve biliyer ağaçla ilişkili sekonder problemlere yol açar. Biliyer ağaç içerisindeki skolekslere bağlı olarak gelişen kolanjit karaciğer KE'sinin sık görülen bir komplikasyonudur (8-11). Belli ve arkadaşları (12) intrabiliyer rüptüre bağlı rekürren kolanjit oranını % 21 olarak bildirmişlerdir.

Karaciğer KE'sinin komplikasyonları; kalsifikasyon, kist kavitesinde enfeksiyon, intrabiliyer rüptür, kolanjit, sarılık ve akut pankreatitdir.



Resim 1. ERCP'de ampulla waterii ağzına oturmuş kist hidatik membranı



Resim 2. Histopatolojik olarak kist hidatik membranı ve skoleksler (Hemotoksilen eozin x100)

Bizim hastamızda hepatik KE ile ilişkili geniş bir komplikasyon spektrumundan nadir görülen akut pankreatit olgusuydu.

Biliyer ağaca rüptürü olan hastalarda kolanjit ve/veya sarılık mevcutsa ERCP tanı ve tedavi için ilk yapılması gereken işlemdir (13).

SONUÇ

Bizim hastamızda ampulla wateriiye oturmuş kist hidatik membranına bağlı sarılık ve akut pankreatit olduğu için ERCP yapıldı ve işlem sonrası hastanın kliniği dramatik olarak düzeldi. Bu olgu sunumu hepatik KE'nin biliyer rüptürü sonucu olan akut pankreatit vakalarında endoskopik sfinkterotominin ve membranların çıkarılmasının seçilecek prosedür olduğunu göstermektedir. Bu hastalarda nihai ve tamamlayıcı tedavinin cerrahi tedavi olduğu unutulmamalıdır (14).

Çıkar Çatışması

Yazarlar herhangi bir çıkar çatışması bildirmemişlerdir.

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Facial Nerve Paralysis Due to Intra-aural *Hyalomma* Tick Infestation

Kulak Kanalı İçinde *Hyalomma* Cinsi Kene Isırığına Bağlı Fasiyal Sinir Paralizi

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ABSTRACT

We present the case of a 33 year-old man from a village of the north-eastern part of central Anatolia admitted to the otolaryngology department of Yeditepe University Hospital with right facial asymmetry and pain on the right ear. A tick of the genus Hyalomma was observed in the external auditory canal of the right ear and it was removed with fine cup forceps under otomicroscopy. We are of the opinion that in patients presenting with sudden acute ear pain and facial palsy, the ear canal should be examined to exclude an infestation by ticks.
(Turkiye Parazitol Derg 2012; 36: 254-7)

Key Words: Facial nerve palsy, tick infestation, ear, external auditory canal
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ÖZET

Bu olguda, Orta Anadolu'nun Kuzey-doğu kesiminde bir köyden gelen, yüzünün sağ tarafında asimetrisi bulunan ve sağ kulak ağrısı şikayeti ile Yeditepe Üniversitesi Hastanesi kulak burun boğaz bölümüne başvuran 33 yaşındaki bir erkek hasta sunulmuştur. Hastanın sağ dış kulak yolunda kene gözlenmiş ve kenenin çıkarılması otomikroskopi altında fine cup forceps ile yapılmıştır. Ani başlayan şiddetli kulak ağrısı ve yüz felci ile başvuran endemik bölgeden gelen hastaların, kulak kanalında kene ısırığı olasılığını dışlamak için dikkatlice incelenmesi gerektiği görüşündeyiz.
(Turkiye Parazitol Derg 2012; 36: 254-7)

Anahtar Sözcükler: Fasiyal sinir paralizi, kene, kulak, dış kulak yolu
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INTRODUCTION

Tick paralysis is a neurological syndrome caused by a potent neurotoxin produced by an attached tick feeding on the host (1). The neurological symptoms may appear immediately after the infestation or progress within days after attachment.

Cases of isolated facial nerve paralysis are less commonly reported (2-5). Usually, the palsy is present at the time of tick detection, and, in the case of facial-nerve palsy, the tick is noticed most often behind the ear or within the external auditory meatus. The recovery usually begins soon after tick removal. Cases of isolated facial palsy may last three days to three weeks (3, 4, 6). If unnoticed, the tick may remain attached, leading to death as a result of respiratory paralysis. Since a delay in the diagnosis may have devastating consequences, physicians must be aware of the basic features of this syndrome.

The bite of some ticks, mainly from the Ornithodoros genus, may lead to local lesions and systemic illness, referred to as tick toxicosis (7). Toxicoses caused by toxic substances secreted by the salivary glands of ticks include: tick paralysis (neurotropic toxin), sweating sickness (dermotropic toxin), and Rhipicephalus appendiculatus toxicosis (leukotropic toxin).

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Approximately 40 species of soft (argasid) and hard (ixodid) ticks secrete salivary toxins that cause paralysis in humans and some animals (8). Tick paralysis is thought to be caused by a toxin secreted in tick saliva during feeding that reduces motor neuron action potentials and the action of acetylcholine, depending on the species of tick.

Several theories may explain the pathophysiology of localised facial nerve palsy in an intra-aural tick infestation. It is likely that a presence of a perforation in the tympanic membrane enables the tick saliva with the toxin to enter the middle ear and reach the facial nerve, probably through a natural dehiscence of the fallopian canal, causing paralysis (9).

Many clinicians may be unfamiliar to this clinical entity; although they are relatively rare, cases of tick paralysis are known from the literature. Tick paralysis has been frequently misdiagnosed, and this envenomation syndrome must be included in the differential diagnoses of any patient presenting with an ascending symmetric paralysis.

Cases with facial paralysis reported to date are mainly due to *Dermacentor* sp. and *Ixodes* spp. because of the geographical distribution of ticks (1-6, 10).

Ticks of the genera Haemaphysalis, Hyalomma, Boophilus, Dermacentor, and Rhipicephalus are widespread throughout Anatolia. Therefore, tick-borne diseases and tick-paralysis are expected to be related to these genera (11). Likewise, Gurbuz et al. (12) and Edussuriya et al. (13) reported tick facial paralysis cases with *Hyalomma* species.

In this report, we present a case of a peripheral facial nerve paralysis which was developed due to a tick of the genus Hyalomma being attached to the external auditory canal.

CASE REPORT

A 33 year-old male patient who lives in the north-eastern part of the Central Anatolia was admitted to the Department of Otolaryngology of the Yeditepe University Hospital. The patient had right facial asymmetry and right ear pain, which had been continuing for ten days. From his detailed history, he noted that his first complaint was a sudden pain in the right ear and not having the ability to close his right eyelid the following day. He also noticed a tingling sensation in his face. There was no history of fever, vomiting, diarrhoea, rash, headache, blurred vision, shortness of breath, cough or respiratory distress.

He was admitted to a local hospital with these complaints and he was diagnosed as 'Bell's palsy'; medical treatment was initiated, including methyl-prednisolone (1 mg/kg/day). After 5 days, since there was no progression in his symptoms, he was admitted to our department. Upon physical examination, the patient was afebrile, alert and cooperative and his vital signs were normal. The neurological examination showed that he was able to close his right eyelid with minimal effort, but he had a continued weakness on the right half of his face while smiling and whistling (puffing up the cheeks), and a slight asymmetry on his face could be observed after a big effort (House-Brackmann score II) (Figure 1).

The right external auditory canal was filled with cerumen, which was removed; at this moment a tick on the posterior edge of tympanic annulus was observed at the entrance of the external auditory canal (Figure 2). The tick was partially engorged and was removed with the help of a fine cup forceps under otomicroscopy without damaging it. After the tick removal, the external auditory canal and the tympanic membrane was examined and, with the exception of a redness at the tick attachment site, no abnormalities were noted. The tick was placed in 70% alcohol and was identified as *Hyalomma* sp. During the examination of the excised tick, it was determined that it belonged to genus *Hyalomma* spp. It was sent to a parasitology laboratory of a regional veterinary hospital, but further identification was not performed. Also, microbiological and molecular examinations to reveal the possession of viruses or bacteria could not be performed.

The laboratory test results, including complete blood cell count, erythrocyte sedimentation rate, chemistry profiles and urinalysis,

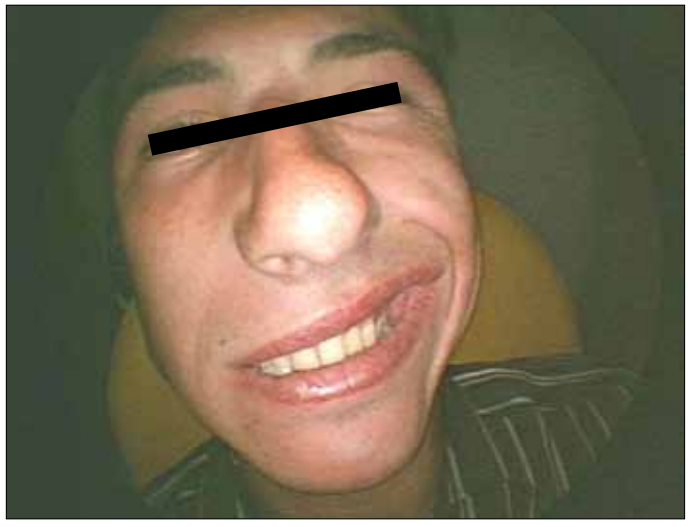

Figure 1. Endoscopic view obtained through a 0 degree telescope demonstrating the right peripheral facial paralysis (H-B Grade II) of the patient

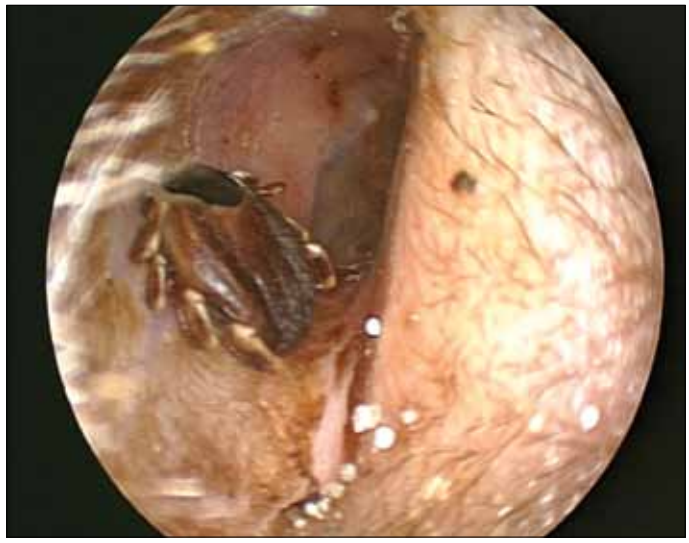

Figure 2. *Genus Hyalomma* spp. tick on the posterior edge of tympanic annulus

were within normal limits. In addition, the patient had no nausea, vomiting, diarrhoea, conjunctival haemorrhage, murmur or cough. No haemorrhagic symptoms such as epistaxis, haematemesis or melena were detected.

DISCUSSION

Tick paralysis has been reported in Australia, North America, Asia and South Africa. In Europe, paralysis cases are relatively rare. Cases of apparent tick toxicosis in humans caused by *Ixodes redikorzevi* were reported in northern Israel (14, 15).

Most of the reported cases of tick paralysis have been caused by either *Dermacentor andersonii* or *Dermacentor variabilis* in the United States (16, 17). In Australia, cases of paralysis caused by the species *Ixodes holocyclus* have been reported. Children aged 1-5 years are most commonly affected, and the tick is usually found in the scalp, often behind the ear (18). In endemic areas, Lyme disease must also be included in the differential diagnosis of acute facial paralysis. Nigrovic et al. (19) identified 313 patients with peripheral facial palsy who were evaluated for Lyme disease. Of these, 106 (34%) had Lyme disease facial palsy.

In Turkey, few cases of tick paralysis have been reported. Gurbuz et al. (12) reported the case of a 3 year-old girl with facial palsy, which was caused by *Hyalomma marginatum marginatum*. Engin et al. (20) described a tick paralysis with atypical presentation with involvement of the upper trunk of brachial plexus. In their case, the tick was not identified.

Although reports of isolated facial paralysis cases due to tick infestation in the ear are rare in the literature, Bell's palsy and Lyme neuroborreliosis are the two most common diagnoses in patients with peripheral facial palsy in areas endemic for *Borrelia burgdorferi* (5, 9, 12, 21, 22). The development of isolated facial paralysis due to ticks can be explained by several theories obtained from animal studies. In tick paralysis, the neurotoxin slows nerve conduction velocity and the amplitude of muscle action potentials inhibits terminal-nerve conduction and acetylcholine release at the pre-synaptic neuromuscular junctions of muscle fibres, and causes total blockade of transmission at myoneural junctions (23-27).

Isolated peripheral nerve palsy may occur simultaneously with tick attachment (5). Following the removal of the tick, the facial palsy improves within hours. In some studies, it has been reported that recovery may be seen in days or weeks (5, 28).

In the differential diagnosis of isolated facial nerve palsy in patients, the involvement of a tick toxicosis should be taken into account. As most of these ticks are also vectors of human and animal diseases, e.g. *Hyalomma* ticks for CCHF) virus, concomitant examinations should be performed to also exclude an infection with viruses and protozoa.

In the literature, many methods of tick removal have been recommended, including traditional methods such as the application of a lighted match, alcohol or petroleum jelly, which are not effective and contra-productive as the salivating tick could more easily infect the host with the pathogenic microorganisms carried in the their saliva (29). Rapidly killing the tick using a pyrethrin-based insecticidal spray or the application of alcohol and local anaesthesia have also been suggested; however they are ineffective (30). Recently, Poirier et al. (1) described how to successfully remove a tick: the tick should be grasped as close to the skin as possible using blunt curved forceps with a gloved hand and then it should be pulled off the skin using a firm steady motion. In our case, the patient came from a region where CCHF prevalence is high; therefore, the tick was removed from the external auditory canal alive under the microscope, so that the possibility of infections carried by ticks to the patient and healthcare personnel was reduced. Although the patient had symptoms for ten days, the tick had enough time to be engorged and release toxins. However, as a general rule and for infection control standards, the tick was removed with caution.

CONCLUSION

In diagnosing tick toxicosis and tick paralysis, a detailed history and physical examination, a high index of suspicion and knowledge of the epidemiology and natural history of tick-borne diseases are essential. We are of the opinion that in patients particularly coming from rural areas with sudden acute ear pain and facial palsy, the ear canal should be examined carefully to exclude the possibility of parasitisation by ticks.

Conflict of Interest

No conflict of interest was declared by the authors.

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Düzeltme / Erratum

Dergimizin "Cilt: 36 Sayı: 3 Eylül 2012" tarihli baskısında yayınlanmış olan "18-45 Yaş Grubu Kadınlarda, Trichomonas vaginalis ve Diğer Mikroorganizmaların Vajinal Akıntı Örneklerinden Mikroskobik Olarak İncelenmesi" isimli yazının yazar kurumları tarafımdan sehven yanlış yazılmıştır. Olması gereken şekli aşağıdaki gibidir. Düzeltir, tüm okuyucularımızdan özür dileriz.

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Eosinophilic Pneumonia Due to Toxocariasis: An Adult Case Report

Erişkin Bir Hastada Toxocariasis'e Bağlı Eozinofilik Pnömoni Olgusu

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ABSTRACT

Toxocara is a roundworm, a common parasite of dogs (*T. canis*) and cats (*T. cati*). Toxocariasis or Visceral larva migrans (VLM) are diseases caused by the larvae of *Toxocara* sp., which may involve many organs, but pulmonary symptoms such as coughing and wheezing and allergic symptoms are seen in more than 80% of patients. It is known that, although the risk of infection is present, the worldwide diagnosis of toxocariasis is difficult since clinical and laboratory data provide insufficient evidence for the diagnosis. Nowadays, the diagnosis of toxocariasis is performed by serologic methods. We describe herein a case of toxocariasis with eosinophilic pneumonia that was diagnosed using serologic methods. *(Turkiye Parazitol Derg 2012; 36: 258-9)*

Key Words: Toxocariasis, eosinophilia, pneumonia

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ÖZET

Toxocariasis veya visseral larva migrans, birçok organ tutulumuyla birlikte olguların %80'inde alerjik akciğer semtomların geliştiği, dünyada yaygın olarak kedi ve köpeklerin paraziti olan *Toxocara canis* veya *T. cati* larvalarının neden olduğu hastalıktır. Dünyada hastalık riskinin yaygın bulunmasına karşın, klinik ve laboratuvar verilerinin hastalığın tanısında zaman zaman yetersiz kalmaktadır. Günümüzde, toxocariasis tanısı geliştirilen serolojik metodlar sağlanmaktadır. Burada eozinofilik pnömoni gelişen ve serolojik metotlar ile toxocariasis tanısı konulan bir olgu sunulmuştur. *(Turkiye Parazitol Derg 2012; 36: 258-9)*

Anahtar Sözcükler: Toxocariasis, eozinofilia, pnömoni

Geliş Tarihi: 12.08.2012 **Kabul Tarihi:** 09.11.2012

INTRODUCTION

Human infection with toxocariasis occurs following ingestion of embryonated eggs from the environment. The larvae penetrate the gut wall and begin a migration through the tissues. This can continue for several years. Sero-prevalence studies suggest that schoolchildren are infected more often than adults. Most disorders consequent to Toxocara infection are due to the damage caused by an inflammatory immune response. Infected subjects often are asymptomatic, but infected children may display cough, fever, abdominal pain, hepatomegaly, and skin lesions. Severe infections are rare but may cause respiratory distress or myocarditis (1, 2). Blood hyper-eosinophilia, which is a frequent sign of tissue invasion by parasites, is inconstant in toxocariasis, and the most reliable diagnostic method is an ELISA which has 98% specificity, since larvae are hardly ever found upon pathological examination (3, 4).

CASE REPORT

The patient is a 52 year-old male with no other significant characteristics except diabetes mellitus, which was controlled by an oral agent for the previous 3 years, and a his-

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tory of cigarette smoking (20 packs per year). The patient was admitted to the outpatient clinic with complaints of malaise, dyspnoea, purulent discharge of sputum and a cough. He had experienced these symptoms for 10 days and a bilateral broncho-pneumonic appearance was present in the middle and lower zones of the chest x-ray, therefore he was hospitalised. In the physical examination, blood pressure was 120/80 mmHg, pulse was 92/min and body temperature was 37.3°C. The respiratory sounds were bilaterally similar and he had bilateral fine rales and an expiratory rhonchus. All other systemic observations were normal. Laboratory investigations were as follows: leukocytes 13970/mm^3, eosinophil 3160/mm^3 (22.6%), sedimentation rate 73 mm/hr, and gamma glutamyl transferase (GGT) 204 U/L. The rest of the routine blood analyses and urine analyses were within the normal limits. Moderate obstruction was detected with the pulmonary function tests. No colonisation was present in the sputum culture and direct microscopy for tuberculosis was negative. Abdomen USG was normal. Endo-bronchial appearance was normal on bronchoscopy. Broncho-alveolar lavage (BAL) was performed through the superior segment of the lower lobe of the right lung. BAL cell ratios were as follows: macrophage 46%, eosinophils 42%, lymphocytes 8%, and neutrophils 4%. Cytological analysis of BAL fluid did not reveal any neoplastic cells. Microbiological investigations for bacteria, mycobacteria, and fungi were negative. In the computerised tomography of the thorax, an appearance reminiscent of pneumonic infiltration was present in the superior lobe, posterior segment of the right lung and the superior segments of the lower lobes of the right and left lungs. Parasitic analyses of the faecal specimen, sputum, and BAL were negative. IgE level was 2034 u/mL following the serological tests performed in order to detect a parasitic disease (toxocariasis, fasciolosis, and cystic echinococcosis). Toxocara serology was positive by ELISA method (The ELISA absorbance value was 2.760). Later, a positive Toxocara specific IgE value was determined also using the same homemade ELISA method.

With these findings, the case was diagnosed as a case of Eosinophilic pneumonia due to toxocariasis; prednisolone 0.75 mg/kg/day and albendazole 15 mg/kg/day was added to the bronchodilator agent the patient had been using. On the third day of the treatment, the pathological physical signs of the patient were diminished, his dyspnoea disappeared and radiological regression was observed with the chest x-ray. In the control after six month, eosinophilia was decreased to a level of 340/mm^3 (2.6%), sedimentation was normal and GGT level was decreased to 87 u/mL.

DISCUSSION

Clinical manifestations of toxocariasis or visceral larva migrans (VLM) are the result of allergic and inflammatory responses of the host, and manifest as airway reactivity, acute pneumonia, and persistent eosinophilia. VLM is a self-limiting disease and specific treatment is rarely necessary. In acute cases, a short course of steroids reduces morbidity and mortality, but preventive measures are more important in curbing Toxocara infection (1, 2). In our patient, intravenous corticosteroid therapy produced a rapid improvement in the clinical picture. Pulmonary infiltration resolved within 10 days after the start of treatment.

Schinkewitch et al. (5) reported a case of bilateral eosinophilic pneumonia in a 33 year-old man due to *T. canis*, and the ELISA index was strongly positive at 2.597. The patient developed a rapidly progressive respiratory failure requiring mechanical ventilation. Intravenous corticosteroid therapy produced a rapid improvement. Roig et al. (6) reported diffuse pulmonary infiltration and 64% eosinophilia in the BAL fluid in a toxocariasis patient who had dyspnoea. They suggested that the routine performance of the ELISA test for Toxocara in the diagnostic approach to pulmonary infiltration with eosinophilia can reveal an undetermined, sometimes unsuspected, number of cases of adult toxocariasis with pulmonary involvement. Bouchard et al. (7) reported a case of acute severe eosinophilic pneumonia the existence of positive *T. canis* serology whose outcome was rapidly favourable following steroid therapy.

Although toxocariasis is a frequent disease in children, the severe clinical manifestations are rarely reported in the literature (diffuse interstitial pneumonia with hypoxaemia and acute severe asthma). In adults, toxocariasis is unusual and infections appear to be mild or subclinical, provoking positive serological tests and sometimes, persistent eosinophilia (occult toxocariasis). Eosinophilic pneumonia seems to be rare in adults with toxocariasis, but there are eosinophilic syndromes that do not have a determined aetiological agent in the literature. However, as described in this case report, this aetiology should be kept in mind when dealing with a case of eosinophilic pneumonia in adults, and serological diagnosis should be considered. Although parasite examination in stool samples has been widely carried out in patients with eosinophilia, tissue parasites are paid insufficient attention in diagnosis (3).

CONCLUSION

The present case established that ELISA for toxocariasis can remove the difficulty of the diagnosis of toxocariasis. If serological methods use routine diagnosis, physicians must understand the advantages of serological diagnosis in the diagnosis of parasitic diseases and the ability of the laboratory performing them as standard.

Conflict of Interest

No conflict of interest was declared by the authors.

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Haemodipsus leporis Blagoveshtchensky, 1966 (Phthiraptera: Anoplura: Polyplacidae) on a Hare (*Lepus europaeus*, L.). New Record for Turkish Phthiraptera fauna

Yabani Bir Tavşanda (*Lepus europaeus*, L.) ilk *Haemodipsus leporis* Blagoveshtchensky, 1966 (Phthiraptera: Anoplura: Polyplacidae). Türkiye Phthiraptera faunası için yeni kayıt

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ABSTRACT

This study was carried out to detect lice species found on hares (*L. europaeus*, L.) in the Konya province. Several lice individuals were collected on a hare and were kept in 70% alcohol. Then, they were cleaned in 10% KOH for 24 hours, washed in distilled water, stored in 70%, 80%, 90% and 96% alcohol for 24 hours for each step. They were mounted on the slides in Canada balsam and examined under a binocular light microscope. Two of the species were identified as *Haemodipsus leporis* Blagoveshtchensky, 1966, while the others were *H. lyriocephalus* (Burmeister, 1839). *H. leporis* was found on *L. europaeus* for the first time in Turkey, and it was also detected on this host species for the first time throughout the world. The morphological characteristics of this species are given in this paper. *(Turkiye Parazitol Derg 2012; 36: 260-3)*

Key Words: Hare, *Lepus europaeus*, *Haemodipsus leporis*, Konya, Turkey

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ÖZET

Bu çalışma Konya yöresindeki yabani tavşanlardaki (*L. europaeus*, L.) bit türlerini belirlemek amacıyla yapılmıştır. Yabani bir tavşanın üzerinden çok sayıda bit toplanmış ve %70 alkol içinde saklanmışlardır. Daha sonra %10 KOH içinde 24 saat bekletilmiş, distile suda 24 saat yıkandıktan sonra 24 saat süreyle %70, %80, %90 ve %96'lık alkol serilerinden geçirilmiştir. Bit örnekleri Kanada balsam ile lam üzerine yapıştırılarak binoküler ışık mikroskobunda incelenmiştir. Bit örneklerinden ikisi *Haemodipsus leporis* Blagoveshtchensky, 1966, diğerleri ise *H. lyriocephalus* (Burmeister, 1839) olarak teşhis edilmiştir. Bu araştırmada *H. leporis*'e Türkiye'deki yaban tavşanlarında ilk kez rastlanmış olup, bu tür *L. europaeus*'da ilk kez tespit edilmiştir. Bu makalede *H. leporis*'in morfolojik özellikleri hakkında bilgi verilmiştir.
(Turkiye Parazitol Derg 2012; 36: 260-3)

Anahtar Sözcükler: Yaban tavşanı, *Lepus europaeus*, *Haemodipsus leporis*, Konya, Türkiye

Geliş Tarihi: 28.02.2012 **Kabul Tarihi:** 29.09.2012

INTRODUCTION

Lepus europaeus belongs to the family Leporidae, order Lagomorpha and is known as the hare. This species is a cosmopolitan species and is found in almost all parts of Turkey (1). About 550 species of Anopluran lice species are known throughout the world. Polyplacidae is the most specious family within the Anoplura; worldwide, 16 genera and about 200 species are currently assigned to this family. Members of *Haemodipsus* are ectoparasites of rabbits and hares throughout much of the world (2, 3). *Haemodipsus conformalis* Blagoveshtchensky, 1965 and *Haemodipsus leporis* Blagoveshtchensky, 1966 were described from hares in Kazakhstan and in Yakutia, respectively (4, 5). Beaucournu (6) stated the morphological characters, hosts, epidemiological roles, distributions and identification keys of 18 Anopluran lice species including *H.lyriocephalus* (Burmeister,

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1839), *H. setoni* Ewing and *H. ventricosus* (Denny, 1842). A checklist of the sucking lice of mammalian hosts in the world was published, and six species, namely *H. africanus* Bedford, *H. conformalis* Blagoveshtchensky, *H. leporis* Blagoveshtchensky, *H. lyriocephalus, H. setoni* and *H. ventricosus,* were reported to have been found in the genus *Haemodipsus* (2). However, Durden and Rausch (3) added to these a seventh species: *Haemodipsus brachylagi* Durden and Rausch, 2007 from Pygmy rabbit; *Brachylagus idahoensis*, (Merriam) in Nevada, USA.

Nevertheless, there are limited studies available on ectoparasites found on hares in Turkey (7-9). Although *H. ventricosus* was recorded from chickens previously; it has not been found in hares in the European part of Turkey (10). It was reported that *Ctenocephalides canis*, *Haemaphysalis otophila, Rhipicephalus bursa, Cheyletiella parasitivorax* and *Trombicula autumnalis* had been found on the hare in the Elazığ province in Turkey (7). However, there are two papers regarding *Haemodipsus* species found on hares in the Konya province (8, 9). In these studies, two *Haemodipsus* species, *H. lyriocephalus* and *H. setoni,* were detected on hares (8, 9); however, *H. leporis* has not been reported on hares in Turkey to date.

The aim of this paper is to discuss the knowledge about the morphological characteristics of *Haemodipsus leporis* found on a hare in Konya province in Turkey.

CASE REPORT

A hare shot by a hunter in Kestel village, Sarayönü, Konya in Middle Anatolia was examined for ectoparasites, and some lice specimens were detected on the hare. They were collected in a tube consisting of 70% alcohol and then cleaned in 10% KOH for a one day. Later, they were washed in distilled water for 24 hours and transferred in alcohol and stored for one day in 70%, 80%, 90% and 96% ethanol on consecutive days. They were mounted on slides in Canada balsam and kept to dry in an incubator for two weeks. They were examined under a binocular light microscope (Leice DM750) and identified as *H. lyriocephalus* (6 ♀, 5 ♂, 8 Nymphs) and *H. leporis* (2 ♂). In this case, information about the morphological characters of male *H. leporis* was given, because morphological characters of *H. lyriocephalus* had been explained in detail before.

***Haemodipsus leporis* Blagoveshtchensky (5)**
Studied materials: 2 ♂♂, *L. europaeus,* February 29th, 2012, Kestel village, Sarayönü, Konya.

Male: a relatively small species (Figure 1). The head is triangular, rounded in front and widened at the temple. It is slightly wider than it is long (Figure 2). The antennae have five segments, and the first segment is clearly thicker than the others. There is a long seta on the temple on each side. The thorax is short and narrowed and is wider than it is long. The legs are strong and their widths are close to equal. The first is relatively short and curved to the anterior; there is a well developed claw on their edges. The sternal plate is hexagonal and relatively large (Figure 3) and the abdomen is wide, with narrowed pleural plates. Genitalia developed well (Figure 4, 5) And the basal plate is rectangular and relatively wide. Parameres are slightly concave and pointed in posterior. Head length: 0.35 mm, head width: 0.39 mm,

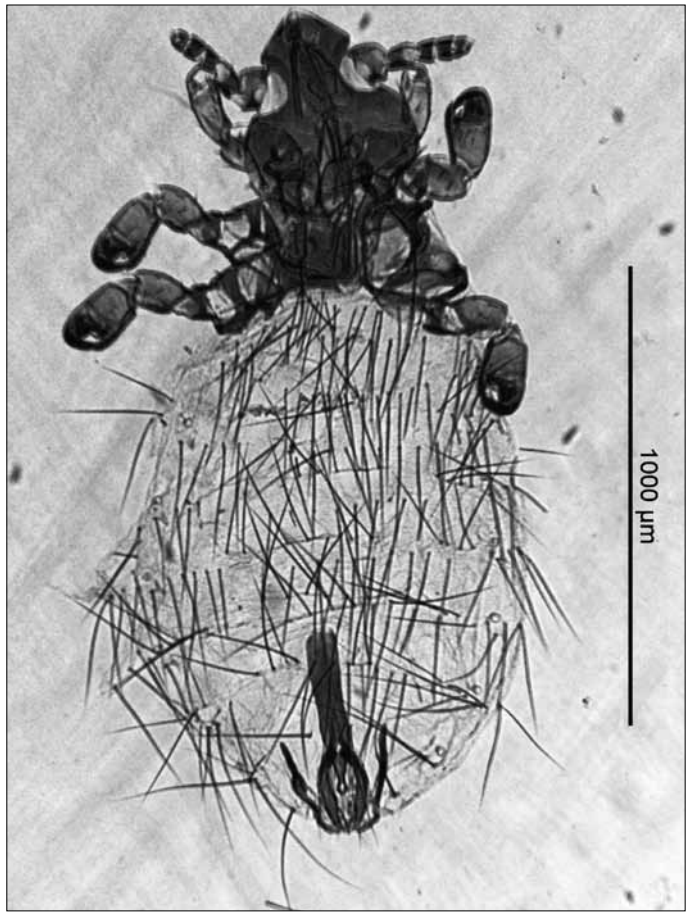

Figure 1. *Haemodipsus leporis*, male, original

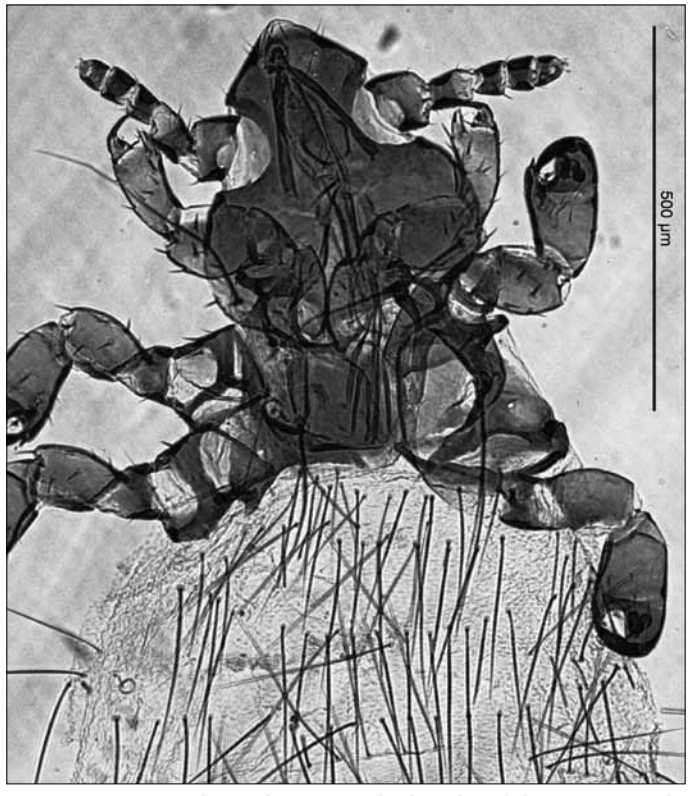

Figure 2. *Haemodipsus leporis*, male, head and thorax, original

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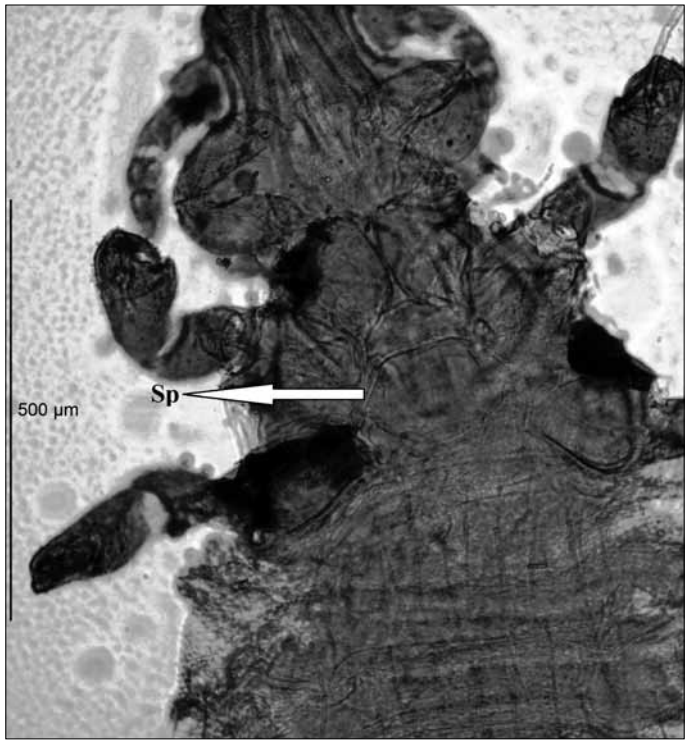

Figure 3. *Haemodipsus leporis*, male, sternal plate, original (Sp, arrowed)

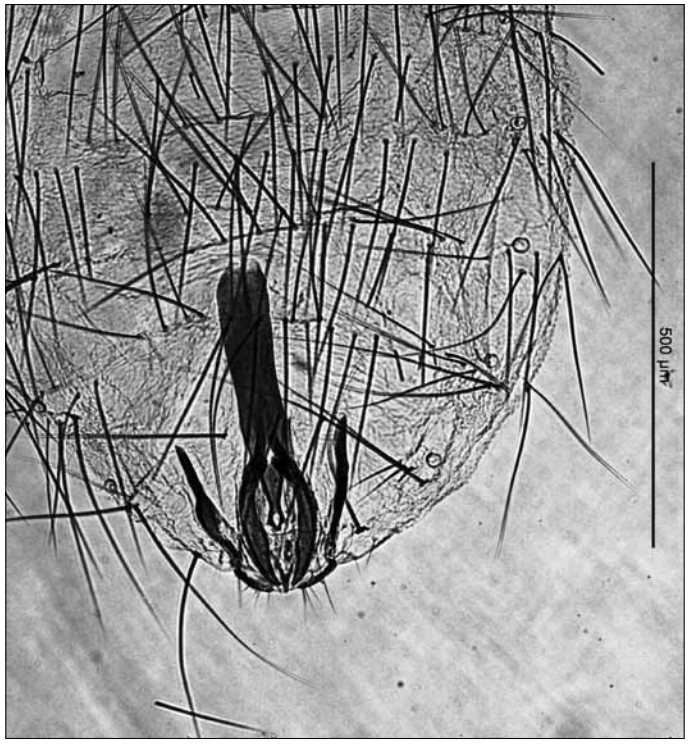

Figure 4. *Haemodipsus leporis*, male, genitalia, original

cephalic index: 0.90, thorax length: 0.32 mm, thorax width: 0.48 mm, abdomen length: 0.90 mm, abdomen width: 0.76 mm, total length: 1.71-1.80 mm.

DISCUSSION

Blagoveshtchensky (5) described *H. leporis* from the mountain hare, *L. timidus,* in Yakutia. This species was also reported on a young hare (*L. timidus*) in Norway (11). According to Blagoveshtchensky (5), the head of *H. leporis* is similar to *H. ventricosus*; however, it is distinguished from the latter species which has a bigger head and a sternal plate that has a hexagonal shape. This author also stated that sternal plate of *H. leporis* is very similar to *H. setoni;* however, it is distinguished from the latter species with the shape of the head and has a slightly narrower projection on the posterior part of the abdomen (5). Two specimens obtained on *L. europaeus* in this study were examined and it was observed that the sternal plate was hexagonal, making it similar to *H. setoni*. However, it is distinguished from the latter species as the head is triangular, there are different male genitalia and the abdominal plate on the posterior is thinner. Two male individuals of *H. leporis* collected from *L. europaeus* in this study have similar morphological characters, such as a triangular head, hexagonal sternal plate and male genitalia according to the original description of *H. leporis*.

Figure 5. *Haemodipsus leporis*, male, genitalia, original

The lice species in the genus *Hemodipsus* live on hares (2, 3, 6). There are seven species in this genus and some authors have stated that *H. africanus* in southern Africa, *H. conformalis* in Central Asia, *H. leporis* and *H. lyriocephalus* in Eurasia, *H. setoni* and *H. brachylagi* in North America and *H. ventricosus* in Europe had been found on both rabbits and hare. Furthermore, some of these species have definitely, or apparently, been introduced into other biogeographical regions (2, 3). According to Durden and Rausch (3), Tenquist and Charleston claimed that *H. lyriocephalus*

had been introduced New Zealand. This species also presented on scrub hares, *L. saxatilis* in South Africa (12). *H. setoni* was recorded from some countries such as England, Switzerland, Poland, and France in Europe (3, 6) and the Asian part of Turkey (8, 9). Some authors reported that earlier authors had been mistakenly identified *H. setoni* as *H. ventricosus* (5, 13). In addition, some authors recorded that the primary host of *H. ventricosus* was *Oryctolagus cuniculus,* and that this species had been found on *L. townsendii, L. saxatilis* and *Sylvilagus audubonii* in error or reflecting accidental host-parasite relationships (3, 6).

There are a few studies available on ectoparasites found on hares in Turkey (7-9). Only two *Haemodipsus* species were detected on the hares in Turkey: *H. lyriocephalus* and *H. setoni* (8, 9). These authors only detected *L. europaeus* and found no other hare species in their studies in Konya province, Turkey (8, 9).

CONCLUSION

H. leporis were recorded for the first time in Turkey. *L. europaeus* is the new host for *H. leporis* throughout the world.

Conflict of Interest

No conflict of interest was declared by the authors.

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