Mehmet Akif Ersoy Üniversitesi
Sağlık Bilimleri Enstitüsü Dergisi

MAKÜ
BURDUR MEHMET AKİF ERSOY ÜNİVERSİTESİ

SBE
SAĞLIK BİLİMLERİ ENSTİTÜSÜ

NİSAN/APRIL **2020**
CİLT/VOLUME **8**
SAYI/ISSUE **1**

Mehmet Akif Ersoy University
Journal of Health Sciences Institute

E-ISSN: 2148-2837

ISSN: 2148-2837

MEHMET AKİF ERSOY ÜNİVERSİTESİ SAĞLIK BİLİMLERİ ENSTİTÜSÜ DERGİSİ

Mehmet Akif Ersoy University Journal of Health Sciences Institute

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Mehmet Akif Ersoy Üniversitesi Sağlık Bilimleri Enstitüsü Dergisi yılda 3 sayı olarak yayımlanır (Aralık-2019 itibariyle). Dergi, *DOAJ, Google Scholar, SciLib, Researchbib, SOBIAD, Türkiye Atıf Dizini* gibi ulusal ve uluslararası indeksler tarafından taranmaktadır.

Yıl/Year: 2020 – Cilt/Volume: 8– Sayı/Issue: 1

Yayın Kurulu / Advisory Board

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Mehmet Akif Ersoy Üniversitesi Sağlık Bilimleri Enstitüsü Dergisi
YAZARLARA BİLGİ

I- Mehmet Akif Ersoy Üniversitesi Sağlık Bilimleri Enstitüsü Dergisi Genel Bilgiler

Mehmet Akif Ersoy Üniversitesi (MAKÜ) Sağlık Bilimleri Enstitüsü Dergisi, Mehmet Akif Ersoy Üniversitesi Sağlık Bilimleri Enstitüsü'nün yayın organıdır. Derginin kısaltılmış adı "MAKÜ Sag. Bil. Enst. Derg" dir. Yılda 2 kez yayınlanır. MAKÜ Sağlık Bilimleri Enstitüsü Dergisi sağlık bilimleri, (veteriner, tıp, diş hekimliği, hemşirelik ve spor bilimleri) alanlarında temel ve klinik hakemli bilim yazılarının yayınlandığı hakem-denetimli bir dergidir. Derginin dili Türkçe ve İngilizce'dir. Dergiye gönderilen yazıların başka herhangi bir dergide yayınlanmamış, yayına kabul edilmemiş ya da yayınlanmak üzere değerlendirme aşamasında olmaması gerekir. Bu kural bilimsel toplantılarda sunulan ve özeti yayınlanan bildiriler için geçerli değildir. Ancak, bu gibi durumlarda bildirinin sunulduğu toplantının adı, tarihi ve yeri bildirilmelidir. Makalelerin formatı "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication (http:/www.icmje.org/)" kurallarına göre düzenlenmelidir.

Gönderilen yazılar yayın kuruluna ulaştıktan sonra öncelikle, yazım kurallarına uygunluğu yönünden değerlendirilir; sonucu yazara dört hafta içinde bildirilir. Yazının, gerek teknik özellikleri gerekse genel kapsamı açısından derginin genel yayın ilkelerine uygun bulunmaması durumunda yazı reddedilir. Ya da, gerekirse, yazar(lar)ın yazıyı yazım kurallarına uygun biçimde yeniden göndermeleri istenebilir. Yeniden gönderilen yazılar benzer bir teknik incelemenin ardından yazım kurallarına uygun ise danışman denetimi sürecine alınır. Yazı, editör ve yardımcı editörler ile yazının başlık sayfasını görmeyen en az iki danışmana gönderilerek incelenir. Yazı, yayın kurulunun belirlediği ve bilimsel içerik ve yazım kuralları açısından değerlendirilir. Editör ve yardımcı editörler gerek gördüğünde makaleyi üçüncü bir danışmana gönderebilir. Hakem belirleme yetkisi tamamen editör ve yardımcı editörler ve yayın kuruluna aittir. Danışmanlar belirlenirken derginin uluslararası yayın danışma kurulundan isimler seçilebileceği gibi yazının konusuna göre ihtiyaç duyulduğunda yurt içinden veya yurt dışından bağımsız danışmanlar da belirlenebilir. Daha sonra, danışman raporları dikkate alınarak ve gerekirse yazar(lar)la tekrar iletişim kurularak yayın kurulunca son redaksiyon yapılır. Yazıların kabulüne editör karar verir.

Editör yayın koşullarına uymayan yazıları; düzeltmek üzere yazarına geri gönderme, biçimce düzenleme veya reddetme yetkisine sahiptir. Yazılarını geri çekmek isteyen yazarlar bunu yazılı olarak editöre bildirmek durumundadır. Editör görülen lüzum halinde bazı makaleler hakkında yayın yürütme kurulunun görüşüne başvurur. Bu değerlendirme süreci dergiye gönderilen yazı türlerinden araştırma yazılarını, olgu sunumlarını ve özgün yazıları kapsar. Diğer yazı türlerindeki yazılar doğrudan yayın kurulunca değerlendirilir. Dergiye gönderilen yazılar yayınlansın ya da yayınlanmasın geri gönderilmez. Tüm yazarlar bilimsel katkı ve 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. Dergide yayınlanan yazılar için herhangi bir ücret ya da karşılık ödenmez.

Yayın kurulu yazar(lar)ın dergiye gönderdikleri yazıları değerlendirme süreci tamamlanmadan başka bir dergiye göndermeyeceklerini taahhüt ettiklerini kabul eder. İnsanlar ve hayvanlar üzerinde yapılan deneysel araştırmaların bildirildiği yazıların gereç ve yöntem bölümünde, bu araştırmanın yapıldığı gönüllü ya da hastalara uygulanan işlemler anlatıldıktan sonra kendilerinin onaylarının alındığını (informed consent) gösterir bir cümle bulunmalıdır. Yazar(lar), bu tür araştırmalarda, uluslararası alanda kabul edilen kılavuzlara (2002 yılında revize edilen 1975 Helsinki Deklarasyonu- http://www.wma.net/e/policy/b3.htm, Guide for the care and use of laboratory animals - www.nap.edu/catalog/5140.html), T.C. Sağlık Bakanlığı tarafından getirilen, 29 Ocak 1993 tarih ve 21480 sayılı Resmi gazetede yayınlanan "İlaç Araştırmaları Hakkında Yönetmelik" ve daha sonra yayınlanan diğer yönetmeliklerde belirtilen hükümlere uyulduğunu belirtmeli ve kurumdan aldıkları Etik Kurul Onayı'nın bir kopyasını göndermelidir. Metin içinde standart kısaltmalar kullanılır, bunlar ilk geçtikleri yerde açık olarak yazılır. İlaç adları kullanımında ilaçların jenerik adları Türkçe okunuşlarıyla yazılır. Ölçüm birimleri metrik sisteme uygun olarak verilir; örneğin, "mg" olarak yazılır, nokta kullanılmaz; ek alırsa („) ile ayrılır. Laboratuar ölçümleri Uluslararası Sistem (US; Systéme International: SI) birimleri ile bildirilir.

Bilimsel sorumluluk

Makalelerin tüm bilimsel sorumluluğu yazarlara aittir. Gönderilen makalede belirtilen yazarların çalışmaya belirli bir oranda katkısının olması gereklidir. Yazarların isim sıralaması ortak verilen bir karar olmalıdır. Sorumlu yazar, yazar sıralamasını "Yazar Sorumluluk ve Yayım Hakkı Devir Formu'nu" doldurarak tüm yazarlar adına kabul etmiş sayılır. Yazarların tümünün ismi makale başlığının altındaki bölümde yer almalıdır.

Yayın Ücretleri

Bu dergide yayın tamamen ücretsizdir. Yayın ücreti, başvuru ücreti, makale işleme ücreti ve bir figürün, rakamın veya tamamlayıcı verinin uzunluğuna göre ek ücret ödenmesi gerekmez. İçerik öğeleri (Editörler, Düzeltmeler, İlaveler, Geri Çekmeler, Mektuplar, Yorumlar vb.) tamamen ücretsizdir.

Etik sorumluluk

Makalelerin etik kurallara uygunluğu yazarların sorumluluğundadır. Hayvanlar üzerinde yapılan deneysel çalışmalarda, çalışma protokolünün çalışmanın yapıldığı kurumdaki hayvan deneyleri etik kurulu tarafından onaylandığı belirtilmelidir. Yazarlar etik kurul onayını makale ile birlikte göndermelidir. Eğer makalede daha önce yayımlanmış alıntı yazı, tablo, resim vs. var ise yazarlar; yayım hakkı sahibi ve yazarlarından yazılı izin alarak bu durumu makalede belirtmek zorundadır. Makalenin değerlendirilmesi aşamasında yayın kurulunun gerek görmesi halinde, makale ile ilgili araştırma verilerinin ve/veya etik kurul onayı belgesinin sunulması yazarlardan talep edilebilir.

İntihal politikası

Mehmet Akif Ersoy Üniversitesi Sağlık Bilimleri Enstitüsü Dergisi'ne (MAKÜ Sag. Bil. Enst. Derg.) Gönderilen yazılar intihal açısından değerlendirilir. Her gönderilen makale, iThenticate ve Turnitin yazılımı ile intihal için kontrol edilir. Makalenin benzerlik oranı %20'nin üzerinde ise, revize edilmesi için ilgili yazara geri gönderilir. Eğer makalenin yayınlanmasından sonra intihal kanıtlanırsa, bu makale derhal web sitesinden kaldırılır ve ilgili yazarlara makalelerinin MAKÜ Sag. Bil. Enst. Derg.'de yayınlanmasının uygun olmadığı bildirilecektir.

II- Dergiye Gönderilecek Yazı Türleri ve Özellikleri

a) Araştırma Makaleleri: Bu yazılar daha önce yayınlanmamış özgün araştırma verilerinin değerlendirildiği net anlam taşıyan bilimsel çalışmaları kapsar. Araştırma makaleleri "Öz, Giriş, Gereç ve Yöntem, Bulgular, Tartışma ve Kaynaklar" bölümlerinden oluşmalıdır. Dergide yayınlanmak üzere gönderilen araştırma makaleleri kapak sayfası hariç en fazla 20 sayfa olmalıdır. Araştırma makalelerinde kullanılacak tablo, çizim ve resim sayısı toplam 10'u geçmemelidir. Yazarlar gerek duydukları takdirde "Tartışma" bölümünden sonra "Teşekkür" bölümü açarak gerekli açıklamaları yapabilirler.
b) Derleme Makaleleri: Derleme makaleleri dergi editör/yayın kurulu tarafından "çağrılı derlemeler" başlığı altında oluşturulan alnında katkı sağlama potansiyeli olan yazıları içerir. Kaynakça bölümü en fazla 30 kaynakçadan oluşturulmalıdır. Derlemelerde kullanılacak tablo, çizim ve resim sayısı toplam 10'u geçmemelidir. Kapak sayfası hariç en fazla 20 sayfa olarak hazırlanmalıdır. Derlemelerde mutlaka "Öz, Giriş, Sonuç ve Kaynaklar" bölümleri bulunmalıdır.
c) Olgu Sunumları: Yazarların, herhangi planlanmış bir araştırmaya dayanmayan ancak karşılaştıkları yeni veya ender gözlemlenen olguların ele alındığı, bilimsel değere sahip bilgileri içeren eserlerdir. Bu eserlerde gereksiz uzatmaları önlemek amacıyla en fazla 15 kaynak kullanılmalı ve bu kaynakların güncel olmasına özen gösterilmelidir. Kapak sayfası hariç en fazla 5 sayfa olmalı; "Öz, Giriş, Olgu, Tartışma ve Kaynaklar" bölümlerinden oluşmalıdır.
d) Kısa Araştırma Raporu: Dar kapsamlı ele alınmış (sınırlı sayıda örneğin analiz edildiği çalışmalar vb.) ancak önemli ve yeni bilgiler sunan bilimsel araştırmaya dayalı makalelerdir. Kısa bildiriler araştırma makalesi formatında hazırlanmalı ve kapak sayfası hariç en fazla 10 sayfa olmalıdır. Bu eserlerde kullanılacak tablo ve şekil sayısı beşi geçmemelidir.
e) Özel Bölümler:
1. Editöre mektuplar: Dergide yayınlanan yazılara ilişkin değerlendirme ve eleştirileri içeren yazılardır. Mümkün olduğunca eleştirilen yazının yazar(lar)ınca verilen yanıtlar ile birlikte yayınlanır. Editöre mektuplar 3 sayfayı geçemez.
2. Toplantı haberleri/izlenimleri: Derginin yayın alanıyla ilgili konularda yapılmış ya da yapılacak olan bilimsel toplantıları tanıtıcı yazılardır. 1 sayfayı geçemez.
3. Dergi haberleri: Derginin yayın alanıyla ilgili konularda yayınlanmakta olan bilimsel dergileri tanıtıcı yazılardır; 1 sayfayı geçemez.
4. Web siteleri tanıtımı: Derginin yayın alanıyla ilgili konulardaki web sitelerini tanıtıcı yazılardır; 1 sayfayı geçemez.
5. Kitap/tez tanıtımı: Derginin yayın alanıyla ilgili konularda yayınlanmış bulunan kitapları/tezleri tanıtan yazılardır; 3 sayfayı geçemez.

III- Makalelerin Düzenlenmesi

Dergiye gönderilecek yazılar türlerine göre, başlık sayfası, İngilizce ve Türkçe özetler, ana metin, kaynaklar, tablo/şekil/resim bölümlerini içerir. Dergiye yayınlanması için gönderilen makalelerde aşağıdaki biçimsel esaslara uyulmalıdır: Yazı Microsoft Word programında Times New Roman yazı stilinde 12 punto büyüklüğünde, siyah renkte, 1,5 satır aralığında hazırlanmalıdır. Kenarlardan 2,5 cm boşluk bırakılmalıdır. Her

sayfaya satır numarası eklenmelidir.

Anatomik terimler Latincede yazıldığı gibi kullanılmalıdır. Günlük tıp diline yerleşmiş terimler ise okundukları gibi Türkçe yazım kurallarına uygun olarak yazılmalıdır. İngilizce veya başka bir yabancı dildeki şekli ile yazılan terimler tırnak içinde belirtilmelidir. Yazının başlık sayfasında, yazının Türkçe ve İngilizce başlığı ve sayfa üstünde kullanılmak üzere boşluklar da dahil 40 karakteri aşmayacak şekilde Türkçe ve İngilizce kısa başlık önerisi bulunmalı. Çalışmaların yapıldığı klinik, anabilim dalı/bilim dalı, enstitü ve kuruluşun adı belirtilmelidir.

a) Başlık Sayfası: Gönderilen makalenin kategorisini, başlığını (Türkçe-İngilizce ve sadece ilk sözcüğün baş harfi büyük), yazarların adlarını (sadece baş harfleri büyük yazılır), çalıştıkları kurumları (rakamla dipnot olarak belirtilmeli), yazışmaların yapılacağı sorumlu yazarın adı, açık adresi, telefon ve faks numaraları ile e-posta adresini içermelidir. Sorumlu yazar yıldız (*) ile belirtilir. Makale daha önce bilimsel bir toplantıda sunulmuş ise toplantının adı, tarihi ve yeri belirtilerek yazılmalıdır.

b) Ana Metin Bölümü: Yazının ana metni Öz ve Anahtar Kelimeler, Giriş, Gereç ve Yöntem, Bulgular ve Tartışma başlıkları içinde düzenlenir. Özler ve anahtar sözcükler: Türkçe ve İngilizce olmak üzere iki dilde yazılır ve yazının başlığını da içerir.

Öz 200 kelimeyi geçmemeli, çalışmanın ana noktaları olan amacını, hayvan ve örnek popülasyonunu, metodunu ve önemli sonuçlarını, çalışmadan elde edilen çıkarımı klinik olarak uygulanabilirliğini içermelidir. Yayını okumadan okuyucular için anlaşılır olmalıdır ve özet içinde kaynaklara atıf yapılmamalıdır. Türkçe ve İngilizce özetler ayrı sayfalarda yazılmalı ve özetlerin sonunda her iki dilden en az 3, en çok 5 anahtar sözcük yer almalıdır. Anahtar kelimeler Index Medicus Medical Subject Headings (MeSH)'e uygun olmalıdır. Anahtar kelimeler için www.nlm.nih.gov/mesh/MBrowser.html adresine başvurulmalıdır.

Giriş bölümünde yazının dayandığı temel bilgilere ve gerekçelere kısaca değinildikten sonra, son paragrafında amaç açık bir anlatımla yer alır. Gereç ve yöntem bölümü gerekirse araştırma/hasta/denek grubu, araçlar, uygulama ve istatistik değerlendirme gibi alt başlıklara göre düzenlenebilir. Bu bölüm çalışmaya katılmayan birisinin de rahatlıkla anlayabileceği açıklıkta yazılmalıdır. Bulgular bölümü çalışmanın sonuçlarını özetler ve temel bulgular gerekirse tablo ve şekillerle desteklenir. Tartışma bölümünde çalışmanın bulguları ilgili yurt içi ve yurt dışı çalışmaların sonuçları bağlamında tartışılır; genel bir gözden geçirmeyi değil, özgün bulguların tartışılmasını içerir. Yayın sisteme yüklenirken ana metin bölümü ana dosya olarak yüklenmelidir.

c) Teşekkür: Yazarlar çalışmalarında vermek istedikleri ek bilgiler ile katkı sağlayan destekçi kurumlara ve/veya şahıslara teşekkür yazılarını bu bölümde belirtebilirler.

d) Kaynaklar: Kaynaklar listesi alfabetik sıraya göre yazılmalıdır. Sadece yayınlanmış veya yayına kabul edilmiş kaynaklar yer almalıdır. Kabul edilmiş ancak henüz yayınlanmamış kaynaklar için "baskıda" ifadesi kullanılmalıdır. Yazarlar kaynaklar listesinde bulunan bütün kaynakların metin içinde kullanılmış olduğunu kontrol etmelidirler.

Yayındaki bütün kaynaklar kullanılmalıdır. Makale içinde referans kullanma şekline örnekler.

Metin içinde doğrudan atıf yapılırken yazar veya yazarların soyadından sonra parantez içinde kaynağın yayın yılı belirtilmelidir.
Örnekler: Bell (2005) tarafından; Nielsen ve Engberg (2006) tarafından; Doyle ve ark. (2007) tarafından
Cümlenin sonunda atıf yapıldığında ise yazar ismi ve yayın yılı parantez içinde belirtilmelidir.
Örnekler: ...bildirilmiştir (Bell, 2005); ....bildirilmiştir (Nielsen ve Engberg, 2006); .....bildirilmiştir (Doyle ve ark., 2007).
Birden çok kaynağa atıf yapılması durumunda kronolojik sıralama yapılmalıdır.
Örnekler: ….bildirilmiştir (Bell, 2005; Nielsen ve Engberg, 2006; Doyle ve ark., 2007).
Aynı yazarın aynı yıl yayınları söz konusu ise her biri "a" harfinden başlayarak küçük harflerle işaretlenmelidir.
Örnek: …. (Bell, 2005a; Bell, 2005b; Bell, 2005c …). Atıf yapılırken aşırı kaynak kullanımından kaçınılmalıdır.

Kaynaklar listesinin düzenlenmesi:

Mendeley programı kullanan yazarlar aşağıda linki verilen dergi format stilini kullanarak çalışmalarını düzenleyebilir:
https://csl.mendeley.com/styles/529990351/makusagbilensderg

Kaynaklar listesinde yazar isimleri ve yayın yılı koyu harflerle yazılmalıdır. Kaynak listesi şu şekilde hazırlanmalıdır:
i) Kaynak makale ise
Yazarların soyadları ve adlarının ilk harfi yazılmalıdır. Devamında sırasıyla makalenin yayın yılı, makalenin adı,

yayınlandığı derginin açık adı, cilt, sayı ve sayfa numaraları belirtilmelidir.
Örnekler:
Cohen, N.D., Vontur, C.A., Rakestraw, P.C., 2000. Risk factors for enterolithiasis among horses in Texas. Journal of the American Veterinary Medical Association 216, 1787-1794.
Rajmohan, S., Dodd, C.E., Waites, W.M., 2002. Enzymes from isolates of *Pseudomonas fluorescens* involved in food spoilage. Journal of Applied Microbiology 93, 205-213.
Ono, K., Yamamoto, K., 1999. Contamination of meat with *Campylobacter jejuni* in Saitama, Japan. International Journal of Food Microbiology 47, 211-219.
Yayınlanmak üzere kabul edilen ve DOI numarası bulunan, ancak henüz basılmamış makaleler için; makale künyesinin sonunda DOI numarası belirtilmelidir.
McGregor, B.A., Butler, K.L., 2014. The value of visual fleece assessment in addition to objective measurements in identifying Angora goats of greater clean mohair production. Small Ruminant Research, in press (DOI: 10.1016/j.smallrumres.2014.04.001).

ii) Kaynak kitap ise

Yazarların (veya editörün) soyadları ve adlarının ilk harfi yazılmalıdır. Devamında sırasıyla kitabın yayın yılı, adı, yayınevi veya yayınlayan kuruluş ve yayınlandığı yer belirtilmelidir. Kaynak, kitaptan bir bölüm ise bölüm yazarlarının isminden sonra sırasıyla kitabın yayın yılı, bölümün adı, editörün soy ismi ve adının ilk harfi, bölümün alındığı kitabın adı, yayınevi veya kuruluş, yayınlandığı yer, bölümün sayfa numaraları yazılmalıdır.
Örnekler:
Combs, G.F., 1992. The Vitamins: Fundamental Aspects in Nutrition and Health. Academic Press, San Diego.
Concannon, P.W., 1986. Physiology and Endocrinology of Cannine Pregnancy. In: Marrow, D.A. (Ed.), Current Therapy in Theriogenology. Philadelphia, W.B. Saunders Company, pp. 491-497.
Perkins, J.B., Pero, J., 2002. Vitamin biosynthesis. In: Sonenshein, A., Hoch, J., Losick, R. (Eds.), Bacillus subtilis and Its Closest Relatives: from Genes to Cells. ASM Press, Washington D.C., pp. 271-286.
Kramer, J.M., Gilbert, R.J., 1989. Bacillus cereus. In: Doyle, M.P. (Ed.), Foodborne Bacterial Pathogens. Marcel Dekker, New York, pp. 22-70.

iii) Kaynak bir tez ise

Tezi yazan kişinin soyadı ve adının ilk harfi koyu olarak yazılmalı, kabul edildiği yıl, tezin başlığı, tezin cinsi (yüksek lisans veya doktora), üniversitesi ve enstitüsü belirtilmelidir.
Örnek:
Bacınoğlu, S., 2002. Boğa spermasında farklı eritme süreleri ve eritme sonrasında oluşturulan soğuk şoklarının spermatolojik özelliklere etkisi. Doktora Tezi, İstanbul Üniversitesi Sağlık Bilimleri Enstitüsü, İstanbul.

iv) Kaynak internette bulunan bir web sitesi ise

Yazarların soyadları ve adının ilk harfi (Yazar adı yoksa web sitesinin veya kaynağın adı) yazılır. Daha sonra sırasıyla yılı, makalenin adı, varsa yayıncı, internet adresi ve erişim tarihi belirtilir.
Örnekler:
FDA, 2001. Effect of the use of antimicrobials in food-producing animals on pathogen load. Systematic review of the published literature. http://www.fda.gov/cvm/antimicrobial/PathRpt.pdf (Erişim 14.12.2001)
Cleveland, C.W., Peterson, D.S., Latimer, K.S., 2005. An Overview of Canine Babesiosis. Clinical Pathology. College of Veterinary Medicine, The University of Georgia: http://www.vet.uga.edu/vpp/clerk/Cleveland (Erişim 17.12.2005).
Thierry, F., 2006. Contagious equine metritis: a review. Equine Reproductive Infections: http://www.equinereproinfections.com (Erişim 07.07.2006].
FSAI, 2008. Report of the Implementation Group on Folic Acid Food Fortification to the Department of Health and Children. Food Safety Authority of Ireland: http://www.fsai.ie/assets/0/86/204/cc3c2261-7dc8-4225-bf79-9a47fbc2287b.pdf (Erişim 20.06.2008)

v) Kaynak bilimsel toplantıda sunulmuş bir bildiri ise

Yazarların soyadı ve adının baş harfinden sonra sırasıyla toplantının yılı, bildirinin başlığı, toplantının adı, toplantı yeri, bildiri kitabındaki sayfa no yazılmalıdır.
Örnekler:
Cardinali, R., Rebollar, P.G., Mugnai, C., Dal Bosco, A., Cuadrado, M., Castellini, C., 2008. Pasture availability and genotype effects in rabbits: 2. development of gastro-intestinal tract and immune function of the vermiphorm appendix. In: Proc. 9th World Rabbit Congress, Verona, Italy, 1159-1164.
Mauget, R., Legendre, X., Comizzoli, P., 1998. Assisted reproductive technology in sika deer: a program to preserve endangered deer subspecies. In: Proc. 4th Int. Deer Biology Congress, Kaspovar, 185-186.

e) Tablolar: Kullanım sırasına göre numaralandırılmalı, kısa başlıklarla ifade edilmeli ve metin içinde tablo numarası verilerek (örneğin Tablo 1) atıfta bulunulmalıdır. Tablo başlıkları tablonun üst bölümüne yazılmalıdır. Tabloda kullanılan kısaltmalar ve gerekli açıklamalar tablo altında verilmelidir.

f) Şekil ve Resimler: Metinde kullanılan fotoğraflar, grafikler ve çizimler metin içinde şekil adı ile kullanılmalıdır. Şekiller kullanım sırasına göre numaralandırmalı ve kısa başlıklarla ifade edilmeli, metin içinde

şekil numarası verilerek (örneğin Şekil 1) atıfta bulunulmalıdır. Şekil başlıkları şekillerin altında yer almalıdır. Şekillerde istenilen noktaya dikkat çekmek amacıyla; üzerlerine işaret konulmalı ve başlıklardan sonra yer alacak olan şekil altı notta kullanılan işaretler belirtilerek gerekli açıklamalar yapılmalıdır.

IV- Makale Süreci (Kör hakemlik)

Makale başvurusu yalnızca online olarak http://dergipark.gov.tr/maeusabed adresi üzerinden kabul edilmektedir. Sorumlu yazar, makale ile birlikte göndereceği tüm dosyaları yukarıdaki internet adresinde bulunan yeni makale gönder ikonunu tıklayarak sisteme ekleyebilir. Yazarlar dergiye gönderi yapmadan önce kayıt olmalıdır. Kaydolduktan sonra, ana sayfadaki Mehmet Akif Ersoy Üniversitesi Sağlık Bilimleri Enstitüsü Dergisi ikonuna tıklayarak; yazım kurallarına göre düzenlenmiş bilimsel çalışmayı dergi panelindeki Makale Gönder kısmından 4 basamaklı (başlarken, yükleme, kaynaklar, önizleme&gönder) gönderi işlemini yapabilir. Gönderilen makalede ön değerlendirme aşaması sırasında yazar künyeleri, çalışmanın yapıldığı kurum, etik kurul ya da özel izin adres bilgileri gibi tanıtıcı bilgiler içermemelidir. Ön değerlendirmeden (bilimsel nitelik, dil, yazım kuralları kontrolü, İntihal kontrolü iThenticate ve Turnitin programı,) geçen bilimsel çalışmaların hakem ataması yapılır. Sorumlu yazar makalenin hangi aşamada olduğunu sistem panelindeki Süreçteki Makaleler kısmından takip edebilir. Atanan hakemlere, kör hakemlik kuralları çerçevesinde çalışmanın tam metni, şekil, tablo, grafik ve resimleri sistem üzerinden yüklenerek e-posta aracılığıyla makale değerlendirme talebi gönderilir. Hakemler e-posta aracılığıyla gönderilen linke tıklayarak talebi kabul ya da reddederler. Kabul eden hakemler, kararlarını sistem üzerinden en fazla 1 ay içinde sebeplerle birlikte yüklemelidirler. Hakemin önerdiği düzeltme var ise tekrar yazara gönderilir. İstenilen düzeltmeler 1 ay içinde tamamlanıp gönderilmediği takdirde makale otomatik olarak iptal edilecektir. Editör, makalelerin yayın değerliliği ve hakemlerin görüşlerine dayanarak yayına kabul veya red kararını verir. İstenilen düzeltmeler yapıldıktan sonra makale yazar tarafından sisteme tekrar yüklenir. Derginin gizlilik bildiriminde belirtildiği gibi, yazarların kimlik bilgileri ve e-posta adresleri hiçbir şekilde başka amaçlar için kullanılmayacaktır.

Bu dergi; bilimsel araştırmaları halka ücretsiz sunmanın bilginin küresel paylaşımını artıracağı ilkesini benimseyerek, içeriğine anında açık erişim sağlamaktadır.

Mehmet Akif Ersoy University Journal of Health Sciences Institute
INSTRUCTIONS TO AUTHORS

I- Mehmet Akif Ersoy University Journal of Health Sciences Institute General Information

Mehmet Akif Ersoy University Journal of Health Sciences Intitute (MAKU J. Health Sci. Inst.) is the publication of Mehmet Akif Ersoy University Health Sciences Institute. It is published two times annualy. The journal is a peer-reviewed scientific journal in which basic and clinical scientific articles in the field of medical sciences (veterinary, medicine, dentistry, nursing and sports sciences) are published. The language of the journal is both Turkish and English. Papers submitted to the journal should not have been previously published, accepted for publication or be in the process of evaluation for publication in any other journal. This rule does not apply to articles presented as bulletins in scientific meetings and whose summaries are published. In such cases, however, the name, date and place of the meeting in which the paper was presented should be notified. The format of the article should be in accordance with the rules of "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication (http:/www.icmje.org/)".

On receipt of the paper by the Editorial Board, the paper is evaluated for compliance with the format rules and the authors are informed about the result in four weeks. In the event that the paper is not found to comply with the general publication principles of the journal from the standpoint of either technical characteristics or general scope, the paper is rejected. Alternatively, the author(s) may be asked to re-submit the paper in accordance with the writing requirements. Papers resubmitted are passed through a similar technical examination and, if found to comply with the rules, are passed on for peer review. The paper is sent, without the title, to two reviewers selected by the board, who then assess the paper for scientific content and format compliance. When necessary the Editorial Advisory Board can send the paper to third reviewers. The selection of reviewers is ultimately at the discretion of the editor, associate Editors and/or the editorial board. The appropriate reviewers can be selected from journal's international database of reviewers listing or, if needed; independent reviewers can be determined from inland or abroad. Thereafter the Editorial Advisory Board carries out the final editing, taking the reports of the reviewers into consideration, and, when necessary, communicating with the author(s).

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The Editorial Board assumes that the author(s) are obliged not to submit the paper to another journal before completion of the assessment process. In the "method" section of articles concerned with experimental research on humans or animals, a sentence showing that the informed consent of patients and volunteers has been obtained following a detailed explanation of the interventions carried out on them. In such studies, authors should clearly state the compliance with internationally accepted guidelines (1975 Helsinki declaration revised in 2002 http://www.wma.net/e/policy/b3.htm, Guide for the care and use of laboratory animals-www.nap.edu/catalog/5140.html) issued by the Republic of Turkey Ministry of Health and published in the Official Journal dated 29 January 1993 number 21480 "Regulations Concerning Drug Research", and other more recently published rules laid out in governing statutes. They should forward a copy of the Ethic Committee Approval received from the relevant institution. Standard abbreviations used in the text are written in full when first mentioned. In the use of drugs, the generic names should be written in their Turkish pronunciation spelling form. Measurement units are given according to the metric system; e.g. written as "mg", no punctuation is used, in the case of extensions (,,) is used as a separator. Laboratory measurements are reported in International System Units (US; Systeme Internationale; SI).

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Manuscripts submitted to Mehmet Akif Ersoy University Journal of Health Sciences Institute is evaluated in terms of plagiarism. Every submitted article is checked for plagiarism through iThenticate and Turnitin software. When Smilarity Index of the article is above %20, it is sent back to the corresponding author to revise it. If plagiarism is proved after publication of the article, that article will be immediately removed from the website and the concerned authors will be considered ineligible for publication of their articles in Mehmet Akif Ersoy University Journal of Health Sciences Institute.

II- Types and Characteristics of Papers to be Submitted to the Journal

a) Research Articles: These articles are prepared in full accordance with the writing style definitions given below, in which previously unpublished original research data are evaluated. The main text section of the research articles should include (Title, Introduction Materials and Methods, Results, Discussion and Conclusion) sections and (excluding title page, bibliography, tables/figures/pictures) should not exceed 20 pages. If some parts of the research data given in these articles have previously been discussed in another paper, this must be notified without fail when sending the paper and, in addition, reference should be made to the relevant paper within the bibliography.

b) Review Articles: Review Articles should cover subjects falling within the scope of the journal which are of active current interest. They may be submitted or invited. Invited reviews will normally be solicited by the Review's Editor, but suggestions for appropriate review topics may be sent to editor.

c) Case Reports: These are articles which present and discuss the characteristics of one or more cases which have special features and scientific importance from the clinical evaluation, observation or other standpoint. Case presentations include the title page, summary, main text (includes introduction, case and discussion), bibliography, table/figure/picture sections; subtitles in the main text are organised according to the text content. Abstracts of the case presentations should have 150 words. The main text (excluding title page, bibliography, table/figure/picture) should not exceed 10 pages.

d) Brief Reports: These are articles in which original ideas dealing with important theoretical or practical problems related to a specific subject are presented and discussed. Original articles include a title page, summary, main text, bibliography, table/figure/picture sections; subtitles in the main text are organised according to the text content. The main text of original articles (excluding title page, bibliography, table/figure/picture) should not exceed 10 pages.

e) Special Sections:

1. Letters to the Editor: These articles include evaluation and criticisms of articles published in the journal. These are published together with the responses of the author(s) of the paper concerned where possible. Letters to the Editor may not exceed 5 pages.

2. Meeting news/notes: These articles introduce scientific meetings held or to be held on subjects within the scope of the journal. The paper may not exceed 1 page.

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4. Introduction of websites: These articles introduce websites relevant to the scope of the journal. These articles may not exceed 1 page.

5. Book/Thesis Section: These articles introduce books/theses published on subjects related to the scope of the journal and may not exceed 3 pages.

III- Preparation of Manuscripts

Papers to be submitted to the journal include the sections of title page, abstract, main text, references and tables/figures/pictures. Articles submitted for publication in the journal should follow the following formal principles: The text should be prepared in Microsoft Word program in Times New Roman font style with a font size of 12 font, black and 1.5 line. All side of the paper, page margins should be as 2.5 cm. Line numbers should be added to the beginning of the page.

Anatomical terms should be used as written in Latin. Running title (not exceed 40 characters) of the manuscript should add to title page. The name of the clinic, department / science, institute and institution should be stated.

a) Title Page: should contain the category, the title (only first letter capital), the names of the authors (only the first letters capital), the institution (s) where they work (indicated with numbered footnotes), corresponding author (address, phone, fax numbers and e-mail address). Corresponding author is indicated by an asterisk (*). If the article was previously presented at a scientific meeting, the name, date and place of the meeting must be stated.

b) Main Text: The main text of the paper is organised under the subtitles of Abstract and Keywords, Introduction, Materials and Methods, Results and Discussion.

Abstract and Keywords: This is written in two languages, Turkish and English, and also includes the title of the paper. The abstract is consists of 200 words. The abstract should bring out the main points of the manuscript and should include the following information: objective, the animals or sample population involved, design, the materials and methods used, the main results, a brief conclusion and clinical relevance, where applicable. They should be comprehensible to readers before they have read the paper, and abbreviations and reference citations should be avoided. At the end of the abstract, at least 3, at most 5 keywords in both languages are included.

In the introduction, following a brief statement of basic information and justifications which constitute the basis of the paper, the objective is clearly given in the last paragraph. If necessary, the “method” section may be organised according to sub-titles such as research/patient/ test group, instruments, application and statistical analysis. This section should be written with clarity so that a person not involved in the study may easily understand. Results summarize the findings of the study and, when necessary, basic findings are supported with tables and figures. In the discussion section, the findings of the study are discussed in the light of relevant national and international studies; this section includes discussion of original findings, not a general review.

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All citations in the text should refer to: the year of publication of the reference should be indicated in parentheses after the surname of the author or authors.
Examples: Bell (2005), Nielsen and Engberg (2006), Doyle et al. (2007) were indicated that.....
The name of the author and the year of publication should be stated in parentheses at the end of the sentence.
Examples: ...were detected as 23% of the samples (Bell, 2005); ....were detected as 23% of the samples (Nielsen and Engberg, 2006); ...were detected as 23% of the samples (Doyle et al., 2007).
In case of more than one reference, references should be arranged chronologically.
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i) Examples of journal articles:
Cohen, N.D., Vontur, C.A., Rakestraw, P.C., 2000. Risk factors for enterolithiasis among horses in Texas. Journal of the American Veterinary Medical Association 216, 1787-1794.

Rajmohan, S., Dodd, C.E., Waites, W.M., 2002. Enzymes from isolates of *Pseudomonas fluorescens* involved in food spoilage. Journal of Applied Microbiology 93, 205-213.
Ono, K., Yamamoto, K., 1999. Contamination of meat with *Campylobacter jejuni* in Saitama, Japan. International Journal of Food Microbiology 47, 211-219.
For articles that are accepted for publication and have a DOI number but not yet published; DOI number must be specified at the end of the article.
McGregor, B.A., Butler, K.L., 2014. The value of visual fleece assessment in addition to objective measurements in identifying Angora goats of greater clean mohair production. Small Ruminant Research, in press (DOI: 10.1016/j.smallrumres.2014.04.001).
ii) Books:
Combs, G.F., 1992. The Vitamins: Fundamental Aspects in Nutrition and Health. Academic Press, San Diego.
Concannon, P.W., 1986. Physiology and Endocrinology of Cannine Pregnancy. In: Marrow, D.A. (Ed.), Current Therapy in Theriogenology. Philadelphia, W.B. Saunders Company, pp. 491-497.
Perkins J.B., Pero, J., 2002. Vitamin biosynthesis. In: Sonenshein, A., Hoch, J., Losick, R. (Eds.), Bacillus subtilis and Its Closest Relatives: from Genes to Cells. ASM Press, Washington D.C., pp. 271-286.
Kramer, J.M., Gilbert, R.J., 1989. Bacillus cereus. In: Doyle, M.P. (Ed.), Foodborne Bacterial Pathogens. Marcel Dekker, New York, pp. 22-70.
iii) Thesis:
Bacınoğlu, S., 2002. Boğa spermasında farklı eritme süreleri ve eritme sonrasında oluşturulan soğuk şoklarının spermatolojik özelliklere etkisi. Doktora Tezi, İstanbul Üniversitesi Sağlık Bilimleri Enstitüsü, İstanbul.
iv) Web site or author is an institution:
FDA, 2001. Effect of the use of antimicrobials in food-producing animals on pathogen load. Systematic review of the published literature. http://www.fda.gov/cvm/antimicrobial/PathRpt.pdf (Accessed: 14.12.2001)
Cleveland, C.W., Peterson, D.S., Latimer, K.S., 2005. An Overview of Canine Babesiosis. Clinical Pathology. College of Veterinary Medicine, The University of Georgia: http://www.vet.uga.edu/vpp/clerk/Cleveland (Accessed: 17.12.2005).
Thierry, F., 2006. Contagious equine metritis: a review. Equine Reproductive Infections: http://www.equinereproinfections.com (Accessed: 07.07.2006).
FSAI, 2008. Report of the Implementation Group on Folic Acid Food Fortification to the Department of Health and Children. Food Safety Authority of Ireland:http://www.fsai.ie/assets/0/86/204/cc3c2261-7dc8-4225-bf79-9a47fbc2287b.pdf (Accessed: 20.06.2008).
v) Paper presented at a scientific meeting
Cardinali, R., Rebollar, P.G., Mugnai, C., Dal Bosco, A., Cuadrado, M., Castellini, C., 2008. Pasture availability and genotype effects in rabbits: 2. development of gastro-intestinal tract and immune function of the vermiphorm appendix. In: Proc. 9th World Rabbit Congress, Verona, Italy, 1159-1164.
Mauget, R., Legendre, X., Comizzoli, P., 1998. Assisted reproductive technology in sika deer: a program to preserve endangered deer subspecies. In: Proc. 4th Int. Deer Biology Congress, Kaspovar, 185-186.
e) Tables: Each table is printed on a separate page and numbered according to the sequence of referral within the text (Table 1). Each table has a title and, when necessary, explanations are given under the table (e.g. abbreviations given in the table). Each table should be understandable without need for referral to the text. Each table should be referred to in the text..
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IV- Submission of Articles (Blind Peer-Review)

The article submission is only accepted online via 'http://dergipark.gov.tr/maeusabed' The Corresponding authors, all the files can be added to the system by clicking the submit new article icon at the above address. Authors must register on Dergipark system before submitting a manuscript. After signing up, clicking Mehmet Akif Ersoy University Journal of Health Sciences icons on the main page, the manuscript written according to the guide for authors is submitted in 4 steps (start, submission, reference, preview & submit). The submitted manuscript must not contain any identifying information, such as author information, institution, ethics committee or special permit address, during the preliminary evaluation phase. The manuscript that pass the preliminary evaluation (paper scientific qualification, language, conformity to Guide for author and checking plagiarism via iThenticate and Turnitin program,) are assigned to the Reviewers. The corresponding author can follow the article evaluation process from the section on the Articles in the Process. According to the blind peer-review rules, the main text, tables, graphics and pictures of the manuscript are uploaded via the system and sent to the appointed reviewers for an article evaluation request via e-mail. The reviewers accept or reject the request by clicking on the link sent via e-mail. The reviewers who accept it have to upload their decisions together with the reasons within a maximum of 1 month via the system. If the correction requested by the Reviewer is sent back to the author. If the requested corrections are not completed within 1 month, the article will be automatically canceled. After the

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Araştırma Makaleleri / Research Articles

Derleme / Review

MAKU J. Health Sci. Inst. 2020, 8(1): 1-10.
doi: 10.24998/maeusabed.688264

Mehmet Akif Ersoy University
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http://dergipark.gov.tr/maeusabed

Research Article/Araştırma Makalesi

Seroprevalance of *Chlamydophila abortus* Infections in Goats in Burdur Province

Burdur İlinde Keçilerde Chlamdophila abortus Enfeksiyonunun Seroprevalansı

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Abstract: *Chlamydophila abortus* is the causative agent of enzootic abortion (OEA) in sheep that causes severe economic loss in sheep and goat breeding worldwide. The aim of this study was to detect the seroprevalence of *C. abortus* infection in goat flocks in Burdur province of Turkey. A total of 384 blood serum samples were collected from two years and older goat in randomly selected 22 goat flocks. The apparent and true seroprevalences of individual, within-flock and between- flocks of the *C. abortus* infection were determined in goats by a commercial the enzyme linked immunosorbent assay (ELISA) kit. The apparent and true seroprevalence of *C. abortus* individual, within- flock and between-flocks was calculated as 19.27%, 22.77%, 86.36% and 19.44%, 23.16% and 90.81%, respectively. The seropositivity of *C. abortus* infection to according to flock size were statistically significant ($p<0.05$) between some goat flocks. There was no statistically significant difference between goat breed and *C. abortus* infection ($p>0.05$). In conclusion, these findings showed that *C. abortus* infection is found high rates in goat flocks in Burdur and the control and eradication programs should be started to prevent the spreading of *C. abortus*.

Keywords: *Chlamydophila abortus*, ELISA, Goat, Seroprevalence.

Öz: *Chlamydophila abortus*, tüm dünyada koyun ve keçilerde ciddi ekonomik kayıplara yol açan, koyunların enzootik abortusunun (OEA) etkenidir. Bu çalışmanın amacı, Türkiye'nin Burdur ilinde keçi sürülerinde *C. abortus* enfeksiyonunun seroprevalansını belirlemektir. Toplam 384 kan serum örneği, rastgele seçilen 22 keçi sürüsünde, 2 ve daha büyük yaşlardaki keçilerden toplandı. *C. abortus* enfeksiyonunun bireysel, sürü içi ve sürüler arası seroprevalansı, ticari bir enzyme linked immunosorbent assay (ELISA) kiti ile belirlendi. *C. abortus*'un görünen ve gerçek bireysel, sürü içi ve sürüler arası seroprevalansı sırasıyla 19.27%, 22.77%, 86.36% ve 19.44%, 23.16%, 90.81% olarak hesaplandı. Sürü büyüklüğüne göre *C. abortus* enfeksiyonunun seropozitifliği bazı sürüler arasında istatistiki olarak önemli ($p<0.05$) bulundu. Keçi ırkları ile *C. abortus* enfeksiyonu arasındaki ilişki önemli bulunmadı ($p>0.05$). Sonuç olarak, *C. abortus* enfeksiyonunun Burdur ilinde bulunan keçi sürülerinde yüksek oranda bulunduğu, *C. abortus* enfeksiyonunun yayılımını önlemek için kontrol ve eradikasyon çalışmalarına hemen başlanması gerektiği kanaatine varıldı.

Anahtar Kelimeler: *Chlamydophila abortus,* ELISA, Keçi, Seroprevalans.

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Geliş tarihi / Received : 12.02.2020 Kabul tarihi / Accepted: 16.03.2020

Introduction

Ovine enzootic abortion (OEA) is a chronic disease caused by *Chlamydophila abortus* (*C. abortus*). *C. abortus* is a compulsory intracellular bacterium that causes abortion, stillbirth and poor offspring in small ruminants. *C. abortus* leads to significant economic losses in sheep and goat flocks in all over the world (Aydın and Paracıkoğlu, 2006). *C. abortus* causes usually abortions in 2-3 weeks before birth (Kalender et al., 2013). Subclinical infection can be transmitted to healthy animals and flocks through the placenta, vaginal discharge and aborted fetus of infected animals (Kalender et al., 2013). *C. abortus* is a zoonotic agent that can cause infections in humans, too. *C. abortus* can cause abortion in pregnant women in close contact with aborted sheep and goats (Kerr et al., 2005; Pospischil 2006). In addition, breeders, veterinarians, slaughterhouse workers, workers in

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ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

the vaccine production and laboratory workers can be infected by inhalation, taking into account the effects of urine, fecal and fetal fluids of infected animals (Hadley et al., 1992; Cislakova et al., 2007; Ortega et al., 2016).

The diagnosis of infection is made by direct and indirect diagnostic methods. Direct diagnostic methods are intended to demonstrate the presence of the agent. The isolation is the gold standard (Cantekin et al., 2015; Essig and Longbottom, 2015). But, *C. abortus* is a compulsory intracellular bacterium and it can not breed in the media, so isolation only involves culturing clinical specimens in laboratory animals, or tissue cultures or embriyonated chicken eggs (Aydın and Paracıkoğlu 2006; Cantekin et al., 2015). The isolation is not routinely performed because of the time consuming as well as the expert staff (Cantekin et al., 2015; OIE 2012, Beckman, 2019). The serologic tests are often used in diagnosis of infection. Complement fixation test (CFT) is a test used in the diagnosis of *C. abortus* infection and recommended by OIE (2012). But, cross reaction is detected between Gram negative bacteria such as *C. abortus*, *C. pecorum* and *Acinetobacter* sp. is low the sensitivity and specificity of test (OIE 2012). ELISA, a more sensitive and specific test than CFT, is frequently used in the detection of *C. abortus* infection in field and experimental studies (Vlahovic et al., 2001; Longbottom and Coulter, 2003). Its application is easy, cheap and at the same time testing a large number of animals and getting the results in a short time is the biggest advantage (Villagro-Blanco et al., 2015, OIE 2012).

The seroprevalence of *C. abortus* infection in sheep has been reported 1.81 %- 32 % in Turkey (Duman and Durak, 1998; Baz and Aydın, 2006; Caya et al., 2006; Küçükkayan et al., 2007; Otlu et al., 2007; Öztürk et al., 2016). In Burdur, there are two studies on the prevalence of *C. abortus* infection in sheep and cattle using ELISA (Öztürk et al., 2012; Öztürk et al., 2016). In these studies, *C. abortus* infection could not detectable in cattle, but the prevalence of individual, within-flocks and between flocks in sheep were 32%, 40% and 80%, respectively. Although presence of *C. abortus* infection in goats has known, the seroprevalence of *C. abortus* in goats had not been investigated in Turkey before.

The aim of the present study was to determine the apparent and true prevalence of *C. abortus* infection in goat flocks in Burdur province of Turkey.

Materials and Methods

Sampling

This study was conducted in between October 2016 and January 2017. The blood serum samples were collected from 22 goat flocks (Hair goat:12, Honamlı goat: 10) found least 20 goat with aged 2 years and older female goats, according to records of the Burdur Association of Sheep and Goat Breeders. The size of the flocks were changed between 40-500 animals. The goats were selected with random sampling method from flocks. The flock information used in this study was given in Table 1. Burdur province, which is located in southwest of Turkey is approximately 7,135 km^2 and a crossing area between Aegean, Middle Anatolia and Mediterranean parts of Turkey. The mean altitude is 1000 m above sea level.

In order to determine the prevalence rate without error, the sample size was determined according to epidemiological criteria. Since there was no study on seroprevalence of *C. abortus* in goats in Burdur province of Turkey, the estimated prevalence was accepted as 50% (Erganiş and Uçan, 2001). The minimum number of samples to be used in the survey according to 95% confidence interval and 5% error margin was determined as 384 (Erganiş and Uçan 2001). The 384 serum samples were collected from 11 to 20 goats in each flocks between October 2016 and January 2017. Blood samples were collected in 10 ml vacutainer tubes from the juguler vein of goats. Blood samples were transported to Burdur Mehmet Akif Ersoy University Faculty of Veterinary Medicine, Department of Microbiology Laboratory. The samples were centrifuged for 5 minutes at 5000 x

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ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

g and the serum samples were stored at -20°C until using.

ELISA

The serum samples were tested for *C. abortus* antibodies using a commercial ELISA kit (IDEXX, Switzerland, Liebefeld-Bern, Switzerland) according to the manufacturer's instructions. The absorbance value (OD) of each well was read in a 450 nm ELISA reader (Microplate Reader RT-2100C, Rayto and Analytical Sciences Co Ltd, PRC). The mean OD values of the positive (PC) and negative controls (NC) were calculated. According to the kit protocol, the test was accepted as valid if the mean value of PC (PCx) was ≤ 2,000, the mean value of NC (NCx) was ≤0,500 and PCx-NCx ≥ 0,300. The ratio S/P (% S / P) for each sera sample was calculated according to the following formula. Example P / S % = Example- NCx- / PCx-NCx- X 100. The S/P ratios of sera samples were evaluated as negative ≤ 30%, 30 % - 40 % suspicious, and ≥ 40 % positive for *C. abortus* antibodies. The flocks in which at least one seropositive animals for *C. abortus* were accepted to "positive flocks".

Table 1. The villages according to numbers of samples and herd size in this study

	Villages	Number of blood samples	Herd sizes
1	Kayis/Central village	20	430
2	Kayis/ Central village	20	160
3	Kayis /Central village	20	300
4	Kayis /Central village	20	350
5	Cine /Central village	12	100
6	Tas kapi/ Central village	20	40
7	MAKU goat farm /Central village	20	250
8	Guneyyayla /Central village	10	40
9	Guneyyayla /Central village	10	40
10	Guneyyayla /Central village	11	45
11	Yarisli /Yesilova	20	230
12	Kayadibi /Yesilova	20	205
13	Kayadibi /Yesilova	20	230
14	Harmanli/Yesilova	20	300
15	Kartalpinar	20	164
16	Bolmepinar/Cavdir	20	300
17	Cavdir	18	500
18	Kizillar / Cavdir	20	350
19	Bayir / Cavdir	19	430
20	Bayir / Cavdir	12	250
21	Karakoy / Cavdir	14	212
22	Cavdir	18	170
	Total	**384**	**5096**

Calculation of appearent prevalence

The appearent individual, within-flock and between- flock prevalence was calculated in 95% confident interval (GA) according to the Wilson binominol estimation method (Brown et al., 2001).

Calculation of true prevalence

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The true individual, within- flock and between-flock prevalence was calculated according to the Rogan-Gladen estimation method (Rogan and Gladen, 1978). In the calculations, Idexx Chlamydiosis Total Ab Test kit manufacturer (IDEXX) was used in 95% sensitivity and 99% specificity which is reported for the goats.

Statistical analysis

First, the data were entered in SPSS computer statistical program and whether serum samples collected from the flocks showed normal distribution was determined by One Sample Kolmogorov-Smirnov test. The association between the seroprevalence of *C. abortus* infection and flock size were calculated using the Chi-square test (χ^2-test). The relation between the flock size and the seroprevalence of infection rate was tested by simple linear regression.

Questionnairs

A questionnaire was carried out with goat owners (flock size, age, breeding and reproductive disorders such as stillbirths and abortions. The village names, sample numbers, size of flock was given in Table 1-2.

Table 2. Distrubition of *C. abortus* seroprevalances in goat herds

Number of herds (N=22)				Number of sample (N=384)			
Positive		Negative		Positive		Negative	
N	%	N	%	N	%	N	%
19	86.36	3	13.64	74	19.27	310	80.73

N: Number of animals

Results

Questionnaire results:

In this study, the serum samples were collected from Hair (n:12) and Honamli (n:10) goat flocks. The age of goats were no known, but blood samples were collected from 384 goat older than 2 years. The flock owners reported that 16 out of 22 flocks had abortions and died within a few days after birth of kids in 15 flocks.

ELISA results

The seroprevalence for *C. abortus* infection in goats were determined to be 19.27 % (74/384) (Table 3). The seropositivity was detected in 19 of 22 goat flocks. The seroprevalence of infection were changed from 5% to 60% in the positive goat flocks (Table 2 and 3). While only five of the positive flocks had one seropositive animal and fourteen flocks had two or more positive animals (Table 3). The seropositivity for *C. abortus* was determined in 4 of the 5 flocks that had no reproductive problems.

In this study, the serum samples were collected from Hair and Honamli goat flocks. Seropositivity for *C. abortus* in Hair and Honamli goats were detected in 15.5 % and 23.34%, respectively (Table 4). The relationship between goat breed and *C. abortus* infection was evaluated statistically.

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Table 3. Sizes, number of aborted fetus and stillbirth and ELISA results in goat herds for *C. abortus*.

Herds no	Herd sizes	Aborted goat		Stillbirth		ELISA positive		ELISA negative		Number of samples
	N	N	%	N	%	N	%	N	%	
1	430	35	8.14	11	2.6	3	15	17	85	20
2	160	10	6.25	20	6.3	1	5	19	95	20
3.	300	10	3.33	20	6.7	1	5	19	95	20
4.	350	20	5.71	10	2.9	5	20	15	80	20
5.	100	15	15	15	15	6	50	6	50	12
6.	40	5	12.5	6	15	2	10	18	90	20
7.	250	0	0	0	0	3	15	17	85	20
8.	40	0	0	0	0	2	20	8	80	10
9.	40	0	0	1	2.5	5	50	5	50	10
10.	45	3	6.7	2	4.4	6	54.55	5	55.45	11
11.	230	0	0	0	0	3	15	17	85	20
12.	205	4	1.9	1	0.5	12	60	8	40	20
13.	300	20	6.7	15	5	2	10	18	90	20
14.	230	6	2.6	10	4.3	11	55	9	45	20
15.	164	2	1.22	0	0	0	0	20	100	20
16.	300	15	5	10	3.3	4	20	16	80	20
17.	500	0	0	0	0	1	5.55	17	94.45	18
18.	350	10	2.9	2	0.6	0	0	20	100	20
19.	430	0	0	0	0	0	0	19	100	19
20.	212	50	23.6	35	16.5	1	7.14	13	92.86	14
21.	170	50	29.4	0	0	1	5.56	17	94.44	18
22.	250	50	20	10	4	5	41.67	7	58.33	12
Total	**5096**	**295**	**5.8**	**168**	**3.3**	**74**	**19.27**	**310**	**80.73**	**384**

N: Number of animals

Table 4. Distribution of *C. abortus* seroprevalence in goats

Goat breed	*C. abortus* positive	
	N	%
Hair goat (N:200)	31	15.5
Honamlı goat (N:184)	43	23.34
Total	**74**	**38.84**

N: The number of animals

Apparent and true prevalence results

In this study, the apparent the individual, within-flock and between- flocks prevalence values for *C. abortus* infection in the goats were calculated to be 19.27% (95% GA: 15.64% -23.51%), 22.77% (95% GA: 18.54-27.63%) and 86.36% 95% GA: 66.67% -95.25%), respectively (Table 5). By the spesificity and sensitivity of *C. abortus* ELISA kit, the true individual, within-flock and between-flock prevalence values for *C. abortus* infection were detected 19.44%(95%GA:15.24-23.63), 23.16%(95% GA:18.31-28.01), 90.81% (95% GA: %75.56-106.07), respectively (Table 5).

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Table 5. The prevalance apparent and true individual, within-herd and between-herd in goats.

			Apparent prevalence		True prevalence	
			Estimated (%)	**% 95 GA**	**Estimated (%)**	**% 95 GA**
Prevalence	Samples	Positive				
Individual	384	74	19.27	15.64-23.51	19.44	15.24-23.63
Within-herd	335	74	22.09	17.98-26.83	22.44	17.71-27.16
Between-herd	22	19	86.36	66.67-95.25	90.81	75.56-106.07

Statistical analysis

The highest prevalence of *C. abortus* infection was 29% in the flock had 120-239 aminals. The lowest prevalence of infection was 9% in the largest flock size group that had over 340 animals. The difference (P<0.05) between the values carrying different letters in the same column was found significant (Table 6).

The range of seropositivity for *C. abortus* was showed differences in goat flocks (Table 4). The difference in the prevalence of *C. abortus* infection between the goat breed was not statistically significant (P>0.05).

Table 6. Seroprevalence of *C. abortus* infection in goats according to flock size in Burdur province.

Size of flock	Number of flocks	Number tested	Seropositives (%)	Rate of seropositive (%)
40-119	5	63	21[a]	10-54.55
120-249	7	132	29[ac]	0-60
250-339	5	92	15[bc]	5-41.67
340-500	5	97	9[b]	0-20
Total	21	384	19.27	0-60

*P<0.05: The difference (P<0.05) between the values carrying different letters in the same column was found significant.

Discussion

Chlamydophila abortus is responsible for abortion, infertility, keratoconjunctivitis, pneumonia, enteritis, mastitis and arthritis in ruminants (Reinhold et al., 2011). The seroprevalence of *C. abortus* infection have been usually investigated in sheep in Turkey (Baz and Aydın, 2006; Duman 1996; Caya et al., 2006; Gokce et al., 2007; Otlu et al., 2007; Muz et al., 2014). Although the presence of *C. abortus* infection has been known for a long time in goats of Turkey (Kalender et al., 2013) there are no studies to determine the seroprevalence of *C. abortus* infection in goats.

In this study, the flock owners reported that 16 out of 22 flocks had abortions and died within a few days after birth of kids in 15 flocks. Out of 22 flocks, 19 were positive for *C. abortus* antibodies. The abortion was detected in 2 out of 3 negative flocks for *C. abortus* antibodies. In Turkey, the high prevalence of abortion cases in small ruminants is due to generally brucellosis (Arda et

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al., 1987; Küçükkayan et al., 2007; Otlu et al., 2007). But, the etiological agents as *C. burnetii*, *T. gondii*, *Leptospira* sp., *Listeria monocytogenes* may cause abortion in small ruminants, too (Arda et al., 1987; Otlu et al., 2007). In this study, the seropositivity were detected in 14 of the flocks with abortion problem and all of the flocks with kid death. ELISA was positive in 4 goat flocks that were considered to be healthy and no reproductive problem such as kid death and abortion. The goat owners were reported that animal movements were high in farms, the animals were taken from random flocks, and history of animals and flocks were unknown. The researchers (Aydın and Paracıkoğlu, 2006) reported that once aborted animals did not abort a second time. However, the animals infected during the late stages of pregnancy can not usually abort, but can develop abortion in next season (Aydın and Paracıkoğlu, 2006).

In this study, the seroprevalence of *C. abortus* infection ranged from 5% to 60% in goat flocks. The highest prevalence of *C. abortus* infection was 29% in the flock had 120-239 aminals. The lowest prevalence of infection was 9% in the largest flock size group that had over 340 animals. However, there was no statistically significant difference between goat breed and *C. abortus* infection ($p>0.05$). Al Qudah et al. (2004) reported that there was correlation between the seroprevalence of *C. abortus* infection and flock size in sheep flocks, but there was no significant correlation in goat flocks. In this study, the apparent and true prevalence of within-flock of *C. abortus* infection was determined 22.77% and 23.16% in goat flocks, respectively. The apparent prevalence of within-flock of *C. abortus* infection were reported between 11.37%-52.9% in goat flocks (Al-Qudah et al., 2004; Masala et al., 2005; Hernandez et al., 2014, Yin et al., 2014). Hernandez et al. (2014) reported that the prevalence of *C. abortus* infection was 4.87% in goats and ranged from 3.44% to 13.51% in flocks. This rate was mostly low from the present study results. This results can be originated from grown together goat and sheep in the same flocks in Burdur province. The researchers (Aydin and Paracikoglu, 2006; Quinn et al., 2009) reported that the sheep can be source of *C. abortus* infection in goat. In the present study, while the apparent prevalence of individual, within flocks and between- flocks for *C. abortus* infection was calculated as 19.27%, 22.09% and 70.37%, respectively, true prevalance of *C. abortus* individual, within- flocks and between- flocks was detected 19.44%, 22.44% and 90.81%, respectively. When these results were compared with the study done in sheep of Burdur province, the prevalence of individual, within-flock and between- flocks the seroprevalence of *C. abortus* in goats was found low. The sheep are more susceptible to *C. abortus* infection than the goats (Quinn et al., 2009). In Burdur, goats and sheep are generally grown together in the same flocks. It can be possible that infections are transmitted from sheep to goats. Furthermore, the high seroprevalence of the infection may be due to the lack of studies on protection and control of *C. abortus* infection.

In this study, the apparent and true prevalence of individual for *C. abortus* infection was calculated as 19.27% and 19.44% respectively. The prevalence of *C. abortus* in goats has been investigated in other countries by different serological tests (20, 48, 54, 82, 83, 94, 97, 101, 104). A Belgian study reported that the seroprevalence of *C. abortus* infection in goats was 18.75% (Yin et al., 2014). The seroprevalence of *C. abortus* infection was reported % 21.2 in Greece (Bisias et al., 2009), 25.6% in Iran (Esmaeili et al., 2015). These results were similar to our study results. But, our study results were found lower than that Taiwan (Wang et al., 2001) and Bosnia-Herzegovina (Krkalic et al., 2015). Our study was conducted in random herds, while both studies were conducted in flocks with abortion problems. While the seroprevalence of *C. abortus* infection in goats was found to be 0.22% in Poland (Czopowicz et al., 2010), 5.8% in Italy (Masala et al., 2005), 7.7% in Slovakia (Cislakova et al., 2007), 11.4% in Jordan (Al-Oudah et al., 2004), 9.3% in Brasil (Santos et al., 2012), 4.87% in Mexico (Hernandez et al., 2014), we was detected 19.27%. The cause of this differences can be differences in

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management of herds, climates, coexistence of sheep and goats, different detection methods, uncontrolled animal moviments and the lack of studies on protection and control of *C. abortus* infection.

In our study, the apparent and true prevalence of between flocks of *C. abortus* infection were 86.36%-90.81%, respectively. Yin et al. (2014) reported that the apparent prevalence of between flocks of the infection was 11.11%, while the apparent prevalence of between flocks of *C. abortus* infection in goats was 48.4% by Masala et al. (2015) and 100% by Al-Quadah et al. (2004). Samkange et al. (2010) reported that the prevalance of between flocks was changed between 17.2%-54%, in goat flocks. However, Czopowicz et al. (2010) was detected 4.2%. In the present study, the prevalence of between flocks of *C. abortus* infection was higher than other studies, while the prevalence of between flocks of *C. abortus* infection was lower than Al-Quadah (2004)'s study. The differences for *C. abortus* infection rate can be probably due to the different diagnostic methods, management, uncontrolled animal movements and it may vary depending on the region where the study was conducted (Rekiki et al., 2002; Masala et al., 2005; Güler et al., 2006; Kalender et al., 2013; Osman, 2013).

In conclusion, In Burdur, the apparent and true prevalence of individual, within-flock and between- flocks seroprevalence of *C. abortus* infection was higher than the other countries. In this context; a control program for *C. abortus* infection should be planned goat breeding in Turkey

Acknowledgement

This study was conducted with the approval of Mehmet Akif Ersoy University Experimental Animals Local Ethics Committee (MAKÜ-HADYEK / 2016-199). Also, this study was derived from the first author's Master Thesis, supported by Burdur Mehmet Akif Ersoy University Scientific Research Projects Coordination Unit (Project Number: 0385-YL-16).

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MAKU J. Health Sci. Inst. 2020, 8(1): 11-17.
doi: 10.24998/maeusabed.695570

Mehmet Akif Ersoy University
Journal of Health Sciences Institute
http://dergipark.gov.tr/maeusabed

Araştırma Makalesi/Research Article

Türkiye'deki Bal Örneklerinde Neonikotinoid Varlığının LC-MS/Q-TOF Yöntemi ile Tespiti

Detection of Neonicotinoids with LC-MS/Q-TOF Method in Honey Samples from Turkey

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Öz: Organofosfatlı ve karbamatlı insektisitlere alternatif olarak geliştirilen neonikotinoidler, günümüzde dünya çapında en yaygın kullanılan insektisit sınıfı olup polen ve nektar dâhil bitkilerin tüm kısımlarına geçerek bu bitkiler tarafından üretilen ürünlere ve hatta arı ürünlerine aktarılabilmektedir. Bu sistemik özellikleriyle neonikotinoidler, bal arıları ve yabani arılar gibi canlıların yanı sıra, insanlar dahil diğer omurgalılar üzerinde de olumsuz etkilere yol açmaktadır. Bu nedenle hangi türevlerinin ne oranda etki oluşturduğuna ilişkin araştırmalar son yıllarda hızla artmakta ve bu çalışma sonuçlarına göre farklı ülkelerde neonikotinoid kullanımlarına yasaklar ve kısıtlamalar getirilmektedir. Bu çalışmanın amacı Türkiye'de üretilen ballarda neonikotinoid varlığının ve dolayısıyla çevredeki kirlilik seviyesinin ölçülmesidir. Bu amaçla, hasat sonrası Türkiye'nin farklı illerindeki arı yetiştiricilerinden direkt temin edilen 44 bal örneği materyal olarak kullanılmıştır. Örneklerde asetamiprid, klotianidin, dinotefuran, imidakloprid, nitenpiram, tiyaloprid ve tiametoksam varlığı Sıvı Kromatografi Kuadrupol Uçuş Zamanlı Kütle Spektrometresi (LC-MS Q-TOF) kullanılarak araştırılmıştır. Analiz edilen örneklerin hiçbirinde neonikotinoid grubu insektisitlere rastlanmamıştır. Analiz edilen örneklerde neonikotinoidlere rastlanmaması Türkiye açısından umut verici bir bulgudur. Neonikotinoidlerin kullanım şekilleri ve canlı organizmalara etkileri üzerine artan araştırmalar yanında, çevresel varlığının da düzenli olarak takip edilmesi ve limitlerin belirlenmesinin, halk sağlığının korunması açısından önemli olduğu düşünülmektedir.

Anahtar Kelimeler: Bal, LC-MS Q-TOF, Neonikotinoid.

Abstract: Neonicotinoids are currently the most widely used class of insecticides worldwide. They are developed as an alternative to organophosphate and carbamate insecticides, and they pass to all parts of plants including pollen and nectar and can be transferred to the products produced by these plants, even to bee products. Recent research has shown that they have negative effects on bees, as well as other vertebrates, including humans. Analytical protocols have been developed to analyze neonicotinoid levels in honey and according to the results of these studies, prohibitions and restrictions are imposed on the use of neonicotinoids in different countries. The aim of this study was to determine the presence of neonicotinoids, in honey produced in Turkey. For this purpose, 44 honey samples obtained after harvesting directly from beekeepers from different provinces were used as material. The presence of acetamiprid, klothianidine, dinotefuran, imidakloprid, nitenpyram, thiacloripid and tiametoksam in the samples were determined by using Liquid Chromatography Quadrupole Flight Time Mass Spectrometer (LC-MS/Q-TOF). Neonicotinoid insecticides were not found in any of the samples analyzed. Detecting no neonicotinoids is a promising finding for Turkey. In conclusion, in addition to increasing research on the use of neonicotinoids and their effects on living organisms, it is also important to monitor the environmental presence regularly and to determine the limits for the protection of public health.

Keywords: Honey, LC-MS Q-TOF, Neonicotinoid.

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Geliş tarihi / Received : 27.02.2020 Kabul tarihi / Accepted: 17.03.2020

To cite this article: Çil Gİ, Korkmaz SD, Ozansoy G, Küplülü Ö. (2020). Türkiye'deki Bal Örneklerinde Neonikotinoid Varlığının LC-MS/Q-TOF Yöntemi ile Tespiti.. MAKU J. Health Sci. Inst., 8(1), 11-17.

ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

Giriş

Neonikotinoidler, tarım ve ev zararlılarına karşı büyük başarı ile kullanılan sentetik, nikotin bazlı bileşiklerden oluşan bir kimyasal sınıftır. İmidakloprid, asetamiprid, klotianidin, tiakloprid, tiamethoksam, dinotefuran ve nitenpiram bu sınıfın üyeleri arasındadır. İlk kez 1980'lerde organofosfatlı ve karbamatlı insektisitlere alternatif olarak geliştirilmiş ve 1990'larda ilk ticari preparat olan imidakloprid ile kullanımları başlamıştır. Neonikotinoidler şu anda dünya çapında en yaygın kullanılan insektisit sınıfıdır (Bonmatin, 2015). Neonikotinoidler, nikotinik asetilkolin reseptörü (nAChR'ler) üzerine nörotoksisite ile insektisit etki gösterirler. İnsektlerin merkezi sinir sisteminin sinyal iletiminde blokaja neden olan çok spesifik bir etki mekanizmasına sahiptirler (Decourtye, 2010).

Neonikotinoid insektisitlerin yağlı tohumlar, tahıllar, meyveler, sebzeler ve süs bitkileri gibi çok çeşitli tarımsal üründe kullanımı vardır. Ayrıca çimlerde, ağaçlarda, yerleşim bölgelerindeki dış mekanlarda da insektisit olarak kullanılırlar. En önemli özellikleri sistemik etki göstermeleridir. Neonikotinoidler suda çözünür ve bitkiler tarafından kökleri veya yaprakları yoluyla kolayca emilirler ve daha sonra bitkinin dokuları boyunca taşınırlar, çiçekler dahil tüm organlara ulaşırlar, böylece polen ve nektar gibi bitki tarafından üretilen kısımlarda da tespit edilmektedirler. Ancak bu sistemik etkilerinin hedef canlılar dışındaki canlılara olumsuz etkidiği görülmüştür (Main, 2014). Neonikotinoidlerin özellikle bal arıları ve yabani arılar gibi tozlaştırıcılar başta olmak üzere insanlar dahil omurgalılar üzerindeki olumsuz etkilerine dair artan endişeler vardır. Bununla birlikte, ana bileşiklerin ve bunların metabolitlerinin çevreye ve besin zincirine aktarılabilmesi de, bu insektisitlerin kullanımı ile ilgili şüphelere neden olmaktadır (Sanches-Hernandez, 2014; Zhang, 2018).

Çevreye ve özellikle bal arılarına verdiği zarar nedeniyle Kanada'da 2015 yılında bütün neonikotinoidlerin, 2018 yılında Avrupa Birliği'nde klotianidin, imidakloprid ve tiamethoksamın kullanımı yasaklanmıştır. Amerika'nın bazı eyaletlerinde sınırlandırmalar getirilmesine rağmen halen neonikotinoid kullanımı devam etmektedir. Türkiye'de ise asetamipridin kullanımına izin verilirken klotianidin yasaklanmış, imidakloprid, tiakloprid ve tiametoksam kullanımlarına ise çeşitli sınırlandırmalar getirilmiştir (PMRA, 2015; EC, 2020).

Neonikotinoid kalıntılarının analizine odaklanan yöntemlerde, meyve ve sebzelerin yanı sıra hayvansal gıdalarda da kalıntı düzeyleri araştırılmaktadır. Neonikotinoidlerin doğada yaygın olarak kullanılması ve bal arılarına etkileri göz önünde bulundurulduğunda, baldaki kalıntılarının varlığı ve miktarının uygun analitik yöntemlerle tespiti gerekliliği dikkati çekmektedir. Ancak çok az sayıda yayın baldaki neonikotinoid kalıntılarının analizini özel olarak hedeflemektedir (Gbylik-Sikorska, 2015). Bu çalışmanın amacı Türkiye'de üretilen ballarda neonikotinoid varlığının ve dolayısıyla çevredeki kirlilik seviyesinin ölçülmesidir.

Gereç ve Yöntem

Gereç

Türkiye'nin farklı illerindeki arı yetiştiricilerinden hasat sonrası direkt temin edilen 44 bal örneği materyal olarak kullanılmıştır. Toplanan süzme bal örnekleri, cam kavanozlara alınarak analiz aşamasına kadar laboratuvarda 20±2 °C'de muhafaza edilmiştir.

Yöntem

Örneklerde asetamiprid, klotianidin, dinotefuran, imidakloprid, nitenpiram, tiakloripid ve

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ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

tiametoksam varlığı Sıvı Kromatografi Kuadrupol Uçuş Zamanlı Kütle Spektrometresi (LC-MS Q-TOF, liquid chromatography time-of-flight mass spectrometry) kullanılarak tespit edilmiştir.

Kimyasallar ve Reaktifler

Asetonitril ve methanol HPLC analizlerine uygun saflıkta Merck'ten (Darmstad-Almanya) temin edilmiştir. Mobil faz tampon için amonyum format (Fluka, USA) ve formik asit (J.T.Baker, Holland) kullanılmıştır. Numune hazırlama işlemi için kullanılan Quechers tuz karışımı olan Quechers EN 15662 Extraction kit (Agilent, USA) içeriğinde; $MgSO_4$, NaCl ve Sitrat tuzları vardır. Analitik saflıktaki pestisit standartları Dr. Ehrenstorfer (Augsburg-Almanya) temin edilmiştir. Ana stok çözeltiler 10.0 ±0.1 mg tartılarak son derişimi 1000 µg/ml olacak şekilde asetonitril içerisinde hazırlanmış, kullanım süresine kadar -18 °C'de muhafaza edilmiştir.

LC-ESI-Q-TOF

Analizler Agilent 1260 serisi HPLC ve Agilent 6550 model iFunnel Q-TOF sistemi ile gerçekleştirilmiştir. HPLC bölümü Degazer, Binary (İkili) pompa, Yüksek performanslı numune örnekleyici ve Kolon fırını modüllerinden oluşmaktadır. Kromatografik ayrım için Agilent Poroshell SB-C18 4.6mm x 150mm x 2.7µ kullanılmıştır. Mobil faz A: 5mM amonyum format + %0,1 formik asit karışımı ve Mobil faz B: methanol (%100 saf) kullanılmıştır. Mobil faz akış değeri 0.6 ml/dk olup Kolon fırını sıcaklığı 40 °C'dir. Tablo 1 ve 2'de kullanılan HPLC'ye ait gradient detayı ve MS sistemine ait analiz parametreleri verilmiştir.

Tablo 1: Kullanılan HPLC gradient program detayları

Süre (dk)	% B (Metanol)
0	10
0.5	10
4	70
6	95
10	95
10.1	10

Ekstraksiyon aşaması

Analiz için hazırlanan örneklerin ekstraksiyon aşaması, QuEChERS EN 15662 yöntemine göre (Lehotay, 2005; Anastassiades, 2013; CEN-EN, 2018) gerçekleştirilmiştir. Buna göre, her bir örnek için 5 gr bal 50 ml'lik plastik santrifüj tüplerine tartılarak 10 ml su ilave edilip homojenize olması için vortex ile karıştırılmıştır. Elde edilen çözeltilere 10 ml asetonitril ve ilk ekstraksiyon tuz karışımı olan MgSO4, NaCl ve sitrat tuzları ilave edilmiştir. Daha sonra 30 saniye kadar vortex ile karıştırılarak 4000 rpm/dk 5 dakika süre santrifüj edilmiştir. Analiz için, üst faz viale alınarak LC-MS sistemine enjekte edilmiştir.

Tablo 2: MS sistemi iyon kaynağı ve analiz parametreleri

İyon Kaynağı parametreleri		**Analiz parametreleri**	
Kurutucu gaz sıcaklığı	225 °C	Kütle aralığı	100-1200
Kurutucu gaz akışı	14 lt/dk	İyonlaşma modu	pozitif
Nebulizer	40 psi	Tarama hızı, MS	1 spektra/saniye
Sheath gaz sıcaklığı	400 °C	Tarama hızı, MS/MS	3 spektra/saniye
Sheath gaz akışı	11 lt/dk	Çarpışma enejisi	10-20-40 V
Kapiler voltajı	3000 V	Referans iyonlar	121.0509 922.0098
Nozzle voltajı	500 V		
Fragmentor voltajı	400 V		

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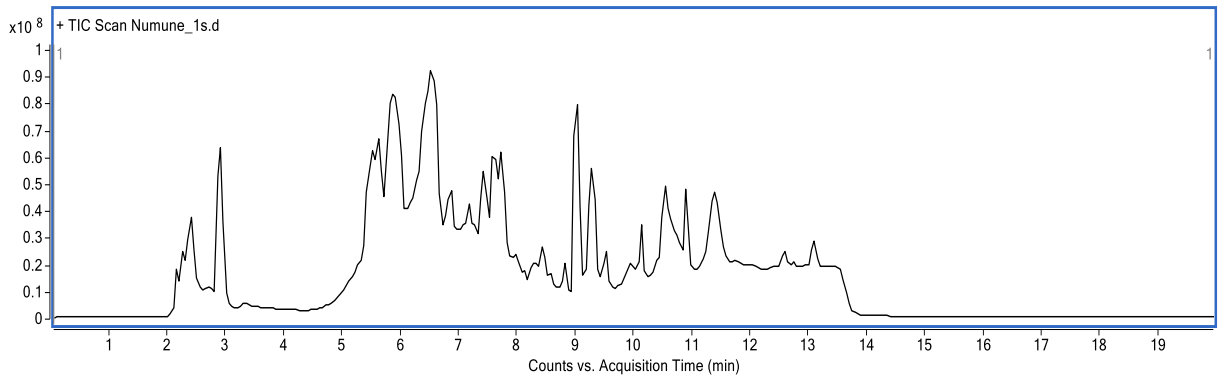
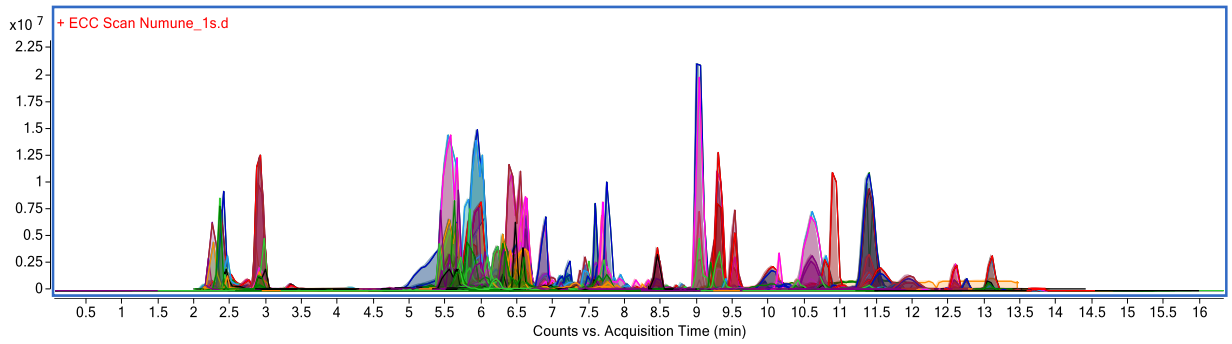
ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

Sonuçlar ve değerlendirme

MS ve MS/MS modunda yapılan cihaz okuma verileri değerlendirilerek olası toksik maddeler ve pestisitler tanımlanmıştır. Elde edilen ana iyonlar, pestisit ve toksikoloji veri tabanlarında taranıp, yüksek skorlu olası sonuçlar MS/MS verileri de değerlendirilerek sonuçlandırılmıştır. Matriks içerisinde hazırlanmış 7 seviye kalibrasyon çözeltileri ile miktarlandırmaları yapılmıştır. Son olarak standart enjeksiyonu ile parçalanma ürünleri ve alıkonma süresi eşleştirmeleri de sağlanmıştır. Şekil 1'de bir çiçek balı örneğine ait MS toplam iyon kromatogramı (pozitif mod) ve Şekil 2'de aynı örneğin toplam iyonları gösterilmiştir.



Şekil 1: Çiçek balı numunesine ait MS toplam iyon kromatogramı, pozitif mod.



Şekil 2: Çiçek balı numunesinin enjeksiyonu sonucu elde edilen toplam iyonları gösteren kromatogram

Bulgular ve Tartışma

Türkiye'nin farklı illerinde yetiştiricilik yapan arıcılardan direkt temin edilen, 44 adet süzme bal örneğinde asetamiprid, klotianidin, dinotefuran, imidakloprid, nitenpiram, tiakloripid ve tiametoksam varlığı LC-MS Q-TOF kullanılarak araştırılmış; ancak örneklerin hiçbirinde neonikotinoid grubu insektisitlere rastlanmamıştır.

Analiz edilen örneklerde neonikotinoid grubu insektisitlere rastlanmaması Türkiye açısından umut verici bir bulgudur. Kanada'da, Saskatoon Şehri'ne 30 km mesafedeki kovanlardan toplanan ballarda yapılan çalışmada, LC-MS/MS ile analiz edilen 26 örneğin %86'sında clothian bulunduğu bildirilmiştir. Tiametoksam da yaygın olarak tespit edilirken, hiçbir örnekte nitenpiram ve asetamiprid saptanmadığı ve sadece bir örnekte thiacloprid, olduğu ortaya konmuştur (Codling, 2016). Lu ve ark. (2016) yaptığı çalışmada Massachusetts'de 62 farklı kovandan aldığı bal numunelerinde 8 farklı neonikotinoid varlığını araştırmıştır. Numunelerin, % 57'sinin en az bir, % 15'inin de iki farklı neonikotinoid içerdiğini bildirmişlerdir.

Başka bir çalışmada, Sırbistan-Voyvodina'nın 7 bölgesinden, farklı orijinlere ait 104 bal örneği

To cite this article: Çil Gİ, Korkmaz SD, Ozansoy G, Küplülü Ö. (2020). Türkiye'deki Bal Örneklerinde Neonikotinoid Varlığının LC-MS/Q-TOF Yöntemi ile Tespiti.. MAKU J. Health Sci. Inst., 8(1), xx-xx.

ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

(ayçiçeği, çiçek, ıhlamur ve akasya) toplanmış ve HPLC-DAD metodu ile neonikotinoid kalıntıları araştırılmıştır. Beş çiçek balı örneğinde tiakloprid, 4 ayçiçeği balı örneğinde imidakloprid tespit edildiği bildirilmiştir. Ancak bu örneklerdeki seviyelerin hepsinin maksimum kalıntı limitlerinin (MRL) altındaki konsantrasyonlarda olduğu da vurgulanmıştır (Jovanov, 2015). Benzer şekilde Avusturya'nın farklı bölgelerinden toplanan 41 bal numunesindeki neonikotinoid kalıntılarının LC-MS/MS ile araştırıldığı çalışmada üç neonikotinoidin varlığı dikkat çekmiştir; tiakloprid (18 numune), asetamiprid (2 numune) ve tiyametoksam (1 numune). Ancak numunelerdeki kalıntı düzeyleri MRL'lerle karşılaştırıldığında, tümünün ilgili sınırların altında olduğu bildirilmiştir (Tanner, 2011).

Mitchell ve ark. (2017) yaptıkları çalışmada dünya çapında bal örnekleri toplayarak neonikotinoid varlığını araştırmıştır. Afrika'dan 37, Asya'dan 41, Avrupa'dan 53, Kuzey Amerika'dan 22, Güney-Orta Amerika ve Karayipler'den 28, Okyanusya ve Pasifik Adaları'ndan 17 olmak üzere toplam 198 bal örneğinde 5 ana neonikotinoid (asetamiprid, klotianidin, imidakloprid, tiakloprid ve tiametoksam) kalıntısını UHPLC-MS/MS metodu ile tespit etmişlerdir. Tüm numunelerin % 75'inde test edilen beş bileşiğin en az birinin, % 45'inde iki veya daha fazlasının ve % 10'unun da dört veya beşinin bulunduğunu ortaya koymuşlardır. Ancak, tespit edilen konsantrasyonların hepsinin insan tüketimi için izin verilen maksimum kalıntı seviyesinin altında olduğunu da bildirmişlerdir. Çalışmada önemli noktalardan biri de sonuçların spesifik neonikotinoid türlerinin kullanımındaki bölgesel farklılıkları yansıtmasıdır. Örneğin, imidakloprid, Afrika ve Güney Amerika'da yaygın şekilde tespit edilirken, Avrupa'da tiakloprid, Asya'da asetamiprid ve Okyanusya ile Kuzey Amerika'da tiametoksam'ın bulunduğu ortaya konmuştur.

Sánchez-Hernández ve ark. (2016) tiametoksam, klotianidin ve imidakloprid neonikotinoidlerini ve bunların metabolitlerini bal örneklerinde LC/Q-TOF-MS ile araştırırken, balların toplandığı lokasyonları özellikle ayçiçeği ve mısır tarlaları yakınlarından seçmiş ve sonuçları da buna göre değerlendirmiştir. Bal örneklerinde 3 ana neonikotinoide rastlanmadığını bildiren araştırıcılar, ayçiçeği bitkilerinin yanındaki arı kovanlarından toplanan tüm bal örneklerinde tiametoksam metabolitleri TM5 ve TM13 bulunduğunu bildirmiştir. Çalışma ayrıca mısır bitkilerinin yakınından toplanan bal örneklerinde ise imidakloprid metabolitleri IMI4 ve IMI-15 varlığını ortaya koymuştur. Bu durum, tarım arazilerine yakın bölgeler ve dağlık alanlarda üretilen ballarda neonikotinoid varlığının farklı olabileceğini de göstermektedir. Buna göre yapılacak benzer çalışmalarda, numune alırken kovanların bulunduğu bölgelerin de göz önünde bulundurulmasının faydalı olacağı düşünülmektedir.

Son zamanlarda, birçok araştırmacı bu insektisitlerin arı, bal, toprak, polen ve işlenmiş tohumlar gibi birçok materyalde tespitini yapmaktadır. Farklı matrislerde pestisit kalıntılarının ölçülmesi, örnek hazırlama (ekstraksiyon ve temizleme) ve enstrümantal analiz olmak üzere iki temel adımı içermektedir. Doğru sonuçlar elde edebilmek için ideal olarak, ekstraksiyon aşamasının hızlı, basit, ucuz ve çevre dostu olması ve temiz örnekler sağlaması önemlidir. 2000'li yıllarda geliştirilen ve ilk olarak 2003 yılında bildirilen QuEChERS tekniği hızlı ve eksiksiz bir ekstraksiyon ve temizleme prosedürüdür (Proietto, 2013). Bu çalışmada bu yöntem tercih edilerek, bal gibi, yoğun şeker konsantrasyonuna sahip matrislerden, güvenilir sonuçlar elde etme şansı arttırılmıştır. Neonikotinoidlerin tespit ve miktar analizlerinde, başlıca gaz kromatografisi veya sıvı kromatografisi gibi kromatografik metodlar yanında, UV/diyot dizisi, floresans, elektrokimyasal veya kütle spektrometrisi gibi farklı birçok teknik kullanılmaktadır (Cicero, 2017; Kocasari, 2018).

Yöntemlerdeki ve özellikle de nicelik sınırlamalarındaki (LOQ) farklılıklar çalışmalar arasında karşılaştırmalar yapmayı zorlaştırmaktadır. Ayrıca neonikotinoid grubu

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ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

insektisitlerin kullanım şekillerini ve canlı organizmalar üzerindeki etkilerini anlamak için artan araştırma çabalarına rağmen, canlılarda görülen riskleri değerlendirmek için dünyada bunlardan kaynaklı kirliliğinin dağılımına dair küresel veriler yeterli düzeyde değildir. Bu nedenle, tarımsal üretimi ve arılar da dahil olmak üzere tüm canlıların ömrünü etkileyebilecek farklı inorganik ve organik kirletici maddeleri göz önünde bulundurarak neonikotinoid grubu insektisitleri değerlendirmek için düzenli izleme yapılması önemlidir.

Bilgi Notu:

Çalışmanın verilerini de içeren sözlü bildiri, 24-27 Ekim Antalya'da düzenlenen 8. Ulusal 2. Uluslararası Veteriner Gıda Hijyeni Kongresinde sunulmuştur.

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MAKU J. Health Sci. Inst. 2020, 8(1): 18-29.
doi: 10.24998/maeusabed.701208

Mehmet Akif Ersoy University
Journal of Health Sciences Institute
http://dergipark.gov.tr/maeusabed

Review / Derleme

Toxicity of *Ortho*-Phenylphenol (OPP) and Sodium *Ortho*-Phenylphenate (SOPP)

Orto-Fenilfenol (OPP) ve Sodyum Orto-Fenilfenatın (SOPP) Toksisitesi

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Abstract: *Ortho*-phenylphenol (OPP) and sodium *ortho*-phenylphenate (SOPP) salt have been used world-wide for decades as fungicides and disinfectants. OPP is generally used as a hospital and household disinfectant, whereas SOPP is used as a fungicide in post-harvest treatment of citrus fruits and vegetables for the prevention of mold. Due to widespread use including many consumer applications, the fate of OPP in the mammalian organism has been the subject of numerous investigations over many years. The aim of this review is to give information about OPP and SOPP including metabolism, general toxicity, carcinogenicity and genotoxicity.

Keywords: *Ortho*-Phenylphenol, Sodium *ortho*-Phenylphenate, Toxicity.

Öz: *Orto*-fenilfenol (OPP) ve sodyum *orto*-fenilfenat (SOPP) yıllardır dünya çapında fungisit ve dezenfektan olarak kullanılmaktadır. OPP genellikle hastane ve ev dezenfektanı olarak kullanılırken, SOPP küf oluşumunun önlenmesi için narenciye ve sebzelerin hasat sonrası korunmasını sağlayan bir fungisit olarak kullanılır. Birçok tüketici uygulaması da dahil olmak üzere yaygın kullanım nedeniyle, OPP'nin memeli organizmasındaki kaderi uzun yıllar boyunca çok sayıda araştırmanın konusu olmuştur. Bu derlemede, OPP ve SOPP'nin metabolizma, genel toksisite, karsinojenite ve genotoksisitesi hakkında bilgi verilmesi amaçlanmıştır.

Anahtar Kelimeler: *Orto*-Fenilfenol, Sodyum *Orto*-Fenilfenat, Toksisite.

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Geliş tarihi / Received : 09.03.2020 Kabul tarihi / Accepted: 17.04.2020

Introduction

Ortho-phenylphenol (OPP) and its sodium salt, sodium *ortho*-phenylphenate (SOPP) are phenolic substances which have wide range of uses (Figure 1). These compounds are used as antibacterial and disinfectant agents and fungicides in a variety of different agricultural, industrial and domestic use (Lambert and Eastmond, 1994; WHO, 2003; Balakrishan and Eastmond, 2006). The main use of OPP and SOPP is the preservation of stored fruits especially citrus. They are also used for disinfection of materials used in storage and applied as a fungistatic wax for the destruction of pathogens on the surface of fruits and vegetables. They can protect the packaged fruits against green mold, blue mold, and sour rot diseases caused by various plant pathogens such as *Penicillium italicum, Diplodia natalenis, Penicillium digitatum* and *Botrytis cinerea* (Lyr, 1995; Appel, 2000). OPP and SOPP are used as disinfectant in hospitals, veterinary clinics, poultry farms, cattle enterprises, home and various workplaces (Grossman, 1995; WHO, 2003; Balakrishan and Eastmond, 2006). These substances are very effective disinfection agents especially in stubborn nosocomial infections caused by *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Jang et al., 2008; Nde et al., 2008). They are

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ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

also used as biocides to provide control of microbial degradation in fibrous or polymeric materials such as leather, rubber, paper and textile products (Appel, 2000).

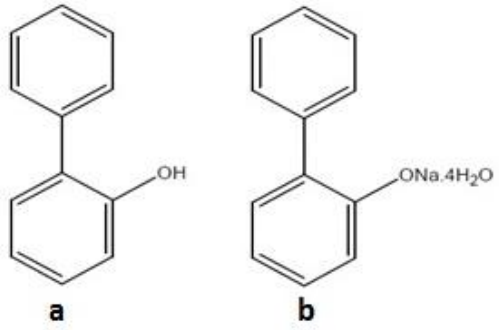

Figure 1. Chemical structures of OPP (a) and SOPP (b) (DPR, 2007).

Due to their widespread usage, living organisms are exposed to OPP and SOPP from many different sources (Kwok and Silva, 2013). Despite these potentially toxic effects, OPP and SOPP are used in applications that come into contact with both humans and animals. The aim of this review is to give information about metabolism, acute and chronic toxicity, carcinogenicity and genotoxicity of OPP and SOPP.

1. Toxicokinetics

Bioavailability of OPP and SOPP in rats and mice after oral administration is very high and their elimination via renal system is dose-independently fast (DPR, 2007). OPP and SOPP are metabolized by cytochrome P450 monooxygenase enzyme system in liver. Both compounds are metabolized to phenyl-hydroquinone (PHQ) and phenylbenzoquinone (PBQ) by oxidation reactions and then conjugate and form OPP-S, OPP-G and PHQ-G conjugates by sulfation or glucuronidation. Metabolites of OPP and SOPP undergo reaction with glucuronic acid (GA) or sulfate (S) and excreted in urine (Bomhard et al., 2002; Brusick, 2005). As a result of the conjugation reactions of OPP, biologically inactive metabolites are formed, and as a result of oxidation reactions, active metabolites (PHQ and PBQ) are formed (Bomhard et al., 2002; Kwok and Silva, 2013). PHQ is a major metabolite formed from OPP and SOPP and it is converted to the corresponding PBQ via reactive phenyl-semiquinone (PSQ) (Nakagawa and Tayama, 1996). PBQ is converted either enzymatically or non-enzymatically. This convertion is occured by the cytochrome P450 monooxygenase enzyme system and prostaglandin H-synthase-mediated oxidation or by pH-dependent autoxidation of PHQ (Kwok and Eastmont, 1997; Balakrishnan and Eastmond, 2006).

In animal studies performed on mainly rats and mice, it was determined that OPP and its main metabolite, PHQ were excreted in low doses as sulfate conjugates (OPP-S and PHQ-S), and in high doses, OPP and PHQ were excreted as GA conjugates (OPP-GA, PHQ-GA) (Ernst, 1965; Bajaj et al., 1976; Ushiyama et al., 1982; Nakao et al., 1983; Ushiyama et al., 1983; Christenson et al., 1996). The toxicities of the main molecule and metabolites *in vitro* and *in vivo* are as PBQ **>** PHQ > OPP/SOPP relatively (Brusick, 2005). It has been reported that the PBQ metabolite is responsible for the damage to the urinary bladder epithelium and hyperplasia (St John et al., 2001; Nde et al., 2008). It has been stated that PHQ is formed at high pH and when it turns into PBQ by oxidation, it causes an increase in the incidence of bladder lesions. Administration of OPP with sodium bicarbonate makes urine alkaline and this resulted in increased carcinogenicity (Fujii et al., 1987; Fukushima et al., 1989; St John et al., 2001). In addition, it has been emphasized that the damage on the bladder is higher due to the higher and faster biotransformation of OPP in male rats (Nakao et al., 1983; Ushiyama et al., 1983).

2. Mechanism of Action

Free radicals are constantly formed as a result of enzymatic and non-enzymatic (between oxygen and organic molecules) reactions in cells. Enzymatic reactions can take place during cellular respiration, with phagocytosis, prostaglandin synthesis and the microsomal enzyme system. Free

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ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

radicals are important compounds in regulating processes involving functions such as maintaining cell homeostasis, signal transduction, gene expression and activation of receptors. However, these free radicals and other ROS can occur in large quantities due to normal basic metabolic processes in the living body or exposure to various xenobiotics (Hussain et al., 2016).

The toxicity caused by oxidative damage has been attributed to the higly reactive hydroxl radical, which can be formed by meta ion-catalysed reaction between super oxide anions and hydrogen peroxide (Brusick, 2005). Many toxic xenobiotics such as OPP, which enter the organism, are activated by cytochrome p450 monooxygenase system to produce toxic intermediates and these products bind irreversibly to cellular macromolecules and cause tissue injuries (Nakagawa and Tayama, 1988).

OPP is converted to PHQ and PBQ by microsomal monoxygenase enzyme system. Oxidative stress and cytotoxicity occur due to excessive formation of reactive oxygen species (ROS) as a result of the bilateral reaction between PBQ and the semiquinone radical. This reaction is catalyzed by cytochrome reductase with an electron reduction. By-products of the reaction that forms semiquinone include super oxide anions, hydroxyl radicals and hydrogen peroxide (Brusick, 2005). In various cellular components, PBQ and PHQ are extremely reactive intermediates which can react with cellular nucleophilic centers in biological components (Nakagawa and Tayama, 1996). These reactive metabolites have the potential to inhibit sulfide-dependent enzymes. They first consume intercellular glutathione (GSH) stores, and then interact with SH-containing structures in cells and tissues. They also change the effectiveness of catalase, superoxide dismutase and other intracellular antioxidant enzymes. Quinones can also binds to proteins, nucleic acids or other macromolecules and cause damage (Nakagawa and Tayama, 1988; Brusick, 2005; Li et al., 2012).

OPP is thought to cause disorders in endocrine systems and this is the basis of genotoxic and cytotoxic effects. Endocrine disrupting activity of OPP was investigated with *in vitro* studies on estrogen receptor binding, estrogen-induced cell proliferation, and estrogen receptor transcription activity. There are also studies that OPP and its metabolites have an inhibitory effect on prostaglandin metabolism. Prostaglandin inhibitors have been reported to cause anomalies (increased resorption and cleft lip) in laboratory animals (Bomhard et al., 2002; Kwok and Silva, 2013).

3. Toxicity

There seems to be no significant difference between animal species in terms of sensitivity to OPP and SOPP (Bomhard et al., 2002). OPP and SOPP belong to the third category of oral toxicants, but SOPP has approximately 3 times more acute toxicity than OPP (DPR, 2007).

In terms of acute and chronic toxicity, mutagenicity, teratogenicity and cytotoxicity, the effects of OPP and SOPP have been extensively investigated *in vitro* and *in vivo* studies (Higara and Fujii, 1981; Ushiyama et al., 1982; Honma et al., 1983; Ushiyama et al., 1983; Higara and Fujii, 1984; Fujii and Higara, 1985; Nakagawa and Tayama, 1988).

3.1. Acute Toxicity

3.1.1. Systemic Toxicity

LD50/LC50 values of OPP and SOPP in animal species are given in Table 1-2. When OPP and SOPP are taken orally, the LD50 is 924-2700 mg/kg. In rats, decrease in respiratory rate, decrease in body temperature, decrease in motor reflexes, coordination disorder, wheezing, cough, increase in urine, depression, exophthalmos, increase in tear secretion, abdominal distension and in mice, clinical signs such as decreased movement, gait disturbance, decreased respiratory rate and depigmentation of hairs were reported (Bomhard et al., 2002; DPR, 2007).

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ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

Nakagawa ve Tayama (1988) gave single dose 700 or 1400 mg/kg b.w OPP orally to F344 rats and they investigated the toxic effects on liver and kidney. Serum transaminase activity and acute hepatocellular necrosis were observed in the group that receiving 1400 mg / kg dose of OPP. In groups given 700 and 1400 mg/kg b.w OPP, glutathione levels decreased rapidly depending on the dose after 6 hours. The researchers then treated the rats with PHQ and PBQ (the metabolites of OPP) at 700 and 1400 mg/kg b.w. doses. They observed that 75% of the animals in the group given PBQ at 1400 mg/kg b.w dose died at the end of 24 hours. It has been stated that serum transaminase activities increase significantly at the doses 1400 mg/kg b.w PHQ and 700 mg/kg b.w PBQ. Hepatocellular necrosis were seen in the group which 700 mg/kg b.w of PBQ applied and it is also have been reported that serum urea nitrogen levels increase in this group of rats. They stated that tubular enlargement in kidneys and renal papillary necrosis were milder in groups receiving 700 mg/kg b.w PBQ. At the higher doses of OPP and its metabolites (PBQ and PHQ), it has been reported that target organs are the liver and kidney. They found that PBQ has a much more toxic effect on liver and kidney than PHQ.

Table 1. Acute toxicity of OPP in rat and mice (LD50/LC50 values) (Bomhard et al., 2002).

Species	Route	Sex	LD50/LC50	References
Rat	Oral	Male	2850 mg/kg b.w	Hasegawa et al.(1989)
		Female	3600 mg/kg b.w	Hasegawa et al. (1989)
		Male/Female	2733 mg/kg b.w	Gilbert and Crissman (1994)
	Dermal	Male/Female	>2000 mg/kg b.w	Bomhard (1991)
	Inhalation (4h)	Male/Female	>36 mg/m^3 (as vapour)	Landry et al. (1992)
Mice	Oral	Male	3499 mg/kg b.w	Tayama et al. (1983)
		Female	3152 mg/kg b.w	Tayama et al. (1983)

Table 2. Acute toxicity of SOPP in rat and mice (LD50/LC50 values) (Bomhard et al., 2002).

Species	Route	Sex	LD50/LC50	References
Rat	Oral	Male	1650 mg/kg b.w	Taniguchi et al. (1981)
		Female	1550 mg/kg b.w	Taniguchi et al. (1981)
		Male/Female	1096 mg/kg b.w	Tayama et al. (1979)
	Inhalation (1h)	Male	>1331 mg/m^3 (aerosol dissolved in water)	Mihail and Kimmerle (1977)
Mice	Oral	Male	1018 mg/kg b.w	Ogata et al. (1979)
		Female	683 mg/kg b.w	Ogata et al. (1979)

3.1.2. Skin İrritation

There are available data on skin irritation caused by OPP and SOPP in rabbits (Norris, 1971a; Schreiber, 1981; Thyssen, 1982; Suberg, 1983; Maertins, 1988; Gilbert, 1994). Skin irritation potential tests were positive for both substances (Table 3). While OPP is a strong irritant on the skin, sodium salt has a corrosive effect (Bomhard et al., 2002).

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ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

3.1.3. Eye İrritation

There are studies on rabbits about the toxic effects of OPP and SOPP on the eye (Norris, 1971a,b; Schreiber, 1981; Pauluhn, 1983; Maertins, 1988). In these studies, permanent opacity in cornea, iritis, conjunctivitis, redness, chemosis, necrosis and exudates were clinically observed. Studies on rabbits have shown that SOPP and OPP are in category I among the toxic substances that affect the eyes (DPR, 2007). While OPP is moderately irritant to the eyes, SOPP has a corrosive effect (Table 4) (Bomhard et al., 2002).

Table 3. Skin irritation of OPP and SOPP in rabbit (Bomhard et al., 2002).

Substance	Exposure duration	Obsevation periyod (day)	Result	References
OPP	4 h	8	Mildly irritant	Norris (1971a)
	4 h	3	Strongly irritant	Thyssen (1982)
	30 min	10	Mildly irritant	Suberg (1983)
	4 h	15	Strongly irritant	Gilbert (1994)
SOPP	4 h	7	Corrosive	Maertins (1988)
	24 h	7	Strongly irritant	Pauluhn (1983)

Table 4. Eye irritation of OPP and SOPP in rabbit (Bomhard et al., 2002).

Substance	Amount (mg)	Post-exposure period (day)	Result	References
OPP	100	7	Moderately irritant	Norris (1971b)
OPP	100	8	Moderately irritant	Schreiber (1981)
SOPP	100	7	Corrosive	Pauluhn (1983)
	40	7	Corrosive	Maertins (1988)

3.2. Subacute, Subchronic and Chronic Toxicity

There are several studies on rats, mice and dogs about the subchronic toxicity of OPP and SOPP. OPP mainly affects the kidney and urinary bladder in rats. In male rats, increase in kidney weight, decrease in kidney function, nephritis, papillary necrosis, pelvis/papilla hyperplasia and increase in the kidney tubular cells are the some of alterations. OPP is thought to affect the kidney (with a decrease in urinary pH and the formation of nephritis) in females, but limited data are available on this subject. SOPP is also affects kidney, urinary bladder and liver. SOPP has several effects on kidneys such as increased organ weight and pyelonephritis in both sexes. There are studies about the effects of OPP and SOPP on chronic toxicity and oncogenicity in rats, mice and dogs, and the triggering effects of SOPP in the urinary tract in guinea pigs and hamsters. The toxicities of OPP and SOPP vary according to gender and species. It has been reported that in rats OPP has an effect on the optic nerves, spleen and heart, primarily in the kidney and urinary tract (Higara and Fujii, 1981; Ushiyama et al., 1982; Honma et al., 1983; Ushiyama et al., 1983; Higara and Fujii, 1984; Fujii and Higara, 1985).

Higara and Fujii (1981) administered SOPP at the rates of % 0, 0.125, 0.25, 0.5, 1, 2 and 4 with feed to male and female rats for 13 weeks. At the end of this period, they reported that a urinary bladder tumor developed in 1 of those given 1% SOPP, 9 of those given 2% SOPP and 1 of those given 4% SOPP in male rats and 2 of those given 4% SOPP

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ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

in female rats. In the 91st week, researchers found that papillary tumors in the kidney and urinary bladder developed in 1 of the rats given 0.5% SOPP, 7 of the rats given 1% SOPP, 20 of the rats given 2% SOPP and 17 of the rats given 4% SOPP.

Higara and Fujii (1984) applied OPP at the rates of 0.156, 0.313, 0.625, 1.25 and 2.5% with feed to male and female rats for 13 weeks. At the end of this period, the authors found that 50% of the animals had urinary bladder tumors in the group receiving 1.25% OPP. At the end of the 91st week, the researchers found that urinary bladder tumors in 96 % of the rats in 1.25% OPP group and 17 % of the rats in 2.5% OPP group were developed.

In another study, 0, 0.25, 0.5, 1 and 2% OPP was given to the group of 15 rats for 12 weeks. In the 4th, 8th and 12th weeks, urinary bladder of 5 rats of each group were examined under light and electron microscope and no change was observed in the group receiving 1% OPP. From 4th week, in the group receiving 2% OPP, damage in the microvilli in the lumen of epithelial cells was detected by Scanning Electron Microscope (TEM) images (Oehme, 1971).

SOPP was given to 20 male F344 rats by adding feed at 0, 0.625, 1.25 and 2.5% concentration for 13 weeks, and in groups fed with 1.25 and 2.5% SOPP, decreases in weight were reported. It was stated that there was no change in biochemical parameters in plasma samples. A decrease in the number of red blood cells, the amount of hemoglobin and the weight of the bladder was reported in the group receiving 2.5% SOPP. In groups given 1.25 and 2.5% OPP, it was reported that the number of rats whose urine pH was acidic increased and urine protein levels decreased in the 2.5% OPP group (Nakamura et al., 1981).

It was reported that SOPP was given to the group consisting of 50 F344 male rats by adding to feed at 0.25, 0.5, 1 and 2% concentration for 36 weeks and samples from 10 rats in each group were examined with light and scanning electron microscopes at 4, 8, 12, 24 and 36 weeks. Slight hyperplasia in the urinary bladder was reported in the group given 2% SOPP, and 40% of the rats examined at 36th week had papillary and nodular (PN) hyperplasia. It was stated that high-grade epithelial surface damages observed in 1% and 2% SOPP groups were detected by scanning electron microscope (Oehme, 1971).

Honma et al. (1983) fed 40 male F344 rats with feed containing 2% SOPP for 50 weeks and at the end of the study researcher found that 86% of rats had PN hyperplasia, 53% of them had papillomas and 39% of them had transitional cell carcinoma. It was reported that in 3 rats papilloma in the pelvis renalis and in 9 rats PN hyperplasia were detected, and there were no tumors in the control group.

Hasegawa et al. (1990) applied 2% SOPP to 30 male F344 rats for 48 weeks, and at the 4th, 6th, 12th, 24th, 36th and 48th weeks, they examined the urinary bladders of groups of 5 rats with light and scanning electron microscope. They reported that there was a pause in body weight increase throughout the study and simple epithelial hyperplasia and pleomorphic microvilli occurred in all groups. At 36 and 48 weeks, PN hyperplasia were observed in all groups of rats and at12th and 48th weeks, they stated that pH of the urine increased and crystaline structures were seen in the urine.

OPP and SOPP were given to 30 male F344 rats by adding feed at 2% doses for 90 days and blood, urine, liver, kidney and urinary bladder samples were taken on the days 3, 7, 14, 30, 65 and 90. It was stated that feed consumption and body weights decreased in both groups, especially in the group which OPP was given. In addition, an increase in mortality was reported in the group receiving OPP between days 7 and 14. In the group which SOPP was given, decrease in feed intake and body weights returned to the normal after two weeks, while this situation continued in animals receiving OPP. In animals receiving 2% OPP, a decrease in urinary density, blood in urine and cysts in kidney were reported on the 65th and

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90th days. It was also stated that urinary bladder tumors were not observed in any of the animals (Okuda, 1986).

OPP was given to 70 F344 rats of both sexes at doses of 0, 0.08, 0.4 and 0.8% to males and 0, 0.08, 0.4 and 1% to females. At the end of the 1-year period, 20 animals were euthanized for further research. It was stated that average body weight decreased at medium and high doses in both sexes, while there was no change in feed consumption and there was a small increase in mortality in male rats. In groups receiving 0.4% and more OPP, changes in urine color were observed. At high doses in male rats, it has been reported that urine samples contain blood. In groups receiving moderate and high doses of OPP, involuntary urination during death and masses in urinary bladder were detected at necropsy. At the end of 1 year, hyperplasia in the bladder in all 20 of the animals, transitional cell carcinoma in 3 of them and papilloma in 6 of them were reported in the group receiving 0.8% OPP. At high doses, the rate of stone formation in kidneys increased in males, while cystic tubular enlargement and chronic ischemia in kidney were detected in females. Simple hyperplasia (84% in males, 12% in females) and PN hyperplasia (86% in males, 2% in females) have been reported in the urinary bladder at high doses (Ushiyama et al., 1982, 1983).

Fujii and Hiraga (1985) administered SOPP to 50 F344 rats. Males at rates of 0%, 0.7% and 2% for 106 weeks, and females at rates of 0, 0.5 and 1% for 104 weeks (2 weeks basal diet) were received SOPP. They found that body weight was lower than other groups in males given 2% SOPP and females given 1% SOPP. They observed that in males given 2% SOPP, blood was observed in the urine from the 40th week and continued to increase until the end of the study. At the end of the 106th week, 47 of 50 males receiving 2% SOPP developed a bladder tumor. They found that carcinomas metastasize to the lung in 15% of males given 2% SOPP. Increased interstitial nephritis and pyleonefritis were observed in females receiving 1% SOPP.

OPP was given to 20 B6C3F1 male mice (4 groups, 13 in each) for 52 weeks by feed at the rates of 0, 6500, 13000 and 26000 ppm. In the 13000 ppm OPP group, death was observed at 40 weeks. In 13000 and 26000 ppm OPP groups, decreases in body weight and feed consumption were observed. It has been determined that OPP has an effect on liver, kidney and spleen in mice. Increase in organ weight in both sexes of the liver, increase in the formation of nonneoplastic (focal necrosis, anisonucleosis, liver cells and pigment residues in phagocytes), preneoplastic (eosinophilic cell foci) and neoplastic (adenoma, hepatoblastoma and carcinoma) lesions, atrophy in the spleen were observed (DPR, 2007). On the other hand, in other studies on OPP and/or SOPP in mice, no histopathological findings were found in the urinary bladder (Savides and Oehme, 1980; Fukushima et al., 1982; Selim, 1996) and they did not cause any toxic effects in urine and changes in blood analysis (Savides and Oehme, 1980; Hasegawa et al., 1990; Selim, 1996).

OPP was given to F344 rats from both sex for 21 days, 5 days a week, once a day at 0, 100, 500 and 1000 mg/kg b.w. It was reported that in group of rats which were given 500 and 1000 mg/kg b.w OPP, skin rashes were detected at the application site and no histopathological changes were observed in any group (Wick and Gschwend, 1998).

OPP (dissolved in acetone) was applied to 50 CD-1 mice 3 times a week for 102 weeks and no effect was detected on the skin. Likewise, SOPP (dissolved in acetone) was applied to 20 female CD-1 mice for 47 weeks twice a week and it was stated that no effect was observed on the skin (Shibata et al., 1985).

3.3. Genotoxicity

Previously some studies reported that OPP and its metabolites showed weak genotoxic effects (Reitz et al., 1983, 1984). However, there are also *in vivo* and *in vitro* studies reporting that OPP, SOPP, PBQ and PHQ may have genotoxic effects (Roy, 1990; Pathak and Roy, 1993; Nakagawa and

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Tayama, 1996). The reactive metabolites have potential for causing damage to biomacromolecules (proteins, peptides and DNA). Reactive PBQ and PHQ metabolites have been shown to bind covalently to both *in vivo* and *in vitro* intracellular biomacromolecules and nucleophilic centers (Pathak and Roy, 1993; Nakagawa and Tayama, 1996). Roy (1990) has demonstrated that P450 microsomal cytochromes catalyze the redox cycle of OPP. Morimoto et al. (1989) showed indirect evidence that reactive metabolites of OPP may cause DNA damage.

Results of S*almonella typhimurium* or *Escherichia coli* tests indicated that OPP (Kojima and Hiraga, 1978; Kojima et al., 1983) and SOPP (Kojima and Hiraga, 1978) did not cause to point mutations in bacteria. However some investigators reported that OPP caused point mutations in mammalian cells (mouse lymphoma, human RSa cells) *in vitro* (Suzuki et al., 1985; NTP, 1986). On the other hand PHQ (Lambert, 1992) and PBQ (Reid et al., 1998) did not induce point mutations in the mammalian cells (PHQ-Chinese hamster V 79 cells, PBQ-AHH-1 human lymphosblastoid cells).

Results of chromosomal damage (i.e., clastogenicity, endoreduplication and aneugenicity) studies indicated that OPP and SOPP caused damages in chromosomes of mammalian cells *in vitro* (Kawachi et al., 1981; Tayama et al., 1989; Tayama and Nakagawa, 1991) and *in vivo* (Tadi-Uppala et al., 1996; Balaskrishnan et al., 2002; Balakrishnan and Easmond, 2006). Tadi-Uppala et al. (1996) administered rats to OPP, SOPP, NaCl or OPP + NaCl in addition to their diet. They reported that micronucleus increases were observed in the bladder cells of animals given OPP and SOPP. Balakrishnan et al. (2002) examined the genotoxic effects of OPP and SOPP with their studies on the rat urinary bladder. It has been stated that the administration of OPP and SOPP together with the diet for 2 years caused tumor induction in the bladder and increased cell proliferation and micronucleus formation from the 2nd week. In addition, Balakrishnan and Eastmond (2006) reported that chromosal breakage and base losses may be the cause of increases in micronucleus formation in the urinary bladder.

OPP irreversibly bind to DNA with covalent bonds (Ushiyama et al., 1992) and rat liver *in vitro* (Pathak and Roy, 1992). Pathak and Roy (1992) investigated the covalent binding of PBQ to rat liver DNA using the ^{32}P-postlabeling technique. Four major and several minor adducts have been revealed. As a result of the chemical reaction between PBQ and deoxyguanosine 3-phosphate oligonucleotides (dGMP), 4 major substances were formed. Modifications resulting from covalent bonds between DNA and the reactive metabolite of OPP precisely determined the presence of *in vitro* genotoxic effects of OPP.

Ushiyama et al. (1992) investigated the formation of DNA adduct caused by OPP. In the presence or absence of rat liver microsomes, they investigated the covalent binding of OPP to calf thymus DNA. It is stated that DNA binding did not occur as a result of incubation without microsomes or when the microsomes are denatured with heat. As a result, it has been suggested that biotransformation of OPP to active metabolites is a prerequisite for DNA binding. Inoue et al. (1990) found that PHQ causes oxidative DNA damage in the presence of copper II (Cu^{2+}).

Murata et al. (1999) reported that PHQ and PBQ can cause oxidative DNA damage in the peripheral blood promyeloblast cells (HL60) via H_2O_2 production resulting in mutation and carcinogenesis due to this damage. As a result of the genotoxicity studies, it has been stated that OPP, SOPP and its metabolites may cause gene mutation, chromosomal and DNA damage.

Conclusion

OPP and SOPP produce toxic effects that are widespread in the phenolic class. Compared with other phenol derivatives, OPP shows a highly lipophilic and electrophilic structure. These physicochemical properties are extremely

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important in terms of transport from membranes and their interaction with target macromolecules (eg DNA, protein, lipids). After OPP and SOPP enter the body, they turn into reactive metabolites. Both these active metabolites (PHQ and PBQ) and the free oxygen radicals produced by the reactions are thought to be accountable for the toxic effects of OPP and SOPP.

In the light of the studies, it has been indicated that rather than acute exposure these substances are subcronically and chronically exposed and cause carcinogenic effects. It has been stated that OPP and SOPP reveal toxic effects on the kidney and liver, especially the urinary tract. In addition, it has been highlighted by studies that genotoxic effects are observed as a result of covalent binding of metabolites to DNA.

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To cite this article: Erdemli Köse SB, Şahindokuyucu Kocasarı F. (2020)). Toxicity of Ortha-Phenylphenol (OPP) and Sodium Ortho-Phenylphenate (SOPP). MAKU J. Health Sci. Inst., 8(1), 18-29.

ISSN: 2148-2837/ MAKU J. Health Sci. Inst.