Mehmet Akif Ersoy Üniversitesi

Sağlık Bilimleri Enstitüsü Dergisi

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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 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 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).

The Editor gives the final decision about the acceptance of the manuscript. The Editorial Board is authorized to publish the paper, return it for correction, or reject it. The assessment process involves research articles, case reports and original articles submitted to the journal. Other types of articles are evaluated directly by the Board. Papers submitted to the journal will not be returned whether they are published or not. The Editor and the Editorial Board have the right to reject, to require additional revision or to revise the format of manuscripts which do not follow the rules. The authors should inform the editorial board if they decide to withdraw the manuscript. The editor may consult editorial executive board about a manuscript if (s) he deems necessary. All the authors should submit a collectively signed statement that there is no conflict of interest regarding scientific contribution or responsibility. The association, establishment, and medication-material supply firms which have given financial, even partial, or material support to the research should be mentioned in a footnote. No fee or compensation will be paid for articles published in the journal.

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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The authors are responsible for their compliance with the ethical rules. In experimental studies on animals, it should be noted that the study protocol has been approved by the animal experiment ethics committee at the institution where the study was conducted. Authors should submit the ethics committee's approval with the article. If there are previously published text, tables, pictures, etc. in the article, the authors have to get written permission from the copyright holder and the authors should specify and indicate the used material in the manuscript. In the course of the manuscript evaluation, the authors may be requested to submit the research data and / or the ethics committee approval document if deemed necessary.

Plagiarism policy

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.

3. Journal news: These articles introduce scientific journals being published within the scope of the journal. The paper may not exceed 1 page.

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.

c) Acknowledgements: When considered necessary, author(s) may add brief acknowledgements in a few sentences to those whose contributions to the paper are not at author level but deserve to be mentioned. Here, the contributions of those acknowledged (e.g. financial or equipment aid, technical support etc) are clearly stated (e.g. "scientific counseling", "editing of the draft", "data collection", "participation in clinical research" etc).

d) Bibliographic References:

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.

Examples: ....were reported that... (Bell, 2005; Nielsen and Engberg, 2006; Doyle et al., 2007).

More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.

Examples: (Bell, 2005a; Bell, 2005b; Bell, 2005c …)

The authors can use below formatted style link in mendeley:

http://csl.mendeley.com/styles/529990351/sagbilensderg

References should be written in alphabetical order. Reference style, the authors' names and year of publication should be written in bold. Source list should be prepared as follows:

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..

f) Figures and Pictures: Figures should be numbered according to the order of use and should be expressed with short titles.Figures should be numbered in the text (Figure 1). Letters, numbers and symbols within the figure should be clear and readable when downsized for printing.Each figure should be referred to in the text..

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

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(Mehmet Akif Ersoy University Journal of Health Sciences Institute)

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Araştırma Makaleleri / Research Articles

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

Erratum / Dizgi Hatası

Sayın yazarlarımız ve okurlarımız;

Dergimizin MAKU J. Health Sci. Inst. 2021, 9(2) numaralı Ağustos sayısındaki kapak sayfasının yayınlanmasında sehven bir hata olmuş ve Ağustos ifadesi Nisan olarak yazılmıştır.

Kullanıcılarımız; dergimizin Aralık sayısı olan bu sayının son sayfasından, Ağustos sayısına ait kapağın düzeltilmiş haline ulaşabilirler.

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

MAKU J. Health Sci. Inst. 2021, 9(3): 29-37.
doi: 10.24998/maeusabed.996891

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

Research Article / Araştırma Makalesi

Evaluation of Some Ecute Phase Proteins, Cytokines and Hepcidin Levels in Naturally Infected Saanen Goats with Paratuberculosis

Doğal Enfekte Paratüberkülozisli Saanen Keçilerinde Bazı Akut Faz Proteinleri, Sitokinler ve Hepsidin Düzeylerinin Değerlendirilmesi

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Abstract: Johne's Disease or paratuberculosis is a mycobacterial infection of ruminants and has a global economical impact. *Mycobacterium avium subsp. paratuberculosis* is the cause of this disease. It reduces milk production, cause chronic weight loss leading death and major losses. Acute phase reactions are defined as minimum 25% increase or decrease in serum concentrations of acute phase proteins which are triggered by pro or antiinflammatory cytokines released from various cells or tissues. The aim of the study was to investigate the effect of Mycobacterium avium subsp paratuberculosis on blood parameters, some acute phase proteins, cytokines and hepcidin in naturally infected goats with paratuberculosis. In this study, total 45 Saanen goats aged 2-5 years from both sex were used as animal material. Study group were included 35 and control group were included 10 animal for evaluation. Complete blood counts were performed on blood taken from all animals. Also interleukin 6, interleukin 10, serum amyloid A, haptoglobin, fibrinogen, and hepcidin levels were evaluated from serum samples. As a result, interleukin 6 ($p<0.01$), interleukin 10 ($p<0.01$) serum amyloid A ($p<0.001$), haptoglobin ($p<0.001$), fibrinogen ($p<0.05$), and hepcidin ($p<0.05$) differences between groups were statistically important and they can be used to support the diagnosis of paratuberculosis.

Keywords: Acute phase proteins, Cytokine, Hepcidin, Paratuberculosis, Saanen goat.

Öz: Johne's hastalığı veya paratüberkülozis, ruminantların mikobakteriyel bir hastalığı olup dünya çapında ekonomik kayıplara yol açmaktadır. Hastalığa neden olan etken *Mycobacterium avium subsp. paratuberculosis*'tir. Süt veriminde azalma, kronik kilo kaybı ve ölümlere neden olmaktadır. Akut faz reaksiyonları, akut faz proteinlerinin hastalık sırasında çeşitli hücre ve dokular tarafından salgılanan pro veya antienflamatuar sitokinlerin stimulasyonu sonucunda serum konsantrasyonunda en az % 25 değişim göstermesi şeklinde tanımlanmıştır. Çalışmanın amacı doğal olarak enfekte olmuş paratüberkülozisli keçilerde, Mycobacterium avium subsp. paratuberculosis etkeninin hayvanların kan parametreleri ve bazı akut faz proteinleri ile sitokinler ve hepsidin üzerindeki etkisini araştırmaktı. Çalışmanın hayvan materyalini, her iki cinsiyetten, 2-5 yaş aralığında toplam 45 adet Saanen ırkı keçi oluşturdu. Çalışma grubunda 35, kontrol grubunda ise 10 hayvan değerlendirildi. Bütün hayvanlardan alınan kanlarda tam kan sayımları yapıldı. Ayrıca toplanan serumlarda interlökin 6, interlökin 10, serum amiloid A, haptoglobin, fibrinojen ve hepsidin değerleri ölçüldü. Sonuç olarak gruplar arası interlökin 6 ($p<0.01$), interlökin 10 ($p<0.01$) serum amiloid A ($p<0.001$), haptoglobin ($p<0.001$), fibrinojen ($p<0.05$) ve hepsidin ($p<0.05$) değerlerindeki farklar istatistiksel açıdan önemli bulunarak paratüberkülozun teşhisini desteklemede belirtilen parametrelerin kullanılabileceği kanaatine varıldı.

Anahtar Kelimeler: Akut faz protein, Sitokin, Hepsidin, Paratüberkülozis, Saanen keçi.

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Introduction

Paratuberculosis is a disease caused by bacteria formerly known as Mycobacterium paratuberculosis. With the molecular characterization developed in the nineties, the causative agent was classified as a subspecies of *Mycobacterium avium* and *M. avium subsp. paratuberculosis* (Map) has been reported (Harris and

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Barletta, 2001). The disease, which occurs sporadically in temperate climates and sporadically in the tropics, has spread as a result of the importation of animals from regions where it is endemic. It is difficult to find sufficient data on the geographical distribution of goat paratuberculosis, as statistics on goat diseases are often reported together with sheep diseases by governments and international agencies. Goats can also become infected as a result of ingestion of the agent at an early age. Goats often show no signs of chronic watery diarrhea unless they are in the last stage of the disease. However, if diarrhea is observed, it has been reported that it may be caused by parasitic factors as well as paratuberculosis. It has been stated that there may be soft stools such as dog feces from time to time, but pellet defecation generally continues (Radostits et al., 2007).

The secretion of cytokines, which are proinflammatory substances, from damaged tissues in response to tissue damage is called the acute phase response, and it can be considered as an early warning system that informs the state of the body (Gruys et al., 2005; Cray et al., 2009; Ceciliani et al., 2012). The aim of the acute phase response is to prevent further damage to an organ, limit the reproduction of the infective organism, remove harmful molecules, and activate the repair processes necessary for the organ to return to normal function (Hirvonen, 2000). Cytokines are first released by macrophages and monocytes in the damaged tissue, go to various tissues and organs through the circulation, cause release from other tissues and organs, and a systemic cytokine release begins (Eckersall, 2000). With the effect of systemic cytokine release, the circulating density of some plasma proteins, generally known as acute phase proteins (AFPs), is increased to support changes from hepatocytes (Eckersall, 2000, Hirvonen, 2000).

Interleukin-6 (IL-6) is a pleiotropic cytokine involved in regulation of acute phase response, cell growth and differentiation as well as metabolic processes (Neurath and Finotto, 2011). Interleukin 10 (IL-10) was first defined as a "cytokine synthesis inhibitory factor" and it was reported to suppress the production of Th1 cytokines such as IL-2 and IFN-γ by acting on antigen-presenting cells (De Silva et al., 2011).

Serum Amyloid A (SAA) as acute phase protein is thought to affect high-density lipoprotein-cholesterol transport. It stimulates inflammatory cells in tissues, prevents leukocytes from losing their structure as a result of oxidation, and manages the immune response. Haptoglobulin (Hp) is not found in healthy bovine serum. This protein has numerous reported functions, but its primary function is to prevent iron loss by forming highly stable complexes with free hemoglobin in the blood (Ceron et al., 2005). Thus, it has been stated that Hp has a bacteriostatic effect by limiting the availability of iron required for bacterial growth (Ceron et al., 2005; Ametaj et al., 2011). Fibrinogen has functions such as homeostasis, providing a substrate for fibrin formation, and providing an interface for the migration of inflammatory cells to the inflammatory region, tissue-repairing, clot-forming, and C3 complement-forming (Gruys et al., 2005).

Hepcidin, discovered in recent years, is a peptide hormone with multiple functions. In the first reported studies, hepcidin was named as an antimicrobial in peptide structure in human blood and urine (Park et al., 2001), but in later studies it was reported that it was a type II acute phase reactant and played a role as a regulator in iron metabolism (Nicolas et al., 2002; Laftah et al., 2004). Studies in humans have reported that hepcidin is mainly released from hepatocytes, but it is also produced by kidney, pancreatic beta cells, adipose tissue and heart tissue (Merle et al., 2007; Kemna et al., 2008; Kulaksiz et al., 2008). It has also been detected in various body fluids, such as urine, bile, pleural and cerebrospinal fluids (Kemna et al., 2008; Arnold et al., 2010).

The aim of the study was to investigate the effect of Mycobacterium avium subsp paratuberculosis on blood parameters, some acute phase proteins, cytokines and hepcidin in naturally infected goats with paratuberculosis.

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Materials and Methods

This research was carried out based on the permission of Mehmet Akif Ersoy University Experimental Animals Local Ethics Committee, dated 09.09.2015 and numbered 143/2015.

Saanen goats bred in farms in different provinces constituted the research material. In this study, blood samples were collected from the animals in the study group, considering the following clinical signs and criteria.

Clinical Symptoms Desired
• Diarrhea (±)
• Progressive weight loss
• Yield loss
• No other obvious symptoms on clinical examination despite the above data.

There were complaints such as weight loss and chronic diarrhea problems in goat herds. Despite treatment, there was no improvement. It has been confirmed that flocks have not been previously vaccinated against paratuberculosis. A total of 750 blood samples were collected according to the criteria mentioned above, and 610 of them were female and 140 male Saanen goats. 105 animals were found to be seropositive for paratuberculosis (14%) and 35 of them constituted the study group. 29 of the animals were female and 6 were male and their ages varied between 2-5 years. The control group consisted of 10 healthy animals negative for paratuberculosis. The sexes of the animals in this group were 7 females and 3 males, and their ages were between 2-5 years old.

Venous blood samples from all goats were taken from vena jugularis into negative pressure tubes with the help of Vacutainer® holder with 21 gauge needle. Plastic tubes with K3-EDTA (2.5 ml) were used for hemogram samples and silicone-based plastic tubes (5 ml) with clot activator for serum samples (BD Vacutainer®). Collected blood samples were centrifuged at 4000 rpm/5 min and blood serums were extracted. The obtained sera were divided into tubes and stored at -20 °C until processed. The collected 750 blood serums were screened for paratuberculosis with the ELISA test (IDEXX MAP Ab ELISA Test, America). Samples were duplicated to increase the reliability of the test. As a result of the ELISA test, 105 of 750 blood samples were positive for paratuberculosis (14%). In the interpretation of the results, the percentage of sample/positivity (s/p %) was calculated for each sample. According to this evaluation, s/p percentage ≤ 45% was evaluated as negative, >45%-<55% as suspicious, and ≥55% as positive. Of the 105 positive samples, 30 serum samples with the highest percentage of positivity were included in the study. The s/p values of the samples in the study ranged between 65.98-171.24. To form the control group, 10 animals were selected from other herds that were healthy as a result of clinical examinations. Serum samples collected from these animals, like the samples in the study group, were examined for paratuberculosis by ELISA method and the s/p percentages were determined as 0.00-0.38.

Thirty-five animals in the study group (naturally paratuberculous animals) and 10 animals in the control group (healthy and paratuberculosis-negative animals) were evaluated. Complete blood counts (Abacus Junior Vet Hematology Analyzer®, Diatron, Hungary) were taken from all animals with EDTA tubes. In addition, serum samples collected from these animals, haptoglobin, serum amyloid A, fibrinogen, interleukin 6, interleukin 10 and hepcidin values were measured in the collected sera. Finally, haptoglobin, serum amyloid A, fibrinogen, interleukin 6, interleukin 10 and hepcidin values of healthy and paratuberculosis animals were compared.

Interleukin 6, interleukin 10, serum amyloid A, haptoglobin, fibrinogen and hepcidin values in blood serum were measured by ELISA (Enzyme-Linked Immunosorbent Assay) method. In the research, goat-specific 96 IL-6 (Catalog no: MBS734666), IL-10 (Catalog no: MBS265401), Fb (Catalog no: MBS735156), of MyBioSource® (MyBioSource Inc., Southern California, San Diego/USA), Hp (Catalog no: MBS280796), SAA (Catalog no: MBS031629) and Hepcidin (Catalog no: MBS044535) ELISA test kits were used.

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Serum samples taken in the study were evaluated with the kits of MyBioSource®. The kits were stored in accordance with the instructions for use on them and then worked again according to the instructions. Microsoft Office® Excel program was used for calculations after ELISA measurements. The data obtained at the end of the procedure were calculated with the calculation method in the kit, curve graphics were created and the samples were grouped and calculated in accordance with this slope.

Statistical analysis

Statistical analysis was performed with the SPSS 19 program (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp). Paired T Test was used while evaluating the obtained hemogram and ELISA results. In the comparison between the groups, values less than $p<0.05$ were found to be statistically significant and included in the study.

Results

Thirty serum samples with the highest positivity percentage out of 105 samples with positive paratuberculosis disease were included in the study. The s/p values of the samples in the study ranged between 65.98-171.24 (Table 1).

To form the control group, 10 animals were selected from other herds that were healthy as a result of clinical examinations. Serum samples collected from these animals, like the samples in the study group, were examined for paratuberculosis by ELISA method and the s/p percentages were determined as 0.00-0.38 (Table 2).

When the hematological parameters of the control and study groups were evaluated, the difference between erythrocyte ($p<0.05$), hemoglobin ($p<0.05$), hematocrit ($p<0.05$), monocytes ($p<0.05$) and leukocyte ($p<0.01$) values was found to be significant. The difference between eosinophil and platelet values was not significant (Table 3).

Table 1. Sample/positive values of paratuberculous (+) animals.

Sample No.	S/P Values
1	145.21
2	123.32
3	114.44
4	130.89
5	65.98
6	125.55
7	167.01
8	102.26
9	170.79
10	127.35
11	171.24
12	115.67
13	148.99
14	136.65
15	129.25
16	114.52
17	142.66
18	161.42
19	94.28
20	123.82
21	144.63
22	108.02
23	141.35
24	87.45
25	137.12
26	90.09
27	134.17
28	105.39
29	96.75
30	132.09
31	132.43
32	107.54
33	99.12
34	98.54
35	83.32

While the difference between hepcidin and fibrinogen values of the control and study groups was found to be statistically insignificant ($p>0.05$), the difference between cytokine IL-6 and IL-10 values was found to be moderately significant ($p<0.01$). According to this table, the highest difference was found between the acute phase

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proteins haptoglobin and serum amyloid A values in animals in the control and study groups ($p<0.001$) (Table 4).

Table 2. Sample/positive values of paratuberculosis (-) animals.

Sample No.	S/P Values
1	0.19
2	0.07
3	0.15
4	0.07
5	0.15
6	0.08
7	0.15
8	0.38
9	0.0
10	0.3

Discussion

Johne's disease or paratuberculosis causes chronic inflammation in the gastrointestinal tract in ruminants and is caused by Mycobacterium avium subsp. paratuberculosis. The disease causes significant economic losses worldwide, with low productivity, sustained weight loss and eventual death in ruminants (Chiodini et al., 1984). All sick animals in the study had a history of progressive weight loss and poor conditioning. The gold standard for diagnosis in eradicating paratuberculosis disease is based on detecting infected animals and preventing the spread of the disease. Undoubtedly, an effective diagnosis and a successful control program are required rather than an expensive treatment. Therefore, additional biochemical markers are essential for the control of paratuberculosis disease.

Table 3. Values of some hematological parameters in the control and study groups.

Parameters	Control Group (n=10) (Ort.±SD)	Study Group (n=35) (Ort.±SD)	P Values
Leukocyte ($X10^9$/L)	7.55 ± 0.86	15.98 ± 2.42	< 0.01**
Neutrophil ($X10^9$/L)	4.54 ± 1.55	8.45 ± 1.36	> 0.05
Lymphocyte ($X10^9$/L)	4.72 ± 0.90	4.42 ± 0.68	> 0.05
Monocyte ($X10^9$/L)	0.13 ± 0.07	0.15 ± 0.10	< 0.05*
Eosinophil ($X10^9$/L)	0.14 ± 0.10	0.19 ± 0.14	> 0.05
Erythrocyte ($X10^{12}$/L)	10.26 ± 0.90	6.57 ± 0.56	< 0.05*
Hemoglobin (g/dl)	9.46 ± 0.94	6.70 ± 0.51	< 0.05*
Hematocrit (%)	31.70 ± 2.11	30.17 ± 3.55	< 0.05*
Platelets ($X10^9$/L)	406.60 ± 24.55	402.77 ± 42.94	> 0.05

*lowly significant, **moderately significant, $p>0.05$ statistically insignificant.

Senturk et al. (Senturk et al., 2009) found that erythrocyte, hemoglobin and hematocrit values in cows with paratuberculosis were lower than healthy cows in the control group. However, they stated that there was no difference in the number of leukocytes, neutrophils, lymphocytes, monocytes, eosinophils and platelets. In the study, erythrocyte, hemoglobin and hematocrit values in goats with paratuberculosis were decreased compared to the values of healthy goats, and the difference was found to be statistically significant ($p<0.05$), and the results were found to be in parallel with the results of the study. The hematocrit value of the study group was lower than the control group, but within normal limits. Although the result of hematocrit value is within normal limits, the difference is statistically significant, it can be concluded that the appetite

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does not decrease in sick goats and dehydration is hidden by continuing fluid consumption. However, according to the results obtained, it was observed that the number of leukocytes ($p<0.01$) and monocytes ($p<0.05$) increased in the animals in the study group compared to the animals in the control group and were found to be statistically significant. Lybeck et al. (Lybeck et al., 2011) showed that anemia occurred in naturally infected goats with paratuberculosis. In the current study, decreases in erythrocyte, hemoglobin and hematocrit values also indicate anemia and show parallelism with other studies. The increase in the number of monocytes in hematological results is thought to be due to the chronic course of the disease.

Table 4. Values of IL-6, IL-10, SAA, Hp, Fb and Hepcidin in the control and study groups.

Parameters	Control Group (n=10)	Study Group (n=35)	P Values
Interleukin-6 (pg/ml)	56.56 ± 10.39	293.66 ± 35.07	< 0.01**
Interleukin-10 (pg/ml)	9.02 ± 1.70	43.81 ± 4.40	< 0.01**
Serum Amiloid A (µg/ml)	5.67 ± 1.19	422.88 ± 118.14	< 0.001***
Haptoglobin (ng/ml)	0.01 ± 0.00	1.95 ± 0.64	< 0.001***
Fibrinogen (ng/ml)	3.11 ± 0.54	7.97 ± 1.24	< 0.05*
Hepcidin (ng/ml)	3.67 ± 0.58	52.03 ± 6.93	< 0.05*

*Low important, **moderately important, ***highly important

Interleukin 6 is a proinflammatory cytokine. It is produced by Th2 cells, acts as a mediator in the formation of the acute phase response and also accelerates the infiltration of inflammatory cells (O'Garra and Murphy, 2009). It has been reported that IL-6 induces hepcidin production during immune activation (Nemeth et al., 2004). In the study, the IL-6 level in the blood serum of the goats in the control group was 56.56±10.39 (pg/ml) and 293.66±35.07 (pg/ml) in the study group. In the statistical evaluation, the difference between these two groups was found to be significant ($p<0.01$). The results obtained were consistent with the results of previous studies. In addition, as mentioned before, the increase in hepcidin value showed parallelism with the increase in IL-6 in the animals in the experimental group. IL-10 produced by monocytes and macrophages is a cytokine that contributes to the regulation of the immune system and is an important balancer between Th1 and Th2 (O'Garra and Murphy, 2009). In previous studies, high levels of IL-10 were detected in goats and sheep with paratuberculosis (Lybeck et al., 2013). In this study, the level of interleukin-10 was found to be 9.02±1.70 (pg/ml) in goats in the control group and 43.81±4.40 (pg/ml) in goats with paratuberculosis. In the statistical evaluation made according to this, it was determined that the difference between IL-10 levels of goats with paratuberculosis and healthy goats was significant ($p<0.01$).

Although the determination of AFPs in goat medicine has been reported to be clinically valuable, there is a lack of information in the veterinary field on this subject. Acute phase proteins are sensitive biomarkers, but their specificity is low against various infectious agents. It is also used in the diagnosis, prognosis and health status screening of animals (Murata et al., 2004). Circulating haptoglobin levels in healthy ruminants are very low and insignificant, but increase 100-fold in animals with compromised immune systems (Conner et al., 1998). Gonzalez et al. (2008) found the highest increase in serum haptoglobin level in infectious conditions in a study they conducted. According to this researcher, haptoglobin and serum amyloid A proteins can be used as an indicator of inflammation in goats. In previous studies on goats, haptoglobin levels were found to be increased in lactating goats (Heller and Johns, 2015), goats with pregnancy toxemia (Albay et al., 2014), turpentine injected goats (Gonzalez et al., 2008), goats with Corynebacterium

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pseudotuberculosis (Jeber et al., 2016) and in many other studies. In this study, a statistically significant difference was found in the mean serum haptoglobin and serum amyloid A values of the animals in the study group compared to the values of the animals in the control group ($p<0.001$). These results were in agreement with the results of other researchers. It has been reported that the level of fibrinogen, which is one of the positive acute phase proteins, increases up to 10 times in cases of inflammation (Eckersall, 2000). Kaneko et al. (2008) found that the blood fibrinogen value in goats varied between 1-4 ng/ml, but this value increased to 9.12 ng/ml after infection. El-Deeb (2013) reported a lower fibrinogen value (3.84±0.21 ng/ml) in goats with gangrenous mastitis. Similar fibrinogen values (9.6 ng/ml) Gonzalez et al. (2008) has also been reported by Experimentally, the fibrinogen level was found to be 9.12 ng/ml in goats with mastitis with Staphylococcus aureus (Fasulkov et al., 2014).

In this study, the mean fibrinogen value in goats in the control group was determined as 3.11± 0.54 ng/ml, which was consistent with the results of previous studies (Hajimohammadi et al., 2013; Fasulkov et al., 2014). The mean fibrinogen level in the animals in the study group was determined as 7.97±1.24 ng/ml, and the statistical difference was found to be significant compared to the control group ($p<0.05$).

Hepcidin, discovered in recent years, is a peptide hormone with multiple functions. Hepcidin was named as an antimicrobial in the first reported studies (Park et al., 2001), but it was reported that it was a type II acute phase reactant and played a role as a regulator in iron metabolism in later studies (Laftah et al., 2004). Kali et al. (2015) reported that hepcidin is triggered by IL-6 during inflammation and can be used as an important marker in sepsis and inflammatory reactions. Although studies on hepcidin in human medicine have increased in recent years, there have not been many studies in veterinary medicine. In this study, hepcidin value was investigated for the first time in goats with paratuberculosis. As a result of the study, hepcidin value was found as 3.67 ± 0.58 (ng/ml) in healthy goats, while it was determined as 52.03 ± 6.93 (g/ml) in goats with paratuberculosis. In this study, the highest levels of serum amyloid A and haptoglobin, which are acute phase proteins, the lowest levels of fibrinogen and hepcidin, and a moderate increase in cytokines levels were determined.

As a result, it was determined that SAA and haptoglobin the highest, IL-6 and IL-10 parameters moderate, and fibrinogen and hepcidin were the least increased parameters in goat paratuberculosis disease.

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References

Albay, M.K., Karakurum, M.C., Sahinduran, S., Sezer, K., Yildiz, R., Buyukoglu, T., 2014. Selected serum biochemical parameters and acute phase protein levels in a herd of Saanen goats showing signs of pregnancy toxaemia. Veterinary Medicine Journal-Czech 59(7), 336-342.

Ametaj, B.N., Hosseini, A., Odhiambo, J.F., Iqbal, S., Sharma, S., Deng, Q., Lam, T.H., Farooq, U., Zebeli, Q., Dunn, S.M., 2011. Application of acute phase proteins for monitoring inflammatory states in cattle. Ed: VEAS F, Acute phase proteins as early non-specific biomarkers of human and veterinary diseases, Croatia 299-354.

Arnold, J., Sangwaiya, A., Manglam, V., Geoghegan, F., Thursz, M., Busbridge, M., 2010. Presence of hepcidin-25 in biological fluids: bile, ascitic and pleural fluids. World Jounal of Gastroenterology 16, 2129–2133.

Ceciliani, F., Ceron, J.J., Eckersall, P.D., Sauerwein, H., 2012. Acute phase proteins in ruminants. Journal of Proteomics 75, 4207-4231.

Ceron, J.J., Eckersall, P.D., Martinez-Subiela, S., 2005. Acute phase proteins in dogs and cats: current knowledge and future perspectives. Veterinary Clinical Pathology 34(2), 85-99.

Chiodini, R.J., Van Kruiningen, H.J., Merkal, R.S., 1984. Ruminant paratuberculosis (Johne's disease): the current status and future prospects. The Cornell Veterinarian 74, 218-262.

Conner, J.G., Eckersall, P.D., Wiseman, A., Aitchison, T.C., Douglas, T.A. 1998. Bovine acute phase response following turpentine injection. Research in Veterinary Science 44, 82–88.

Cray, C., Zaias, J., Altman, N.H., 2009. Acute phase response in animals: A review. Comparative Medicine 59(6), 517-526.

De Silva, K., Begg, D., Whittington, R., 2011. The interleukin 10 response in ovine Johne's disease. Veterinary Immunology and Immunopathology 139, 10–16.

Eckersall, P.D., 2000. Recent advances and future prospects for the use of acute phase proteins as markers of disease in animals. Revue de Médecine Vétérinaire-Toulouse., 151(7), 577-584.

El-Deeb, W.M., 2013. Clinicobiochemical investigations of gangrenous mastitis in does: immunological responses and oxidative stress biomarkers. Journal of Zhejiang University. Science. B 14, 33–39.

Fasulkov, I., Karadaev, M., Vasilev, N., Urumova, V., Mircheva, T., 2014. Determination of plasma fibrinogen and haptoglobin, hematological and blood biochemical changes in Bulgarian local goats with experimentally induced Staphylococcus aureus mastitis. Turkish Journal of Veterinary & Animal Sciences 38, 439-444.

Gonzalez, F.H.D., Tacles, F., Martinez-Subiela, S., Tvarijonaviciute, A., Soler, L., Ceron, J.J., 2008. Acute phase protein response in goats. J Vet Diagn Invest., 20, 580–584.

Gruys, E., Toussaint. M.J.M., Niewold, T.A., Koopmans, S.J., 2005. Acute phase reaction and acute phase proteins. Journal of Zhejiang University. Science A 6 (11), 1045-1056.

Hajimohammadi, A., Nazifi, S., Ansari-Lari, M., Khoshmanzar, M.R., Bigdeli, S.M., 2013. Identifying relationships among acute phase proteins (haptoglobin, serum amyloid A, fibrinogen, ceruloplasmin) and clinical findings in dairy calf diarrhea. Comparative Clinical Pathology 22(2), 227-232.

Harris, N.B., Barletta, R.G., 2001. Mycobacterium avium subsp. paratuberculosis in veterinary medicine. Clinical Microbiology Reviews 14(3), 489–512.

Heller, M.C., Johns, J.L., 2015. Acute phase proteins in healthy goats: establishment of reference intervals. Journal of Veterinary Diagnostic Investigation 27(2), 177-181.

Hirvonen, J., 2000. Hirvonen's thesis on acute phase response in dairy cattle, Ed: Pyörälä S, University of Helsinki Faculty of Veterinary Medicine Publications, Helsinki 7-67.

Jeber, Z.K.H., Mohdjin, Z., Jesse, F.F., Saharee, A.A., Sabri, J., Yusoff, R., Wahid, H., 2016. Influence of Corynebacterium pseudotuberculosis infection on level of acute phase proteins in goats. BMC Veterinary Research., 12(48), 1-5.

Kali, A., Charles, M.V.P., Seetharam, R.S.K., 2015. Hepcidin-A novel biomarker with changing trends. Pharmacognosy Reviews 9(17), 35-40.

Kaneko, J.J., Harvey, J.W., Bruss, M.L., 2008. Clinical Biochemistry of Domestic Animals, 6th edition. Saunders, Philadelphia, USA.

Kemna, E.H., Tjalsma, H., Willems, H.L., Swinkels, D.W., 2008. Hepcidin: from discovery to differential diagnosis. Haematologica 93, 90–97.

Kulaksiz, H., Fein, E., Redecker, P., Stremmel, W., Adler, G., Cetin, Y., 2008. Pancreatic beta-cells express hepcidin, an iron-uptake regulatory peptide. Journal of Endocrinology 197, 241-249.

Laftah, A.H., Ramesh, B., Simpson, R.J., Solanky N, Bahram S, Schumann K, Debnam ES, Sari SKS (2004): Effect of hepcidin on intestinal iron absorption in mice. Blood 103, 3940–3944.

Lybeck, K.R., Storset, A.K., Djønne, B., Valheim, M., Olsen, I., 2011. Faecal shedding detected earlier than immune responses in goats naturally infected with Mycobacterium avium subsp. paratuberculosis. Research in Veterinary Science 91, 32–39.

Lybeck, K.R., Løvoll, M., Johansen, T.B., Olsen, I., Storset, A.K., Valheim, M., 2013. Intestinal strictures, fibrous adhesions and high local interleukin-10 levels in goats infected naturally with Mycobacterium avium subsp. paratuberculosis. Journal of Comparative Pathology 148, 157-172.

Merle, U., Fein, E., Gehrke, S.G., Stremmel, W., Kulaksiz, H., 2007. The iron regulatory peptide hepcidin is expressed in the heart and regulated by hypoxia and inflammation. Endocrinology 148, 2663–2668.

Murata, H., Shimada, N., Yoshioka, M., 2004. Current research on acute phase proteins in veterinary diagnosis: an overview. Veterinary Journal 168, 28–40.

Nemeth, E., Rivera, S., Gabayan, V., Keller. C., Taudorf, S., Pedersen, B.K., Ganz, T., 2004. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. Journal of Clinical Investigation 113(9), 1271-1276.

Neurath, M.F., Finotto, S., 2011. IL-6 signaling in autoimmunity, chronic inflammation and inflammation-associated cancer. Cytokine and Growth Factor Reviews 22, 83–89.

Nicolas, G., Viatte, L., Bennoun, M., Kahn, A., Vaulont, S., 2002. Hepcidin, a new iron regulatory peptide. Blood Cells, Molecules and Diseases 29, 327–335.

O'Garra, A., Murphy, K.M., 2009. From IL-10 to IL-12: how pathogens and their products stimulate APCs to induce T(H)1 development. Nature Immunology 10, 929–932.

Park, C.H., Valore, E.V., Waring, A.J., Ganz, T., 2001. Hepcidin, a urinary antimicrobial peptide synthesized in the liver. Journal of Biological Chemistry 276, 7806–7810.

Radostits, O.M., Gay, C.C., Hinchcliff, K.W., Constable, P.D., 2007. Veterinary Medicine. A textbook of the diseases of cattle, horse, sheep, pigs and goats, 10th Ed, Saunders Elsevier, Edinburgh.

Senturk, S., Metcitoglu, Z., Ulgen, M., Borum, E., Temizel, E., Kasap, S., 2009. Evaluation of serum iron and iron binding capacity in cows with paratuberculosis. Tierarztliche Praxis Ausgabe Grobtiere Nutztiere 37(6), 375-378.

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Research Article / Araştırma Makalesi

Investigation of Bovine Coronavirus and Bovine Rotavirus in Calves with Neonatal Diarrhea in Kırıkkale and Surrounding Provinces

Kırıkkale ve Çevre İllerindeki Neonatal İshalli Buzağılarda Sığır Koronavirüs ve Sığır Rotavirüs Varlığının Araştırılması

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Abstract: Bovine coronavirus (BCoV) and bovine rotavirus (BRV) infections are very common in neonatal diarrhoea of calves which are of the important problems in cattle breeding. In the present study 110 calves with neonatal diarrhoea from Kirikkale and surrounding provinces were investigated for BCoV and BRV presence by RT-PCR in stool samples and positive BRV samples were genotyped by PCR based on VP4 and VP7 genes. In total, 41 samples were BCoV positive (37.27%) and 41 samples were BRV positive (37.27%), whereas 20 samples were positive for both BCoV and BRV (18.18%). According to the results of the study, BCoV and BRV are the neonatal calf diarrhoea agents in calves reared in Kirikkale, Kirsehir, Ankara, Cankiri, Corum, and Yozgat provinces. Genotyping results of positive BRV samples indicated that G6P[5], G10P[5], G10P[11], and G6P[11] genotypes, commonly seen in Turkey, are circulating among these provinces. Detection of these genotypes indicated importance of vaccination against neonatal diarrhoea and selection of vaccine strain.

Keywords: Bovine coronavirus, Bovine rotavirus, Kırıkkale, RT-PCR.

Öz: Sığır koronavirüs (BCoV) ve sığır rotavirüs (BRV) enfeksiyonları, sığır yetiştiriciliğindeki önemli sorunlardan biri olan neonatal buzağı ishallerinin en sık rastlanan viral etkenleri arasında yer almaktadır. Bu çalışmada, Kırıkkale ve çevre illerindeki neonatal ishalli 110 buzağıda BCoV ve BRV etkenleri RT-PCR ile araştırıldı ve pozitif BRV örnekleri PCR ile VP4 ve VP7 genleri temelli olarak genotiplendirildi. RT-PCR sonuçlarına göre toplamda 41 örnek BCoV pozitif (%37,27), 41 örnek BRV pozitif (%37,27) ve 20 örnek hem BCoV hem de BRV pozitif (%18,18) olarak belirlendi. Bu sonuçlara göre Kırıkkale, Kırşehir, Çankırı, Çorum, Yozgat ve Ankara illerinde yetiştirilen buzağılarda BCoV ve BRV, neonatal buzağı ishallerinin etkenleri olarak tespit edildi. BRV genotiplendirme sonuçlarına göre ise bu illerde ülkemizde yaygın olarak görülen genotipler olan G6P[5], G10P[5], G10P[11] ve G6P[11] genotiplerinin sirküle olduğu belirlendi. Bu genotiplerin buzağılarda saptanması, neonatal ishallere karşı aşılamanın ve aşılarda kullanılacak olan suş seçiminin önemini göstermektedir.

Anahtar Kelimeler: Sığır koronavirüs, Sığır rotavirüs, Kırıkkale, RT-PCR.

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Introduction

Neonatal calf diarrhoea is one of the most challenging global problems for dairy and beef cattle industry which is caused by viruses, bacteria and protozoa. Common infectious causes of neonatal calf diarrhoea are Enterotoxic *Escherichia coli, Cryptosporidium parvum*, bovine rotavirus (BRV) and bovine coronavirus (BCoV) (Foster and Smith, 2009; Lorenz and ark., 2011; Azkur and Aksoy, 2018). In a study investigating the diarrhoea of beef and dairy calves, 36.2% of

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enteric pathogens were found to be viral agents (Cho et al., 2013). Among these viral agents, BRV and BCoV are detected in diarrheic calves as 7-80% and 3-79%, respectively (Gomez and Weese, 2017). BCoV and BRV infections, usually affect calves less than 3 weeks old, are found to be responsible for economical losses in cattle breeding including significant effects on the body weight gain of calves (Torres-Medina et al., 1985; Foster and Smith, 2009; Renaud et al., 2020). The most common cause of calf mortality was reported as diarrhoea in Kars province of Turkey (Erdoğan et al., 2009).

BRV is classified in *Rotavirus A* species, *Rotavirus* genus, *Sedoreovirinae* subfamily of *Reoviridae* family, *Reovirales* order, *Riboviria* realm (ICTV, 2020). BRV has 11 segments of double-stranded RNA which encodes 6 structural (VP1, VP2, VP3, VP4, VP6, VP7) and 5 non-structural (NSP1-NSP5/6) proteins. The classifcation system commonly used for Rotaviruses is based on the sequences of the VP7 (G genotype) and VP4 (P genotype) genes (Matthijnssesns et al., 2011). To date 28 G genotypes and 39 P genotypes are determined in Rotavirus A species (Desselberger, 2017), among them G6, G8, G10 and P[1], P[5], P[11] are regarded as the common bovine genotypes worldwide. In Turkey, presence of G6, G8, G10, P[5], P[11] genotypes of BRV were hitherto reported in cattle (Alkan et al., 2010; Karayel et al., 2017; Aydın and Timurkan, 2018).

BCoV is a member of *Betacoronavirus* genus, *Coronaviridae* family of *Nidovirales* order in *Riboviria* realm (ICTV, 2020). BCoV is enveloped, positive-sense single-stranded RNA virus which is associated with respiratory disease in cattle and severe diarrhoea in neonatal calves (Azkur and Aksoy, 2018; Franzo et al., 2020). BCoV infection was reported in Turkey in both enteric and respiratory diseases of cattle as well as calves (Hasoksuz et al., 2005; Pestil et al., 2016; Aydın and Timurkan, 2018).

The aim of the present study was to investigate prevalence of BCoV and BRV in neonatal diarrhoea of calves which are reared in Kirikkale, Kirsehir, Ankara, Cankiri, Corum, and Yozgat provinces and to determine which BRV genotypes are circulating in these provinces.

Materials and Methods

Sampling

The study includes 110 neonatal calves (0-30 days old) which were already patients of Animal Hospital of Kirikkale University that were reared in Kirikkale, Ankara, Kirsehir, Corum, Cankiri, and Yozgat provinces and have acute diarrhoea. Following clinical examination of calves, stool samples were taken in specimen containers from calves by rectal provocation for defecation for routine clinical diagnosis by parasitological examination and rapid diagnostic kits. The stool samples that were taken for routine clinical diagnosis and stored at -20°C were used in the present study.

RNA isolation from stool samples

One gram of each stool samples was taken in sterile centrifuge tubes, diluted 1:5 in phosphate buffered saline (PBS) and homogenized by vortexing. Samples were centrifuged at 2000 rpm for 20 minutes at 4°C (Allegra X100, Beckman Coulter, USA). After centrifugation, the supernatants were transferred into sterile microcentrifuge tubes and stored at -20°C. RNA isolation from supernatants was performed with commercial kit (740956.50, Macherey-Nagel) by following the instructions of the manufacturer. RNA samples were stored at -80°C until use (DF590, Nüve, Turkey).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

The cDNAs from the RNA samples were synthesized within 3 steps. In first step, 4 µl RNA and 2.8 µl dimethylsulphoxide (DMSO) was incubated in 95°C for 5 minutes. In second step, 6 µl sterile distilled water and 1 µl random hexamer primer (PM-301S, Jena Bioscience) were added to the mixture and incubated in 70°C for 5 minutes. In the last step, the tubes were put into cracked ice and 2 µl dNTPs (DN001-0250, GeneDirex), 4 µl

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RT buffer and 1 µl reverse transcriptase (M0253, New England Biolabs) were added to the mixture, and incubated in 25°C for 10 minutes, 37°C for 1 hour and 70°C for 5 minutes.

The cDNA samples were used as template in PCR that is carried out with primers specific for bovine coronavirus (Nucleocapsid protein gene), bovine rotavirus (VP6 gene), and bovine glyceraldehyde 3-phosphate dehydrogenase (GAPDH) genes (Table 1). PCR mixture was containing 3 µl template, 1U Taq DNA polymerase (MB101-0500, GeneDirex), 1.25 mM dNTPs (DN001-0250, GeneDirex), 25 mM MgCl2, 10× PCR buffer, 10 pmol forward primer, 10 pmol reverse primer in 50 µl total volume. PCR was carried out in 35 cycles as following conditon: 94°C for 3 minutes, 94°C for 50 second, 55°C for 50 second, 72°C for 1 minute and 72°C for 10 minutes for final extention. PCR products were visualized under UV transluminator after ethidium bromide stained gel electrophoresis.

Genotyping of Bovine Rotavirus

Rotavirus PCR-positive samples were tested for VP4 ([P] genotyping) and VP7 (G genotyping) genes to determine genotypes.

Rotavirus VP4 ([P] genotyping) genotyping were carried out in 2 steps. For first round for VP4 genotyping, 3 µl cDNA, 1U Taq DNA polymerase, 1.25 mM dNTPs, 25 mM MgCl2, 10× PCR buffer, 10 pmol Con3 forward primer, 10 pmol Con2 reverse primer were mixed in 50 µl total volume. According to electrophoresis results, the samples have 877 bp product were determined as positive whereas the samples do not have product were determined as negative. In the second round of PCR, 1 µl (from VP4 positive samples) or 5 µl (from VP4 negative samples) first round PCR product, 1U Taq DNA polymerase, 1.25 mM dNTPs, 25 mM MgCl2, 10× PCR buffer, 10 pmol P1-P5-P11 forward primer mixture, and 10 pmol Con2 reverse primer were used. All optimized PCR conditions were shown in Table 2. Samples were genotyped as P[1], P[5] and P[11] if 624 bp, 552 bp, and 314 bp products were seen, respectively.

Rotavirus VP7 (G genotyping) genotyping were carried out in 2 steps. For the first round of VP7 genotyping, 3 µl cDNA, 1U Taq DNA polymerase, 1.25 mM dNTPs, 25 mM MgCl2, 10× PCR buffer, 10 pmol INI forward primer, and 10 pmol FIN reverse primer were used in first round PCR. According to electrophoresis results, the samples have 1062 bp product were determined as positive whereas the samples do not have product were determined as negative. In the second round PCR, 1 µl (from VP7 positive samples) or 5 µl (from VP7 negative samples) first round PCR product, 1U Taq DNA polymerase, 1.25 mM dNTPs, 25 mM MgCl2, 10× PCR buffer, 10 pmol DT6 primer, 10 pmol HT8 primer, 10 pmol ET10 primer, and 10 pmol INI primer were mixed. All optimized PCR conditions were shown in Table 2. Samples were genotyped as G6, G8 and G10 if 499 bp, 273 bp, and 714 bp products were seen, respectively.

Table 1. Primers used for detection of bovine rotavirus, bovine coronavirus and bovine GAPDH.

Target	Primer	Sequence (5'→3')	Product
Bovine rotavirus	BRV - F	GTTTTCCAAGAGTDATHAHYTCAGC	214 bp
	BRV - R	ACCGCTGGTGTCATGTTTGG	
Bovine coronavirus	BCoV - F	CGATCAGTCCGACCAATCTA	597 bp
	BCoV - R	GAGGTAGGGGTTCTGTTGCC	
Bovine GAPDH	GAPDH - F	GGTCACCAGGGCTGCTTTTA	222 bp
	GAPDH - R	CCAGCATCACCCCACTTGAT	

F: Forward primer, R: Reverse primer, bp: base pair.

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Table 2. Primers used for genotyping of bovine rotavirus and PCR conditions.

Gene	Primer	Sequence (5'→3')	Product	PCR round and number of cycles	PCR conditions
VP4	Con3 - F	TGGCTTCGCTCATTTATAGACA	877 bp	1st 35 cycles	94°C 3 min 94°C 1 min 54°C 1 min 72°C 1 min 72°C 10 min
	Con2 - R	ATTTCGGACCATTTATAACC			
	P1- F	ACCAACGAACGCGGGGGTG	624 bp	2nd 30 cycles	94°C 3 min 94°C 30 sec 47°C 30 sec 72°C 45 sec 72°C 10 min
	P5 - F	RCCAGGTGTCRCATCAGAG	552 bp		
	P11 - F	GGAACGTATTCTAATCCGGTG	314 bp		
VP7	INI - F	GGCTTTAAAAGMGAGAAWTT	1062 bp	1st 35 cycles	94°C 3 min 94°C 1 min 47°C 1 min 72°C 2 min 72°C 10 min
	FIN - R	GGTCWCATCATACAAYTCT			
	DT6 - R	CTAGTTCCTGTGTAGAATC	499 bp	2nd 30 cycles	94°C 3 min 94°C 30 sec 42°C 30 sec 72°C 45 sec 72°C 10 min
	HT8 - R	CGGTTCCGGATTAGACAC	273 bp		
	ET10 - R	TTCAGCCGTTGCGACTTC	714 bp		

F: Forward primer, R: Reverse primer, bp: base pair, min: minutes, sec: seconds.

Table 3. Sampling provinces and cattle breeds included in the study.

		Kirikkale	Kirsehir	Cankiri	Corum	Yozgat	Ankara	Total
Breed	**Simmental**	43	13	4	9	1	8	78
	Montofon	5	5	0	1	1	0	12
	Holstein	3	0	1	0	0	1	5
	Crossbred	4	4	4	0	2	1	15
	Total	**55**	**22**	**9**	**10**	**4**	**10**	**110**

Results

A total of 110 stool samples were included in the present study from calves with neonatal diarrhea which were brought from Kirikkale, Kirsehir, Ankara, Cankiri, Corum, and Yozgat provinces to the Kirikkale University Veterinary Faculty Animal Hospital (Table 3).

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RT-PCR was carried out for screening of BCoV (nucleocapsid protein gene) and BRV (VP6 gene) from 110 stool samples. Samples have 597 bp and 214 bp products were evaluated as BCoV- and BRV-positive, respectively (Figure 1). Forty-one of 110 samples were determined as BCoV positive (37.27%) and 41 samples were BRV positive (37.27%). Distribution of BCoV and BRV positive samples among provinces were given in Table 4. Total of 20 samples were detected as BCoV and BRV double positive (18.18%).

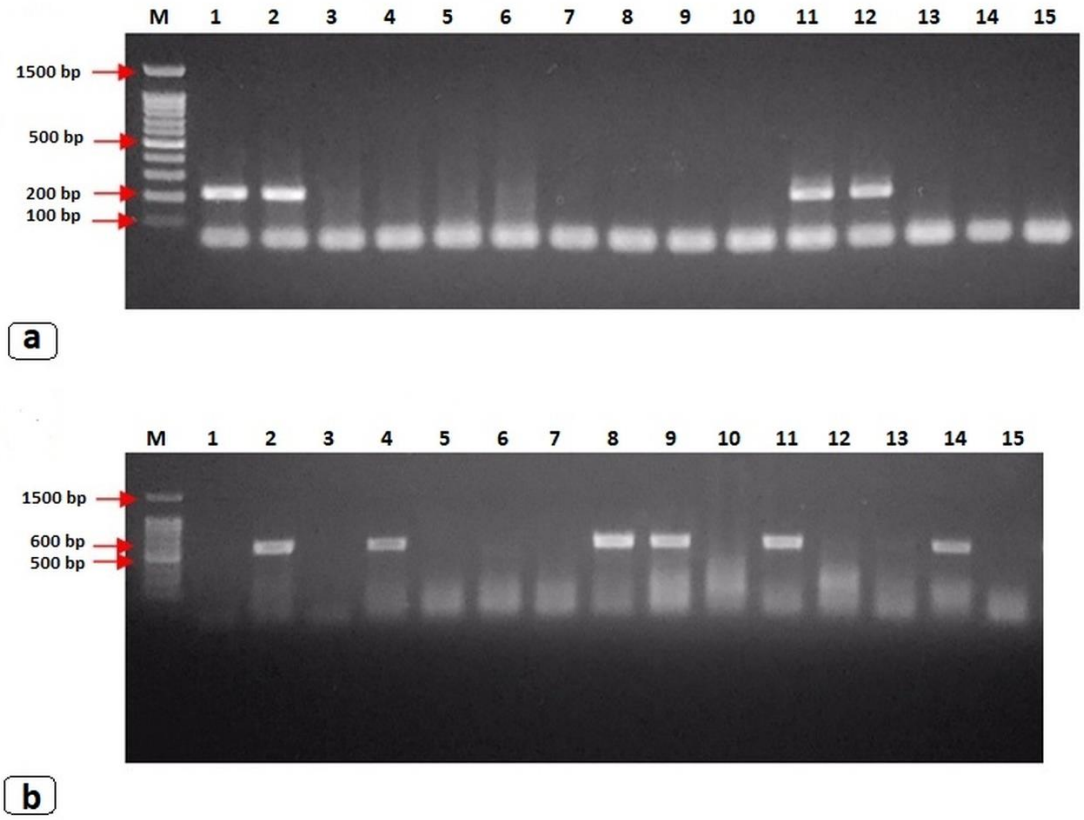

Figure 1. RT-PCR results of **a:** bovine rotavirus (214 bp) and **b:** bovine coronavirus (597 bp). M: DNA marker, 1-15: samples.

Table 4. Number of positive samples for bovine rotavirus (BRV) and bovine coronavirus (BCoV) among provinces.

	Kirikkale	Kirsehir	Cankiri	Corum	Yozgat	Ankara	Total
BRV	19	6	4	5	2	5	41
BCoV	23	8	1	3	1	5	41

Following screening of samples for presence of BCoV and BRV by RT-PCR, BRV-positive samples were analysed for genotyping based on VP4 and VP7 genes. Firstly P genotyping based on VP4 gene was carried out and whole VP4 gene of BRV was amplified (877 bp). The samples have 624 bp, 552 bp, and 314 bp products were characterised as P[1], P[5], and P[11], respectively. According to results of VP4 genotyping, 31 samples were P[5], 7 samples were P[11], 1 sample was P[5]+P[11], and 2 samples were suspected out

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of 41 BRV-positive samples (**Figure 2**). None of the samples were genotype P[1].

VP7 gene based PCR to determine G genotypes of BRV-positive samples were implemented and whole length VP7 gene (1062 bp) was amplified. Following amplification of VP7 gene, the samples have 499 bp, 273 bp, and 714 bp products were determined as G6, G8, and G10, respectively. According to results of VP7 genotyping, 13 samples were G6, 6 samples were G10, 21 samples were G6+G10, 1 sample was suspected out of 41 BRV-positive samples (Figure 3). G8 genotype was not determined in any samples.

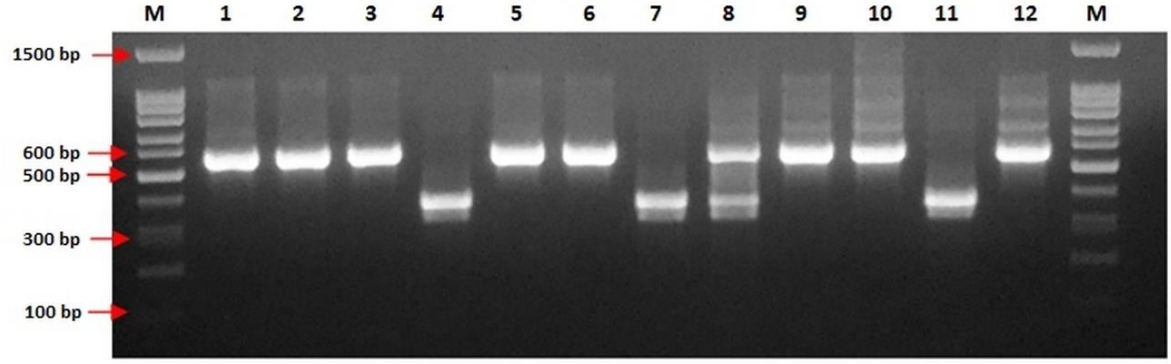

Figure 2. Bovine rotavirus VP4 genotyping results. The samples have 552 bp and 314 bp products were determined as P[5] and P[11], respectively. M: DNA marker, 1-12: samples.

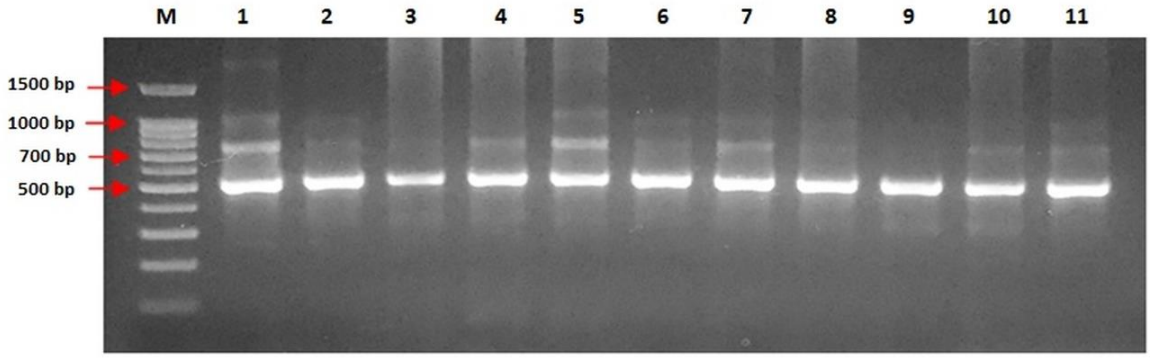

Figure 3. Bovine rotavirus VP7 genotyping results. The samples have 499 bp and 714 bp products were determined as G6 and G10, respectively. M: DNA marker, 1-11: samples.

Genotyping results of BRV-positive samples indicated that 11 samples were G6P[5] and 6 samples were G10P[11]. According to results some samples have two genotypes (19 samples were G6P[5]+G10P[5], 1 sample was G6P[11]+G10P[11]) and one sample has four genotypes (G6P[5] + G10P[5] + G6P[11] + G10P[11]). Three samples were determined as suspected by PCR, 2 out of 3 suspected samples were only genotyped as G6, and 1 sample was only genotyped as P[5].

As a result of the study, it was determined that G6P[5], G6P[11], G10P[5] and G10P[11] genotypes were circulated in diarrhea cases of neonatal calves reared in Kirikkale, Kirsehir, Corum, Cankiri, Yozgat and Ankara provinces (Table 5). In total BRV genotype G6P[5], G6P[11], G10P[5], and G10P[11] were detected in 31, 2, 20, and 8 samples in consideration of double- and multi-positive samples.

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Table 5. Distribution of bovine rotavirus (BRV) genotypes among provinces.

		Kirikkale	Kirsehir	Cankiri	Corum	Yozgat	Ankara	Total
BRV genotypes	**G6P[5]**	17	4	3	4	1	2	31
	G10P[5]	10	4	2	3	1	-	20
	G10P[11]	2	2	1	1	1	1	8
	G6P[11]	1	1	-	-	-	-	2
	G6P[?]*	-	1	-	-	-	1	2
	G?P[5]**	-	-	-	-	-	1	1

**VP4 genotyping can not be determined. **VP7 genotyping can not be determined.

Discussion

Bovine rotavirus (BRV) and bovine coronavirus (BCoV) infections are the most important viral agents of neonatal calf diarrhoea which is economically significant disease for cattle industry because of mortality, cost of medication, labour needed to treat sick calves, delayed growth of calves, etc (Azkur and Aksoy 2018). Due to importance of BRV and BCoV in neonatal calf diarrhoea, in the present study, BRV and BCoV presence was investigated by RT-PCR in stool samples of neonatal diarrhoeic calves reared in Kirikkale, Kirsehir, Ankara, Cankiri, Corum, and Yozgat and circulating genotypes of BRV were determined.

BRV and BCoV presence in Turkey have been reported in many researches, determining prevalence of these infections by serological and molecular methods in numerous provinces. Some researchers used ELISA to detect BRV and/or BCoV in diarrhoea of neonatal calves (Hasoksuz et al., 2005; Çabalar et al., 2007), whereas rapid diagnostic kits are another tool for antibody detection against BRV and BCoV (Al and Balıkçı, 2012; Altuğ et al., 2013). RT-PCR and sequencing are molecular methods for both detection and phylogenetic studies of BRV and BCoV, and many studies had been submitted local strains of BRV and BCoV to Genbank (Alkan et al., 2010; Karayel et al., 2017; Aydın and Timurkan, 2018). In this study, RT-PCR were carried out to determine BRV and BCoV in stool samples of calves and which genotypes of BRV were circulating in the study area, however positive BRV and BCoV samples were not sequenced due to financial reasons.

Studies for BRV and BCoV infection in Turkey showed wide range of prevalence rates. In a study conducted in Erzurum, BRV positivity was found to be 6.1% and BCoV positivity was 12.1% (Aydın and Timurkan, 2018). BCoV positivity in many provinces of Marmara region was reported as 2% (Pestil et al., 2016). BCoV infection in diarrhoeic calves was determined as 10.8% in a study involving many provinces of Turkey including Ankara which is also studied in the present study (Alkan et al., 2011). In the present study, both BCoV positivity and BRV positivity was determined as 37.27% and 18.18% of samples were detected as positive for both BCoV and BRV. The higher rate of positivity could be affected by many factors, such as sampling region, season, sample size, vaccination status of cows, housing of the calves, etc.

The previous studies showed that main genotypes of BRV in many provinces of Turkey are G6P[5], G6P[11], G10P[5], G10P[11] (Alkan et al., 2010; Aydın and Timurkan, 2018), and distinctively G8P[5] genotype of BRV was reported in Amasya (Karayel et al., 2017). In the present study, genotyping of BRV indicated that G6P[5], G6P[11], G10P[5], and G10P[11] genotypes are circulating among Kirikkale, Kirsehir, Ankara, Cankiri, Corum, and Yozgat provinces, consistent

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with the results of the previous studies (Alkan et al., 2010; Aydın and Timurkan, 2018).

Although natural infection of BRV and BCoV in field are very common and most of cows are found to be seropositive, studies showed that antibody titers in milk decrease to non-protective levels. Thus, maternal antibody level in colostrum can be increased by vaccination of cows against BRoV and BCoV in order to protect newborn calves. BRV and BCoV vaccination, usually in combination with *E. coli*, is administrated to pregnant cows in order to induce immunity and maternal antibody production in dams which is then transferred to calves with colostrum. Protection level of the vaccine depends on identity of the vaccine strain and the field strain, suggesting that if the vaccines do not contain field strains, vaccination may not be protective against circulating strains (Gomez and Weese 2017). Vaccines used for BRV and BCoV in Turkey contain G6, G10, P[1], P[5] genotypes of BRV however circulating BRV genotypes in Turkey are G6, G8, G10, P[5], and P[11], which means vaccines in practice could not cover the circulating genotypes and may not be protective for natural field infection.

In the conclusion, BRV and BCoV infections are widely distributed in Kirikkale, Kirsehir, Ankara, Cankiri, Corum, and Yozgat provinces and seem very important agents of neonatal calf diarrhoea in this region. Circulating BRV genotypes are G6P[5], G6P[11], G10P[5], and G10P[11] in these provinces, and this data showed that field strains are different from vaccine strains. Considering the strains available in the BRV and BCoV vaccines used against calf diarrhea in Turkey, this study shows that the vaccine strains are not compatible with the circulating strains in the field and therefore the calves cannot be protected against neonatal calf diarrhea even if they take colostrum. In this context, the fact that the vaccines used in Turkey should contain up-to-date field strains which will prevent calf diarrhea and accompanying economic losses.

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References

Al, M., Balıkçı, E., 2012. Neonatal ishalli buzağılarda rotavirus, Coronavirus, E. Coli K99 ve Cryptosporodium parvum'un hızlı test kitleri ile teşhisi ve enterojen ile maternal immünite ilişkisi. Fırat Üniversitesi Sağlık Bilimleri Dergisi 26, 73-78.

Alkan, F., Ozkul, A., Bilge-Dagalp, S., Karaoglu, T., Oguzoglu, T.C., Caliskan, E., Burgu, I., 2011. The detection and genetic characterization based on the S1 gene region of BCoVs from respiratory and enteric infections in Turkey. Transboundary and Emerging Diseases 58, 179-185.

Alkan, F., Ozkul, A., Oguzoglu, T.C., Timurkan, M.O., Caliskan, E., Martella, V., Burgu, I., 2010. Distribution of G (VP7) and P (VP4) genotypes of group A bovine rotaviruses from Turkish calves with diarrhea, 1997-2008. Veterinary Microbiology 141, 231-237.

Altuğ, N., Yüksek, N., Özkan, C., Keleş, İ., Başbuğan, Y., Ağaoğlu, Z.T., Kaya, A., Akgül, Y., 2013. Neonatal buzağı ishallerinin immunokromotografik test kitleri ile hızlı etiyolojik teşhisi. Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi 24, 123-128.

Aydın, H., Timurkan, M.Ö., 2018. Buzağı ishallerinde coronavirusun nukleoprotein gen ve rtavirusun VP7/VP4 gen bölgelerinin kısmi sekansı ve filogenetik analizi. Atatürk Üniversitesi Veteriner Bilimleri Dergisi 13, 211-218.

Azkur, A.K., Aksoy, E., 2018. Buzağı hastalıklarında koruyucu önlemler. Lalahan Hayvancılık Araştırma Enstitüsü Dergisi 58, 56-63.

Cho, Y.I., Han, J.I., Wang, C., Cooper, V., Schwartz, K., Engelken, T., Yoon, K-J., 2013. Case-control study of microbiological etiology associated with calf diarrhea. Veterinary Microbiology 166, 375-385.

Çabalar, M., Kaya, A., Arslan, S., 2007. Yeni doğan buzağıların ishal olgularında rotavirus ve coronavirus araştırılması. Veteriner Bilimleri Dergisi Eurasian Journal of Veterinary Sciences 23, 103-106.

Desselberger, U., 2017. Differences of rotavirus vaccine effectiveness by country: likely causes and contributing factors. Pathogens 6, 65.

Erdoğan, H.M., Ünver, A., Çitil, M., Güneş, V., Arslan, M.Ö., Tuzcu, M., Gökce, H.İ., 2009. Dairy farming in Kars district, Turkey: III. Neonatal calf health. Turkish Journal of Veterinary and Animal Sciences 33, 185-192.

Foster, D.M., Smith, G.W., 2009. Pathophysiology of diarrhea in calves. Veterinary Clinics of North America: Food Animal Practice 25, 13-36.

Franzo, G., Drigo, M., Legnardi, M., Grassi, L., Pasotto, D., Menandro, M.L., Cecchinato, M., Tucciarone, C.M., 2020. Bovine coronavirus: Variability, evolution, and dispersal patterns of a no longer neglected Betacoronavirus. Viruses 12, 1285.

Gomez, D.E., Weese, J.S., 2017. Viral enteritis in calves. Canadian Veterinary Journal 58, 1267-1274.

Hasoksuz, M., Kayar, A., Dodurka, T., Ilgaz, A.A., 2005. Detection of respiratory and enteric shedding of bovine coronaviruses in cattle in Northwestern Turkey. Acta Veterinaria Hungarica 53, 137-146.

ICTV, 2020. Virus Taxonomy: 2020 Release. https://talk.ictvonline.org/taxonomy/ (Erişim 15.10.2021)

Karayel, I., Fehér, E., Marton, S., Coskun, N., Bányai, K., Alkan, F., 2017. Putative vaccine breakthrough event associated with heterotypic rotavirüs infection in newborn calves, Turkey, 2015. Veterinary Microbiology 201, 7-13.

Lorenz, I., Fagan, J., More, S.J., 2011. Calf health from birth to weaning. II. Management of diarrhoea in pre-weaned calves. Irish Veterinary Journal 64, 9.

Matthijnssens, J., Ciarlet, M., McDonald, S.M., Attoui, H., Bányai, K., Brister, J.R., Buesa, J., Esona, M.D., Estes, M.K., Gentsch, J.R., Iturriza-Gómara, M., Johne, R., Kirkwood, C.D., Martella, V., Mertens, P.P., Nakagomi, O., Parreño, V., Rahman, M., Ruggeri, F.M., Saif, L.J., Santos, N., Steyer, A., Taniguchi, K., Patton, J.T., Desselberger, U., Van Ranst, M., 2011. Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). Archives of Virology 156, 1397-1413.

Pestil, Z., Gülyaz, V., Hasöksüz, M., 2016. The determination and pathogenicity of bovine coronavirus in newborn calves with diarrhea in Marmara region. Etlik Veteriner Mikrobiyoloji Dergisi 27, 16-20.

Renaud, D.L., Rot, C., Marshall, J., Steele, M.A., 2020. The effect of Cryptosporidium parvum, rotavirus, and coronavirus infection on the health and performance of male dairy calves. Journal of Dairy Science S0022-0302, 31037-7.

Torres-Medina, A., Schlafer, D.H., Mebus, C.A., 1985. Rotaviral and coronaviral diarrhea. Veterinary Clinics of North America: Food Animal Practice 1, 471-493.

MAKU J. Health Sci. Inst. 2021, 9(3): 47-52.
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Research Article / Araştırma Makalesi

Effects of Sucrose (Sugar) as Inoculant on Physical Quality, Fermentation Profile and Relative Feed Value of Alfalfa Silage at Different Ensiling Time

Sükroz (Şeker) İnokulantının Farklı Silolama Dönemlerindeki Yonca Silajının Fiziksel Kalitesi, Fermantasyon Profili ve Nispi Yem Değeri Üzerine Etkisi

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Abstract: This study was conducted to determine effects of sucrose (sugar) addition on physical quality, fermentation profile and relative feed value of alfalfa silage at different ensiling time. Silage quality was evaluated based on inoculant supplementation (C; no additive and S: sucrose additive) and four ensiling periods (7, 14, 30 and 60 days). The color and structure scores were unchanged but the smell score increased significantly by sucrose inoculant. Sucrose supplementation significantly decreased pH value in comparison to the control group. Significant change in Flieg point of alfalfa silage was observed between the groups. Relative feed value significantly increased in the sucrose group at d 7 and 14 of ensiling, but remained unaffected at d 30 and 60 of ensiling. As a result, addition of sucrose improved silage quality at different fermentation time.

Keywords: Alfalfa silage, Silage quality, Sucrose.

Öz: Bu çalışma, sükroz (şeker) ilavesinin farklı fermantasyon zamanlarda yonca silajının fiziksel kalite, fermantasyon profile ve nispi yem değerleri üzerine olan etkilerini belirlemek üzere yapılmıştır. Silaj kalitesi iki grup (C: katkısız; S: şeker ilaveli) ve dört farklı silolama zamanı (7, 14, 30 and 60 günler) olarak değerlendirilmiştir. Sükroz ilavesi ile koku ve strüktür skoru etkilenmemiş ancak koku skoru önemli düzeyde artmıştır. Kontrol grubu ile karşılaştırıldığında sükroz ilavesi ile pH değeri önemli düzeyde azalmıştır. Gruplar arasında Flieg puanın değişim önemli bulunmuştur. Nispi yem değeri sükroz grubunda silolamanın 7 ve 14. gününde önemli düzeyde artmış, ancak silolamanın 30 ve 60. günlerinde etkilenmemiştir. Sonuç olarak, sükroz ilavesi farklı zamanlarda silaj kalitesini iyileştirmiştir.

Anahtar Kelimeler: Yonca silajı, Silaj kalitesi, Sükroz..

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Introduction

Alfalfa (*Medicago sativa* L.) is a perennial plant and can supply green fodder continuously for 4-5 years from the same sowing. Alfalfa is flavorfully consumed by all kinds of livestock, since it yields nutritious and palatable green fodder, providing with 16–25% crude protein (with 72% digestibility) and 20-30% fiber. It is naturally high in many essential minerals and vitamins, including; calcium (1.5%), magnesium, potassium, iron, A, D, E, K, and B vitamins (Patra and Paul, 2019). According to the Turkish Statistical Institute, an average of 19.290 million tons of alfalfa green hay were produced in 2020 in Turkey (TUIK, 2020). Alfalfa hay or silage is used in ruminants rations as a forage and it is important for the animal nutrition because of its high protein value (Aktürk and Gümüş, 2020). However, the protein value of alfalfa hay can decrease in the production, transport and storage processes (Gao et al., 2021). Due to the irregular rainfall, the wilting of legumes such as alfalfa hay, clover hay, trefoil hay etc., has become gradually difficult (Unal et al., 2012); therefore, making of alfalfa silage is an alternative method. Ensiling is a natural fermentation process of forage conservation to improve the nutritional value and extend the storage time (Ni et al., 2017). During this process, water-soluble carbohydrates (WSC) are converted into lactic acid (LA) via

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epiphytic lactic acid bacteria (LAB) resulting in a decline of pH (Yan et al., 2019). It is difficult to preserve quality of alfalfa silage due to its high protein content, greater buffer capacity and quite low sugar content (Ergin and Gümüş, 2020). Silage additives such as glucose, sucrose, and molasses have been used to decrease pH level that increased the number substrates for the growth of lactic acid bacteria (LAB) as well as improved lactic acid concentration in the silage (Li et al, 2014). This study aimed to examine the effects of sucrose (sugar) addition on physical quality, fermentation profile and relative feed value of alfalfa silage at different ensiling time.

Materials and Methods

The study was conducted at the Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, located at 36° 53' North latitude and 30° 53' East longitude and 950 m above sea level. Burdur is the transition region in the inner part of the western Mediterranean. The experiment was conducted in a completely randomized design using two experimental groups as CON (control), with no inoculants; S (Sucrose), with 3% sucrose as an inoculant (calculated based on the fresh weight of alfalfa). The inclusion levels of sucrose inoculant were based on a proper review of the existing literature. Following harvesting, the fresh alfalfa samples were chopped into small pieces (~1.5-2 cm) by pruning shears for ensiling and the sucrose was applied to fresh alfalfa in the plastic basin. About 900 g of chopped alfalfa (fresh weight) was compressed by hand into a 1-L jar (100 mm diameter × 170 mm height). A total of 10 jars (5 jars per experimental group) were prepared and stored at ambient temperature (16 ± 2ºC). Five silos from each group were opened for the analysis of physical quality (smell, color, structure of alfalfa silage), fermentation quality (pH and Flieg point), nutritive value (DDM, DMI, RFV) on 7, 14, 30, and 60 d of ensiling. Physical quality analysis was assessed by using DLG scoring system (DLG, 1997). Each alfalfa silage samples were carefully opened and scored by 3 experts in terms of colour point (0-2), structure point (0-4), and scent point (0-14) of the silage. According to score; silage was divided into the quality classes as Excellent (16-20 points); Good (10-15); Mid (5-9), and too bad (0-4). For the assessment of pH value, a 25 g fresh silage sample was blended with 100 ml distilled water in a mixer for 4-5 min and filtered through a cheesecloth. The pH value was measured with a glass electrode pH meter (*ECPlaza, Guro-gu, Seoul, Korea*). Fleig point (Dong et al., 2017) was calculated by using pH and DM values of alfalfa silage at different days of ensiling with the following equation: Flieg's point = 220 + (2 × DM – 15) – (40 × pH). Digestible dry matter (DDM) was determined by using ADF content of alfalfa silage [DDM%= 88.9 – (0.779 x ADF%)]. Then dry matter intake (DMI) was measured by using NDF content of alfalfa silage [DMI%=120/NDF %)]. Relative feed value was calculated (Redfarn and Zhang, 2014) by using DDM and DMI [RFV = DDM% x DMI% x 0.775]. The statistical analyses were conducted to the International Business Machines (IBM) for the Social Sciences (SPSS), version 22 (IBM, SPSS Statistics, 2022). The data were analyzed by independent-samples t-test to determine the effects of sucrose (sugar) addition on physical quality, fermentation profile and relative feed value of alfalfa silage at different fermentation time. The level of significance was taken as $P<0.05$ (Dawson and Trapp, 2001).

Results

The smell score of silage was affected ($P<0.05$) by sugar inoculant at d 14, 30 and 60 except at d 7 of ensiling. Colour score of silage remained unaffected all treatments d of ensiling ($P<0.05$). Structure score of silage was not affected ($P>0.05$) by sucrose inoculant regardless of the days. The total score of silage was significantly increased by sucrose addition at d 14, 30 and 60 of ensiling, but it was not affected at d 7 of ensiling (Figure 1). The quality of alfalfa silage treated with sucrose was determined as 'excellent' all days. It was defined on 7^{th}, 30^{th} and 60^{th} days as "excellent", but on 14^{th} d as "good". There was a significantly different effect ($P<0.01$) between groups for silage pH. The pH value was in parallel decreases in all silages during the fermentation. Regardless of the days, the pH value was lower in the sucrose group in comparison to the CON group. Irrespective of the days, sugar additives remarkably increased

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(P<0.01) the Flieg point due to the increases in DM and decreases in pH. At the end of the experiment, Flieg point of silage in the CON and S group were 25.97 and 76.52, respectively (Figure 2). At d 7 and 14 of ensiling, RFV was significantly higher in silages prepared with sugar inoculant, but unaffected at d 30 and 60 of ensiling (Figure 3).

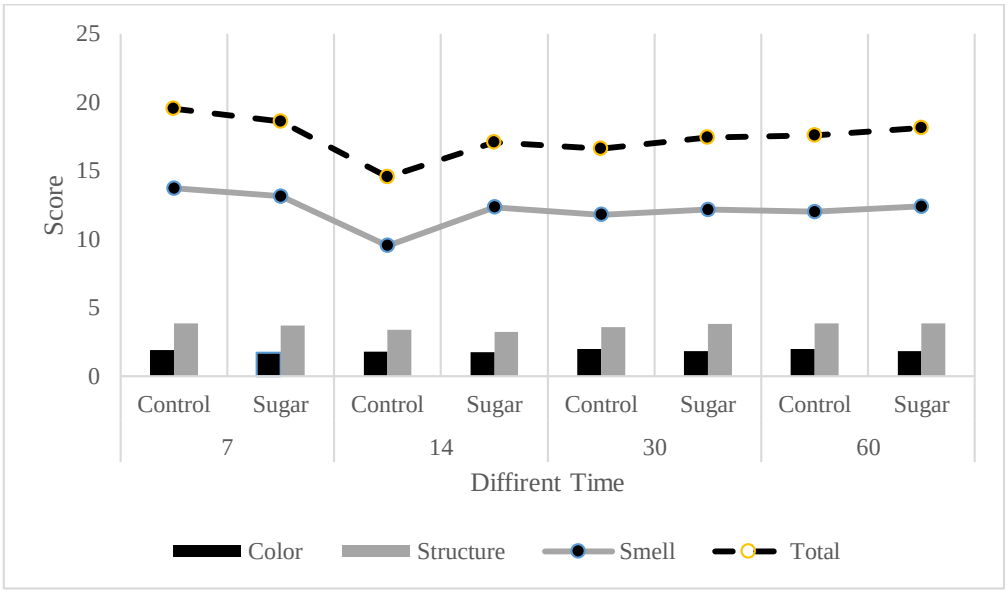

Figure 1. Color, structure, smell and total score of alfalfa silage

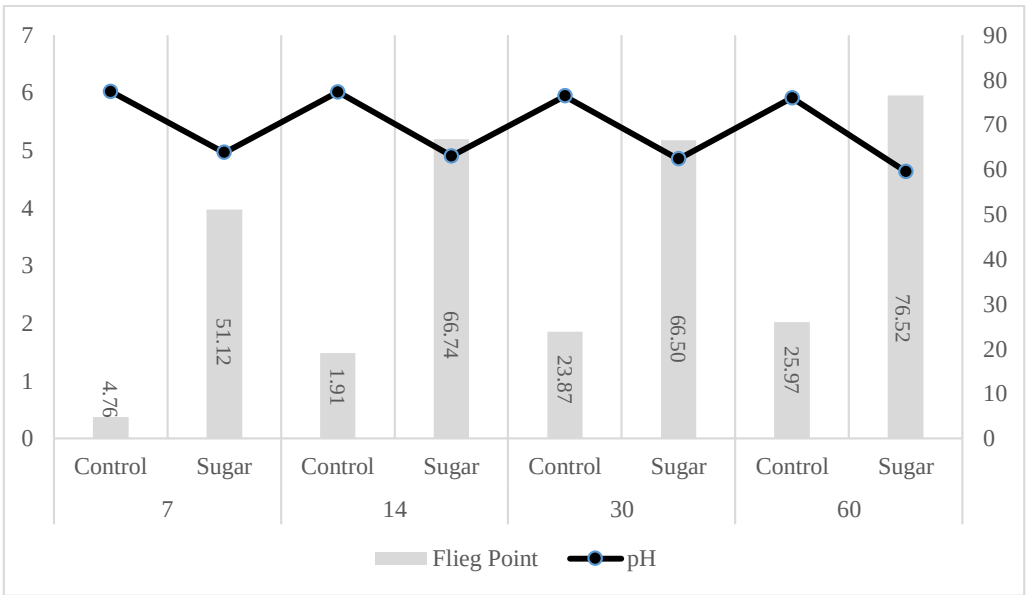

Figure 2. Flieg point and pH value of alfalfa silage

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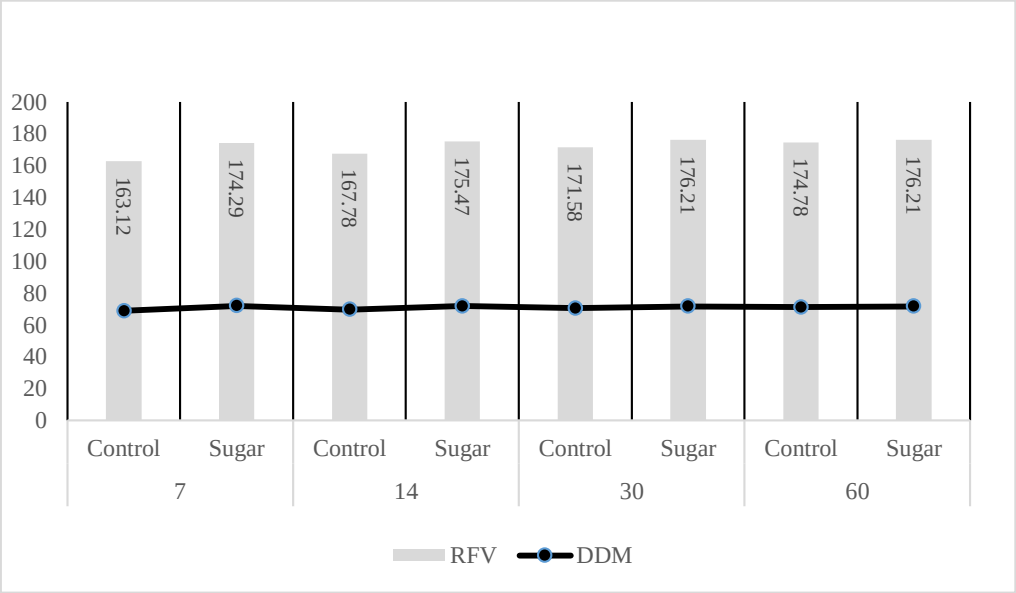

Figure 3. Relative feed value and digestibility dry matter of alfalfa silage

Discussion

Silage making is a significant technique in animal nutrition and it allows for longer storage time of forage. The assessment of smell, color, and structure score of silages are the best simple methods to evaluate the physical quality of silage during the fermentation process. This evaluation system has been commonly used for years in silage because it is an inexpensive and quick method (Zhao et al., 2019). Open green color, aromatic scent, such as bouquet and good structure, are desired in a high-quality silage (Muck et al., 2018). In the present study, smell score of alfalfa silage was significantly improved by sucrose inoculant throughout the ensiling period (Figure 1). Ergin and Gümüş (2020) stated that LAB inoculant significantly increased the smell score at d 60 of ensiling. Turan and Önenç (2018) stated that no change in smell score of alfalfa silage was observed between cumin essential oil supplemented and unsupplemented silages. The smell score of silage ranged from 12.75-14.00 point (Topçu and Özkan, 2021). Color and structure score of alfalfa silage remained unaffected irrespective of the days (Figure 1). Also, the mean value of color score is in accordance with those verified in other silage study by addition sugar under similar conditions of the current study, where the values ranged from 1.74 and 2.00 point (Gümüş et al., 2020). Addition of sucrose did not affect the structure score. The findings of this study were in agreement with results of previous studies conducted on alfalfa silage (Turan and önenç, 2018; Ergin and Gümüş, 2020) and lenox silage (Gümüş et al., 2020). As expected, the increase in the total score in the sucrose-supplemented silages may be associated with the higher smell score. Gümüş et al. (2020) also reported similar finding for total score in silages with treated sucrose. Silage quality depends on certain factors such as forage and inoculant type, environmental temperature, nutritive value, and type of silage (Yan et al., 2019). Acidity profile and power (pK_a) are an essential factor to reflect a good silage fermentation. There was a significantly decrease in the pH value in S-silages in comparison to that of C-silages throughout the research (Figure 2). The outcomes of the current study were in line with Li et al. (2014), who found significant influence of sucrose addition on pH value. The addition of sucrose in silage leads to a higher availability of WSC (Kung et al., 2018), which may have positive effects on the pH value of silage, increasing the LAB number and improving the LAB growth. Lower pH value due to increased LAB population by supplementation of sucrose contributed to higher Flieg point (Figure 2). A strong linear relationship between Flieg point and silage quality has been identified in previous results (Toruk and Kayışlıoğlu, 2008; Turan and Önenç, 2018). Flieg point was positively correlated with dry matter; whereas, it was negatively correlated

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with pH value of silage (Gümüş et al., 2020). This theory might be supportive of the present study, namely increased DM in silage treated with sucrose, where the values ranged from 28.31% and 28.42% of DM at d 60 of ensiling. RFV has been commonly used by the United States to determine the feed value of alfalfa. Sucrose additive significantly increased RFV at d 7 and 14 of ensiling; however, it was unaffected at d 30 and 60 of ensiling (Figure 3). These outcomes were in line with the results of reported by Baba et al. (2018) that the addition of molasses to the silages improved RFV. Importantly, positive effects were stated with addition of silage additives to alfalfa silage. Supplemented sucrose in silage has been indicated to be advantageous due to increased silage WSC density (Kung et al., 2018), reduced NH_3-N content by reducing breakdown of protein, (Gümüş et al., 2020), and improved LAB growth and number.

Conclusion

The addition of sucrose improved the silage quality and affected fermentation profile and relative feed value of alfalfa silage. Silages treated with sucrose were well in color, aromatic scent, and good structure compared to control. Lower pH value and higher Flieg point and RFV were observed in silages treated with sugar. This research showed the positive effects of sucrose addition on silage quality.

References

Aktürk B., Gümüş H. 2020. Effects of lactic acid bacteria inoculant on quality, fermentation profile and nutritive value of alfalfa silage at different ensiling period. Ankara Üniversitesi Veteriner Fakültesi Dergisi 37, 281-287

Baba, M., Nasir, A., Kabiru, A., Umar, G. A. 2018. Effects of additives and their levels of inclusion on nutritive value of silage made from elephant grass (Pennisetum purpureum). Nigerian Journal of Animal Production 45(2), 352-362.

Dawson, B., Trapp, R.G. 2001. Basic and clinical biostatistics. 3rd ed., Lange 305 Medical Books/ McGraw-Hill Medical Publishing Division, New York, USA, 89, 306 131-153.

DLG, 1997. DLG-Schlussel zur Beurteilung der Garqualita ̈t von Grunfuttersilagen auf der Basis der chemischen Untersuchung. [DLG-pattern for the Evaluation of the Fermentation Quality of Grass Silages on the basis of Chemical Analyses]. Frankfurta. M.: Deutsche Landwirtschafts-Gesellschaft.

Dong, Z., Yuan, X., Wen, A., Desta, S. T., Shao, T. 2017. Effects of calcium propionate on the fermentation quality and aerobic stability of alfalfa silage. Asian-Australasian Journal of Animal Sciences 30(9), 1278.

Ergin S., Gümüş H. 2020. Silage quality, fermentation dynamics and chemical composition of alfalfa silage prepared with salt and lactic acid bacteria inoculants. Animal Nutrition and Feed Technology 20, 367-380

Gao, R., Wang B., Jia T., Luo Y., Yu Z. 2021. Effects of different carbohydrate sources on alfalfa silage quality at different ensiling days. Agriculture 11(1), 58.

Gümüş, H., Oğuz, F.K., Oğuz, M.N., Buğdaycı, K.E., Kuter, E. 2020. The Effects of Different Additives on the Fermentation and Physical Characteristics of Lenox Silage. Journal of Faculty of Veterinary Medicine, Erciyes University 17, 39-44.

Kung Jr, L., Shaver, R.D., Grant, R.J., Schmidt, R.J. 2018. Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. Journal of Dairy Science 101(5), 4020-4033.

Li, M., Zi, X., Zhou, H., Hou, G., Cai, Y. 2014. Effects of sucrose, glucose, molasses and cellulase on fermentation quality and in vitro gas production of king grass silage. Animal Feed Science and Technology 197, 206-212.

Muck, R.E., Nadeau, E.M.G., McAllister, T.A., Contreras-Govea, F.E., Santos, M.C., Kung Jr, L. 2018. Silage review: Recent advances and future uses of silage additives. Journal of Dairy Science 101(5), 3980-4000.

Ni, K., Wang, F., Zhu, B., Yang, J., Zhou, G., Pan, Y., Tao, Y. and Zhong, J. 2017. Effects of lactic acid bacteria and molasses additives on the microbial community and fermentation quality of soybean silage. Bioresource Technology 238, 706-715.

Patra, S.P, Paul T. 2019. Lucerne. In: Hedayetullah M., Zaman P. (Eds.), Forage Crops of the World. Apple Academic Press, Canada, pp. 231-243

Redfearn, D., Zhang, H., Caddel, J. 2014. Forage Quality Interpretations. Oklahoma Cooperative Extension Service, PSS-2117. Available at http://factsheets.okstate.edu/ (Accessed October 2, 2021).

Topçu, G.D., Özkan, S.S. 2021. Quality properties of sunn hemp *(Crotalaria juncea L.)* and maize (Zea mays L.) silages. Tropical Grasslands-Forrajes Tropicales 9(3), 315–320.

Toruk, F., Kayişoğlu, B. 2008. Effect of applied vacuum of silage package machine on silage quality. Journal of Agricultural Mach Sciences 4, 355-60.

TUIK. 2020. Turkish Statistical Institute. www.tuik.gov.tr. (Erişim 17.10.2021)

Turan A., Önenç S.S. 2018. Effect of cumin essential oil usage on fermentation quality, aerobic stability and in vitro digetibility of alfalfa silage. Asian-Australasian Journal of Animal Sciences 31, 1252-1258.

Unal, Y.S., Deniz, A., Toros, H. and Incecik, S. 2012. Temporal and spatial patterns of precipitation variability for annual, wet, and dry seasons in Turkey. International Journal of Climatology 32, 392-405.

Yan, Y., Li, X., Guan, H., Huang, L., Ma, X., Peng, Y., Li, Z., Nie, G., Zhou, J., Yang, W., Cai, Y. and Zhang, X. 2019. Microbial community and fermentation characteristic of Italian ryegrass silage prepared with corn stover and lactic acid bacteria. Bioresource Technology 279, 166-173.

Zhao, J., Dong, Z., Li, J., Chen, L., Bai, Y., Jia, Y., Shao, T. 2019. Effects of lactic acid bacteria and molasses on fermentation dynamics, structural and nonstructural carbohydrate composition and in vitro ruminal fermentation of rice straw silage. Asian-Australasian Journal of Animal Sciences 32(6), 783-791

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Research Article / Araştırma Makalesi

Seroepidemiology of Akabane Virus Infection in Honamlı Goat Breed

Honamlı Keçi Irkında Akabane Virus Enfeksiyonunun Seroepidemiyolojisi

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Abstract: The goal of this study was to conduct a seroepidemiological investigation of Akabane Virus (AKAV) in Honamlı goats with pure breed characteristics in the hands of the public in Burdur region. For this purpose, blood was collected from 425 goats bred in the mentioned region, unvaccinated, 6 months and older, male and female clinically healthy and was tested for antibodies against AKAV usingCompetitive Enzyme Linked Immunosorbent Assay (C-ELISA) method. The results showed that 9 goat (2.12%) of the 425 were found antibody positive for AKAV infection. Seropositivity rates were found to be between 6.67% and 0% according to the settlements. The distribution of positivity according to age is 3.66% (3/82) in goats in the 4 age group, 3.70% (3/81) in the goats in the 5 age group, 3.45% (2/58) in the 6-year-old goats, and 3.45% (2/58) in the 7-year-old goats. was determined at a rate of 3.33% (1/30) in goats. It was determined that the difference between the seropositivity rates determined in different age groups, males and females and in the districts was statistically insignificant (*P>0.05*). This study is the first serological study to determine seroprevalence of AKAV infection in Honamlı goat in the Burdur Region of Turkey.

Keywords: Akabane Virus (AKAV), ELISA, Honamlı Goat, Seroprevalence.

Öz: Bu araştırmada, Burdur yöresinde halk elinde bulunan saf ırk özelliğine sahip Honamlı keçilerinde Akabane Virus (AKAV) enfeksiyonunun varlığının/yaygınlığının belirlenmesi amaçlanmıştır. Bu amaçla söz konusu yörede yetiştiriciliği yapılan 6 ay ve üzeri, dişi ve erkek sağlıklı görünüme sahip 425 keçiden kan örneklemesi yapıldı. Toplanan kan numuneleri Enzyme Linked Immunosorbent Assay (ELISA) yöntemiyle AKAV antikorları yönünden kontrol edildi. Test edilen 425 keçi kan serumunun 9'u (%2,12) seropozitif olarak belirlendi. Yerleşim yerlerine göre seropozitiflik oranları %6,67-%0 arasında tespit edildi. Pozitifliğin yaşa göre dağılımı ise 4 yaş grubundaki keçilerde %3,66 (3/82), 5 yaş grubundaki keçilerde %3,70 (3/81), 6 yaş grubundaki keçilerde %3,45 (2/58) ve 7 yaş grubundaki keçilerde de %3,33 (1/30) oranında belirlendi. Farklı yaş gruplarında, erkek ve dişilerde ve ilçeler de belirlenen seropozitiflik oranları arasındaki farklılığın istatistik olarak önemsiz (*P>0,05*) olduğu tespit edildi. Bu araştırma sonucunda ilk defa Honamlı keçi ırkında AKAV enfeksiyonunun varlığı ve yaygınlığı serolojik olarak ortaya konulmuştur.

Anahtar Kelimeler: Akabane Virus (AKAV), ELISA, Honamlı Keçi, Seroprevalans.

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Introduction

As one of the important infectious agents in cattles, sheeps and goats, Akabane virus (AKAV) causes various of symptoms including abortion, mummified fetus, stillbirth; blindness, congenital arthrogriposis and hydranencephaly (AH) in offsprings. AKAV is member of *Orthobunyavirus* genus in *Bunyaviridae* family in taxonomy. AKAV is classified in Simbu serogroup which has close relationship with other orthobunyaviruses. The very first agent called OBE-1 was isolated from a naturally infected bovine fetus (Ishihara, 2016) in Japan in 1959 and named after the region (Matsuyama et al. 1960; Kinney and Calisher 1981). Virus carries icosahedral, membraned, 3-segmented ssRNA morphologically (Ludwig, 1991; Sharma ve Adlakha, 2009; Ishıhara, 2016). There are many cell lines developed for the isolation and production of the virus, however, BHK-21, VERO and HmLu-1 cell lines of mammalian origin were used for the production of

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the agent in vitro most of the time. In recent years, insect derivated cell lines are preferred due to detection of more intensive virus reproduction in these cells (Elliot and Blakqori, 2011).

Culicoides spp. biting midges plays the important role in the transmission of the disease and the distribution of these vectors may differ geographically. *Culicoides oxystoma* is common in Japan, *C. brevitarsis* in Australia, *C. milnei* and *C. imicola* is seen in Africa mostly. In addition, other *Culicoides species* seen in North America such as *C. variipennis*, is infected experimentally in laboratory studies. On the other hand, vertical transmission is also important when it comes to epidemiology of Akabane disease. Transmission occurs from non-immune mothers to their offsprings and causes congenital anomalies (Spicler, 2017).

The pathogenesis of Akabane infection differs in pregnant and non-pregnant animals. When AKAV infects pregnant cattle, sheep or goats; it causes various congenital anomalies in the fetus. The incidence and severity of these anomalies depend on the gestational period. This is particularly evident in cattles due to longer gestation period than small ruminants. Abortions, stillbirths and premature births may be the first signs of an Akabane epidemic. Aborted fetuses may appear normal in first examination but disease may be diagnosed with examination of joints. In addition, severe hydranencephaly may be detected if skull is opened (Spicler, 2017). Blindness, nystagmus, deafness, dullness, slow sucking, paralysis and incoordination develop in offsprings born with hydranencephaly. These offsprings can survive for several months if they are fed properly. In adults, infection often occurs subclinically and animals living in endemic areas gain immunity to the disease from early ages (Mellor and Kirkland, 2008).

If clinical symptoms are present and Akabane disease is suspected, final diagnosis should be confirmed by using either serological or virological tests. Enzyme linked immunosorbent assay (ELISA), serum neutralization test (SNT) and as golden standard for diagnosis, polymerase chain reaction (PCR) are used for this purpose (OIE, 2014; OIE, 2016).

In this study, it was aimed to determine the presence of Akabane virus infection serologically by using competitive ELISA method and to obtain information about the prevalence of the Akabane virus in Honamlı goats that unvaccinated, different groups of age and sexin Burdur province. Additionally, due to limited number of studies in Turkey and lack of studies on Honamli goat breed in the world, it was aimed to contribute academic literature of science.

Materials and Methods

Ethics Statement

This research was conducted after the approval of Burdur Mehmet Akif Ersoy University Animal Testing Local Ethics Council (Approval Number: 18.09.2019/547)

Sampled Animals

In this study, blood samples were collected from 425 Honamlı goats that were not immunized against AKAV infection, aged 6 months and older, of different sexes and ages, bred in private small scale family production units in 10 districts (Table 1) of Burdur region (Figure 1).

Table 1. Distribution of collected blood samples by districts and gender

Sampling Districts	Male	Female	Total
Karamanlı/Burdur	18	57	75
Ağlasun/Burdur	2	54	56
Çeltikçi/Burdur	10	45	55
Bucak/Burdur	7	43	50
Tefenni/Burdur	4	21	25
Gölhisar/Burdur	1	23	24
Çavdır/Burdur	14	13	27
Yeşilova/Burdur	13	32	45
Altınyayla/Burdur	1	29	30
Kemer/Burdur	3	35	38
Total	**73**	**352**	**425**

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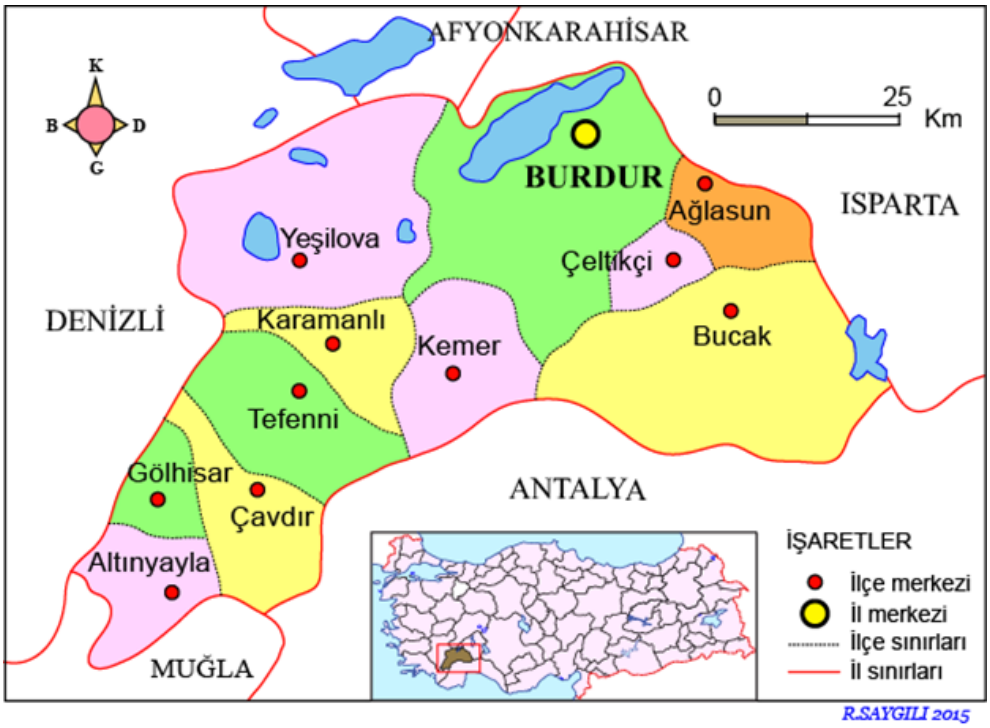

Figure 1. Political map of Burdur province.

Determining the number of samples to be used in the research; At 99.9% confidence level, 99% confidence interval, 5% margin of error, population size of 30598 (Number of pure Honamlı breed goats in Burdur province: 30598 (2020 TR Ministry of Agriculture and Forestry data) (https://hbs.tarbil.gov.tr) in the herd, considering the other studies conducted in Turkey, the possible prevalence of AKAV infection was calculated as 20%, and the sample size was calculated as 425 (Erganiş, 1993).

Serum Samples

The blood samples used in the study were taken from the vena jugularis into sterile coagulated tubes and brought to the laboratory under cold chain conditions. After the blood samples were centrifuged at 3000-4000 rpm for 20 minutes and then separated serum was transferred to sterile eppendorfs and stored in a deep freezer at -20˚C until the ELISA test.

Competitive Enzyme Linked Immunosorbent Assay (C-ELISA)

The presence of anti-G specific antibodies against the structural G protein of Akabane virus was investigated using C-ELISA in sera obtained from blood samples from Honamlı goats. This method works on the principle of solid phase indirect competitive ELISA. Blood serum samples are placed in the wells of microtiter plates coated with AKA antigen and if AKAV antibodies are present in the serum, they are bound to the antigens. If specific AKAV immunoglobulins are present in the test sera, the conjugate binds to the wells containing the viral antigen and is enzyme catalyzed, transforming into a colorless chromogen pigmented compound. For this purpose, ID Screen® Akabane Competition ELISA (ID Vet, Product Code: AKAC, Lot No: F42, France) commercial kit produced by ID Vet company was used. The test was performed according to the kit procedure reported by the manufacturer (ID Vet). The results were evaluated in an ELISA reader (Mindray MR-96A, Hamburg-Germany) using a 450 nm filter. These absorbance values obtained were calculated as specified in the kit's protocol.

In the evaluation of the samples; The result was calculated by multiplying the ratio of the optical density (OD_{Sample}) value of the plate eye on which the sample was placed to the negative control optical density (OD_{NC}) value by 100, and the result

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was evaluated according to the table specified in the test procedure. This process was calculated for each sample separately and the status of each sample was determined (positive/negative) according to the values specified in the table (S/N % = (OD_{Sample} ÷ OD_{NC}) x 100). Using the table below according to the test procedure, each blood serum sample was evaluated. The sample was considered negative if the result was greater than or equal to 30%. The sample was considered positive if it was less than 30% (Table 2).

Table 2. AKAV C-ELISA test results and evaluation.

Result	Evaluation
S/N < 30%	Positive
S/N ≥ 30%	Negative

Statistical Analysis

Statistical analysis was carried out with Statistical Package for Social Sciences software (IBM SPSS 21 Software, USA). Chi-square (chi-square -χ2) test was used to evaluate the statistical significance of the difference between the seropositive rates detected in the sampling districts, the difference between the seropositive rates determined in males and females, and the difference between the AKAV seropositive rates determined in age groups. A p-value < *0*.05 was regarded as significant difference.

Results

The seroprevalence rate of AKAV infection was determined as 2.12% (9/425) in the blood serum of 425 pure Honamli goats tested in the study. When the antibody positivity rates were evaluated on the basis of districts, the highest seropositivity was found in Karamanlı with a rate of 6.67% (5/75). Among its other districts, 4% (2/50) seropositivity was found in Bucak, 1.82% (1/55) in Çeltikçi, and 1.79% (1/56) in Ağlasun (Figure 2). No positivity was detected in other centers where sampling was performed.

The distribution of 9 seropositive samples by gender was found to be 1.37% (1/73) in males and 2.27% (8/352) in females (Table 3).

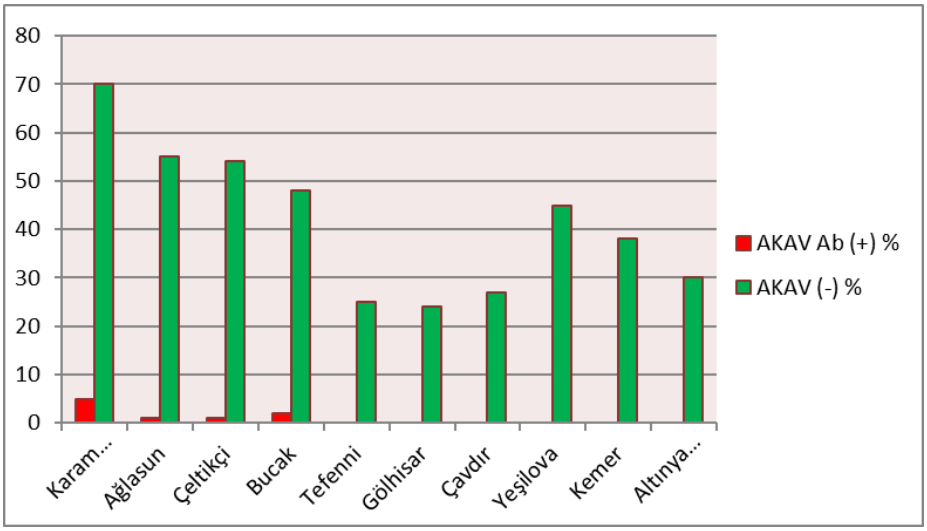

Figure 2. Districts and seropositivity rates in Burdur province

AKAV seropositivity rates were determined according to the age of Honamli goats sampled in the study. Accordingly, 3.66% (3/82) in 4 year old goats, 3.70% (3/81) in 5 year old goats, 3.45% (2/58) in 6 year old goats and 3% in 7 year old goats. Antibody positivity was detected at a rate of 3.33 (1/30). Seropositivity was not determined in one, two, and three-year-old animals (Table 4.)

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In the evaluation of the barn condition in which the goat herds belonging to the sampled private small scale family production units are housed, wall/roof: present, absent criteria; floor quality: with low, moderate and good grades; drinker status: low, moderate and good; insect control: done, not done criteria; Separate housing compartment for goats, kids and goats: evaluated with the criteria of present, absent, and the determined results are shown in Table 5. In addition, it was determined that there were occasional abortion cases in all of the sampled goat herds and the natural breeding method was used in insemination.

Statistical Analysis Results

As a result of the statistical analysis, it was determined that the difference between the AKAV seropositivity rates in the districts of Burdur, where the samples were collected, was statistically insignificant *(p>0.05)* ($\chi2$=2.894, p=0.408). Again, it was determined that the difference in positivity rates in males and females and the differences in positivity rates in different age groups were statistically insignificant *(p>0.05)* (Male/female $\chi2$=0.238, p=0.626; age groups $\chi2$=0.013, p=1,000).

Discussion

Various of diseases threatening human and animal health (Akabane, Crimean Congo fever, West Nile virus, Sandfly fever) are usually transmitted by arthropods feeding on the blood such as mosquites, ticks and sandflies. Due to rapid development of modern transport systems, global warming, demographic changes, the disappearance of natural ecological borders because of fast urbanization and the emergence of new areas increasing contact between vector species and their hosts, very important health problems start to occur in new millennium such as arboviruses.

Table 3. AKAV seroprevalence rates by gender

Gender	Number of samples (n)	AKAV Ab		AKAV Ab	
		n (+)	%	n (-)	%
Male	73	1	1.37	72	98.63
Female	352	8	2.27	344	97.73
Total	**425**	**9**	**2.12**	**416**	**97.88**

Table 4. AKAV seropositivity rates by age groups

Age	Number of samples (n)	AKAV Ab			
		n (+)	%	n (-)	%
6-12 month	38	0	0	38	100
2 age	62	0	0	62	100
3 age	74	0	0	74	100
4 age	82	3	3.66	79	96.34
5 age	81	3	3.70	78	96.30
6 age	58	2	3.45	56	96.55
7 age	30	1	3.33	29	96.67
Total	**425**	**9**	**2.12**	**416**	**97.88**

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Table 5. Evaluation of the Conditions of the Barns Housing the Sampled Honamli Goat Herds

Sampling Districts	Wall-roof availability	Floor quality	Drinker status	Insect control	Separate compartment (for goats, kids and goats)
Karamanlı/Burdur	Absent	Low	Low	No	Absent
Ağlasun/Burdur	Present	Moderate	Low	No	Absent
Çeltikçi/Burdur	Absent	Low	Low	No	Absent
Bucak/Burdur	Absent	Low	Low	No	Absent
Tefenni/Burdur	Present	Good	Good	No	Present
Gölhisar/Burdur	Present	Good	Good	No	Present
Çavdır/Burdur	Present	Good	Good	No	Present
Yeşilova/Burdur	Present	Good	Good	No	Present
Altınyayla/Burdur	Absent	Moderate	Moderate	No	Present
Kemer/Burdur	Present	High	High	No	Present

When viewed from this aspect, geographical localization of the research area is extremely important. Burdur province, where the study was conducted, is in West of Mediterranean Region of Turkey, located at 37°43' North 30°17' East coordinates and its altitude is 950 meters. There are rich water sources, rivers and lakes in the province, hence the area is called the region of lakes. Next to the city of Burdur, "Burdur Lake" is located and is the seventh largest lake of Turkey with coordinates 37°45' North, 30°12' East and an area of 250 km² (2013 DSİ). Moreover, there are various kinds of natural lakes and dam lakes established for irrigation (Karatas Lake, Karamanli Lake, Onac Lake, etc…) in the region. Likewise, irrigation-based agriculture is actively applied in the region as well as animal husbandry. Considering all these geographical aspects, it is safe to say Burdur province is ideal environment for vector mosquitoes' habitats due to the epidemiological cycle of AKAV.

The climatic features of the region are also suitable for AKAV. The annual average temperature is 13.2°C and the annual average number of rainy days is 89 (Hızel et al., 2010). It is thought that the changes in the annual average temperature and the annual average number of rainy days are the result of the altitude and topographic structure. The area, where the study conducted, provides a suitable habitat for mosquito larvae with its wide variety of wetlands and contains different type of hosts that adults can feed on due to intensive livestock activities. All this features makes the region potential risky area for Akabane virus infection.

Furthermore, Antalya province, the capital of tourism, next to Burdur province, has suitable habitats for AKAV vectors. Due to the fact that Antalya International Airport, marina, highway connections of the coastline passing through this region and the climate of the region provides an appropriate enviroment, direct transfer of virus-infected vector mosquitoes from other regions/countries to these regions poses a potential risk. Thus, there is a possibility of transmission of Akabane virus from close provinces to Burdur province.

There are many studies on Akabane infection in Turkey and in the world. In one study, AKAV seropositivity was determined as 87% (47/54) in the blood serum of 54 cattle with AH syndrome in Israel. Same study also reported that AKAV seropositivity in farms without AH syndrome was 3.7% (1/27). According to the aforementioned study, AKAV vector mosquites were active between August and December, thus spreading AKAV (Brenner et al., 2004). General prevalence

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rates of AKAV antibodies in dairy cattles in Sudan was reported as 29.4% and prevalence rate was between 69.6% and 3.3%among the states. Similarly, the prevalence of AKAV antibodies was higher in crossbred animals than in domestic animals, and a higher rate of positivity was detected in females than males. At the same time, the prevalence of AKAV has been reported to be high in infertility and abortion cases (Elhassan et al., 2014). In another study, 210 blood serum samples collected from affected cattle during the cattle enzootic encephalomyelitis epidemic in 2010 were analysed for AKAV antibody by using serum neutralization test (SNT) and ELISA. As a result, seropositivity rates of SNT and ELISA were determined as 90.0% and 85.2%, respectively (Oem et al., 2014).

The very first report of Akabane disease in Turkey was delivered by Urman et al. in 1980. In the following years, many studies have been carried out to evaluate the status and prevalence of Akabane infection. In two different studies, AKAV seroprevalence rate in cattles was determined as 13.7% (Çabalar and Dağalp, 2006) and 27.98% (Özgünlük, 2003) in Southeastern Anatolia region. Moreover, Akabane seropositivity was reported at a rate of 22% in the Black Sea region (Albayrak and Özan, 2010) and 0.14% in the Thrace region (Karaoğlu et al., 2007). Although AKAV seropositivity of cattle was reported as 9.72% in Aydın province in the Aegean region (Özgünlük et al., 2013), seropositivity was not found in sheep, goats and cattle in another study conducted in the same province (Koç, 2014). Similarly, meanwhile in a study related to Akabane infection in small ruminants (sheep and goats) seropositivity was reported as 1.1% in goats in Aydın province of Aegean region, seropositivity could not be detected in Muğla (Tan and Bilge, 2000). The seropositivity of Akabane infection was reported as 0.08% in sheep in the Marmara region (Pestil, 2014) and 44.9% in sheep in the Mediterranean region (Şevik, 2017). In a seroprevalence study conducted in Hatay, seropositivity rates were found as 42.41% in cattle, 16.19% in sheep, and 7.46% in goat (Doğan, 2018).

In this study, seroprevalence of AKAV infection was determined as 2.12 % (9/425). In the districts where the research was conducted, these seropositivity rates were 6.67% in Karamanlı, 1.79% in Ağlasun, 1.82% in Çeltikçi, and 4.00% in Bucak seperately. In addition, the distribution of seropositivity in our study by gender was 1.37% in males and 2.27% in females.

In husbandries with seropositivity, it was identified that wall, roof and floor condition of barns where animals live in, are uneven, sloppy and dirty; feeders and water sources are not hygienic; there is no pest control administration, there is no separate section for offsprings and all animals are living together. The seropositivity rate of Honamli goat breed in our study is coherent with previous studies conducted on small ruminants, thus results are significant and valuable. The Mediterranean region has a suitable geographical location and climatic conditions for the survival of *Clucoides species*, which are an important vector in the transmission and transmission of the agent.

Depending on breeding purpose of goats (meat, milk or mixed), the status of AKAV infection may vary. It has been determined that the transmission of Akabane virus from mother to offspring during pregnancy is more common in beef breed goats. Likewise, AKAV infection transmission by mosquitoes and spreading among animals are more likely in dairy goats raised in crowded, closed and cramped enviroments and not left outside for grazing. Thus barn conditions, husbandry practices and indoor goat breeding have an important role in spreading the infection.

The increase of greenhouse gas density in the atmosphere in the last century results in the melting of glaciers and droughts associated with the global warming. Thus these effects of the global warming cause changes in habitats of animals and forces them either to adapt the current situations or to live in higher regions. Due to all these reasons, the potential of mosquitoes to spread the disease, which is the vector of Akabane virus as a result of atmospheric changes, is increasing day by day.

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Consequently, considering the data of this study and presence/prevalence of Akabane infection in the region where the study was conducted, it is possible to report that the disease is important both in Burdur and other regions of Turkey in terms of animal husbandry, and serious measures should be taken to prevent infection. The most important factor in preventing the spread of the disease is the struggle against vectors. Traps to be set in areas where biting midges are common will both prevent the spread of infection and enable more detailed research to be carried out by identifying the species of the caught flies. In addition, it has been concluded that the vaccination studies against AKAV, which is applied in some countries where the disease is seen but not yet implemented in our country, will be useful to define the places where the infection is seen in our country.

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References

Albayrak, H., Özan, E., 2010. Orta karadeniz bölgesinde ruminant ve tek tırnaklılarda kan emici sineklerle nakledilen bazı arboviral enfeksiyonların seroprevalansı. Kafkas Universitesi Veteriner Fakultesi Dergisi 16, 33-36.

Brenner, J., Tsuda, T., Yadın, H., Kato, T., 2004. Serological Evidence of Akabane Virus Infection in Northern Israel in 2001. The Journal of Veterinary Medical Science 66(4), 441-443.

Çabalar, M., Bilge Dağalp, S., 2006. Seroprevalence of Bluetongue and Akabane diseases in dairy cattle in South-East Turkey. Slovenian Veterinary Research 43(10), 296-297.

Doğan, F., 2018. Hatay İlinde Ruminantlarda Bazı Arboviral (Akabane virus, mavidil Virus, Schmallenberg Virus) enfeksiyonların epidemiyolojisinin araştırılması ve olası vektörlerin belirlenmesi. Doktora Tezi, Ankara Üniversitesi Sağlık Bilimleri Enstitüsü, Ankara.

Elhassan, A.M., Mansour, M.E., Shamon. A.A., El Hussein, A.M., 2014. A serological survey of Akabane virus infection in cattle in Sudan. ISRN Veterinary Science, 123904, p. 4.

Elliott, R.M., Blakqori, G., 2011. Molecular biology of orthobunyaviruses. In: Plyusnin A, Elliott RM (Eds). Bunyaviridae. Norfolk: Caister Academic Press, Corpus ID: 80812852, p. 1-39.

Erganiş, O., 1993. Veteriner Epidemiyoloji (Temel Bilgiler). Mimoza Yayınları:16, Sağlık bilimleri dizisi: 2, Konya, ISBN: 975-543-018-0, p.74-79.

Hızel, K., Yenicesu, İ., Erdal, B., Yeşilyurt, E., Fidan, I., Kalkancı, A., Dilsiz, G., 2010. Investigation of West Nile virus seroprevalence in healthy blood donors. Mikrobiyoloji bülteni 44, 425-430.

Ishihara, Y., Shıoda, C., Bangphoomi, N., Sugiura, K., Saeki, K., Tsuda, S., Iwanaga, T., Takenaka-Uema, A., Kato, K., Murakamı, S., Uchida, K., Akashi, H., Horimoto, T., 2016. Akabane virus nonstructural protein NSm regulates viral growth and pathogenicity in a mouse model. The Journal of Veterinary Medical Science 79, 1391–1397.

Karaoğlu, T., Özgünlük, İ., Demir, B., Ozkul, A., Burgu, I., 2007. Seroprevalence of culicoides borne disease in cattle in European Turkey. Ankara Üniversitese Veteriner Fakültesi Dergisi 54, 121-125.

Kinney, R.M., Calisher, C.H., 1981. Antigenic relationships among Simbu serogroup (Bunyaviridae) viruses. The American journal of tropical medicine and hygiene, 30(6), 1307–1318.

Kirkland, P.D., 2015. Akabane virus infection. Revue scientifique et technique (International Office of Epizootics) 34(2), 403-410.

Koç, B.T., 2014. Muğla ve Aydın İllerinde Akabane Virus (AKAV) Enfeksiyonunun Serolojik Olarak Araştırıması. Yüksek Lisans Tezi, Adnan Menderes Üniverstesi Sağlık Bilimleri Enstitüsü, Aydın.

Ludwig, G.V., Israel, B.A., Christensen, B.M., Yuill, T.M., Schultz, K.T., 1991. Role of La Crosse virus glycoproteins in attachment of virus to host cells. Virology 181, 564-571.

Matsuyama, T., Oya, A., Ogata, T., Kobayashi, I., Nakamura, T., Takahashi, M., Kitaoka, M., 1960. Isolation of arboviruses from mosquitoes collected at livestock pens in Gumma prefecture in 1959. Japanese journal of medical science & biology 13, 191-198.

Mellor, P.S., Kirkland, P.D., 2008. Akabane virus. In: Mahy BWJ, Van Regenmortel MHV (eds). Desk Encyclopedia of Animal and Bacterial Virology. Jordan Hill, Oxford. Academic Press, p. 18-22.

Oem, J.K., Kim, Y.H., Kim, S.H., Lee, M.H., Lee, K.K., 2014. Serological characteristics of affected cattle

during an outbreak of bovine enzootic encephalomyelitis caused by Akabane virus. Tropical Animal Health And Production 46, 261-263.

OIE (2014). Bunyaviral diseases of animals. p. 1-16. In: Manuals of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE ed.). Office International des Epizooties, Paris.

OIE (2016). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Akabane Disease. Veterinary Diagnostic Investigation 12, 518-24.

Özgünlük, İ., 2003. Güneydoğu Anadolu Projesi (GAP) kapsamındaki bölgede sığırlarda mavidil, akabane ve ibaraki enfeksiyonlarının seroepidemiyolojisi. Doktora Tezi, Ankara Üniversitesi Sağlık Bilimleri Enstitüsü, Ankara.

Özgünlük, İ., Yıldırım, Y., Gür, S., Tan, M.T., 2013. Aydın Yöresindeki Sığırlarda Akabane Virus (AKAV) ve İbaraki Virus (IBAV) Enfeksiyonlarının Seroprevalansı. Harran Üniversitesi Veteriner Fakültesi Dergisi 2, 36-41.

Pestil, Z., 2014. Marmara bölgesinde koyunlardan alınan abort ve postnatal örneklerde viral etkenlerin (Pestivirus, Mavidil ve Akabane Virus) araştırılması. Doktora Tezi, Fırat Üniversitesi Sağlık Bilimler Enstitüsü, Elazığ.

Şevik, M., 2017. Molecular and serological survey of Akabane virus infection in sheep in the Mediterranean Region of Turkey. Small Ruminant Research, 156, 1-6.

Sharma, S.N., Adlakha, S.C., 2009. Textbook of Veterinary Virology. First Press. Delhi International Book Distributing Co., p. 347-354.

Spicler, A.R., 2017. Akabane Disease. http://www.cfsph.iastate.edu/Factsheets/pdfs/akabane.pdf (Erişim 14.05.2021).

Tan, M.T., Bilge, S., 2000. Serological investigation of Akabane infection in goat with congenital abnormalities in Turkey. Pendik Veteriner Mikrobiyoloji Dergisi 31, 65-66.

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