ISSN : 0377 - 6395
e- ISSN : 2651 - 4214

Veteriner Hekimler Derneği Dergisi

Journal of the Turkish Veterinary Medical Society

Cilt / Volume : 92 Sayı / Issue: 1 Yıl / Year: 2021

92(1)

ISSN : 0377 - 6395
e-ISSN : 2651 - 4214

Veteriner Hekimler Derneği Dergisi

Journal of the Turkish Veterinary Medical Society

Cilt / Volume : 92 *Sayı / Issue:* 1 *Yıl / Year :* 2021

92(1)

Veteriner Hekimler Derneği Dergisi

Journal of the Turkish Veterinary Medical Society

Cilt / *Volume:* 92 **Sayı** / *Issue:* **1** **Yıl** / *Year:* 2021

Altı ayda bir yayımlanır / *Published bi-annually* • Yayın Türü: Yerel Süreli Yayın

http://dergipark.org.tr/vetheder

ISSN : 0377 -6395 **e-ISSN: 2651-4214**

Veteriner Hekimler Derneği Adına Sahibi

Dr. Hüseyin Yalçın KÖKSAL

Ziya Gökalp Caddesi No:16/7 Kızılay, Ankara

Yazı İşleri Müdürü

Dr. Gülay KABASAKAL ERTÜRK

Ziya Gökalp Caddesi No: 16/7 Kızılay, Ankara

Hakemli Dergidir / Peer-Reviewed Journal

Bu dergi, ULAKBİM (Yaşam Bilimleri), CAB Abstracts, Google Scholar ve Türkiye Atıf Dizini tarafından indekslenmektedir.

This journal is covered by ULAKBIM (Life Sciences), CAB Abstracts, Google Scholar and Turkiye Citation Index database systems.

VETERİNER HEKİMLER DERNEĞİ

Adres: Ziya Gökalp Caddesi No:16/7 Kızılay, Ankara • **Tel:** +90 312 431 62 74 • **Faks:** +90 312 435 79 14

e-ileti: info@veteriner.org.tr • **web adresi:** www.veteriner.org.tr

Derneğin Kuruluş Tarihi: 6 Şubat 1930

Derginin İlk Yayın Tarihi: 1 Ekim 1930

Yayımlanma Tarihi / Publication Date: 15.01.2021

VETERİNER HEKİMLER DERNEĞİ DERGİSİ
JOURNAL OF THE TURKISH VETERINARY MEDICAL SOCIETY
ISSN: 0377-6395
e-ISSN: 2651-4214
Dergi ana sayfası:
Journal homepage:
http://dergipark.org.tr/vetheder

DOI: 10.33188/vetheder.713559

Araştırma Makalesi / Research Article

Investigation of the antimicrobial effect of endemic *Sideritis galatica* plant

Ulaş ACARÖZ [1, a*], Sinan İNCE [2, b], Recep KARA [1, c], Zeki GÜRLER [1, d], Ali SOYLU [1, e]

[1] Afyon Kocatepe University, Veterinary Faculty, Department of Food Hygiene and Technology, Afyonkarahisar, Turkey
[2] Afyon Kocatepe University, Veterinary Faculty, Department of Pharmacology and Toxicology, Afyonkarahisar, Turkey
ORCID: 0000-0002-1533-4519 [a]; 0000-0002-1915-9797 [b]; 0000-0002-9257-7506 [c]; 0000-0002-9037-2945 [d]; 0000-0003-3199-782X [e]

MAKALE BİLGİSİ / ARTICLE INFORMATION:

Geliş / Received:
04 Nisan 20
04 April 20

Kabul / Accepted:
11 Temmuz 20
11 July 20

Keywords:
Antimicrobial activity
Herbal tea
Sideritis galatica

Anahtar Sözcükler:
Antimikrobiyal aktivite
Bitkisel çay
Sideritis galatica

ABSTRACT:

Herbal teas are widely consumed in Turkey. This study aimed to evaluate the antimicrobial activity of methanol, acetone, and aqueous extracts of endemic *Sideritis galatica* plant collected from Afyonkarahisar. For this purpose, the effect of different concentrations (2.5%, 5%, 10%, 20%) of these extracts on *L. monocytogenes, B. cereus, S. aureus, E. faecalis, E. coli, E. coli O157, S. typhimurium, C. albicans* were investigated by the disk diffusion method. Methanol extract showed antimicrobial activity against all of the tested microorganisms. Also, acetone and aqueous extracts showed antimicrobial activity against all of the tested microorganisms except *E. faecalis* (for acetone extract), *E. coli* and *E. coli O157* (for aqueous extract). Consequently, *S. galatica* as an endemic plant showed antimicrobial activity especially at high concentrations against tested microorganisms.

Endemik Sideritis galatica bitkisinin antimikrobiyal etkisinin araştırılması

ÖZET:

Bitkisel çaylar Türkiye'de yaygın olarak tüketilmektedir. Bu çalışmada Afyonkarahisar'dan toplanan endemik *Sideritis galatica* bitkisinin metanol, aseton ve sulu ekstraktlarının antimikrobiyal aktivitesi değerlendirildi. Bu amaçla, bu ekstraktların farklı konsantrasyonlarının (% 2,5, % 5, % 10, % 20) *L. monocytogenes, B. cereus, S. aureus, E. faecalis, E. coli, E. coli O157, S. typhimurium, C. albicans* disk difüzyon yöntemi ile araştırıldı. Metanol ekstraktı test edilen tüm mikroorganizmalara karşı antimikrobiyal aktivite gösterdi. Ayrıca, aseton ve sulu ekstraktlar, *E. faecalis* (aseton ekstraktı için), *E. coli* ve *E. coli O157* (sulu ekstrakt için) hariç test edilen tüm mikroorganizmalara karşı antimikrobiyal aktivite gösterdi. Sonuç olarak, endemik bir bitki olarak *Sideritis galatica*, test edilen mikroorganizmalara karşı özellikle yüksek konsantrasyonlarda antimikrobiyal aktivite gösterdi.

How to cite this article: **Acaröz U, İnce S, Kara R, Gürler Z, Soylu A**: Investigation of the antimicrobial effect of endemic Sideritis galatica plant. *Veteriner Hekimler Dernegi Dergisi*, 92(1): 1-6, 2021, DOI: 10.33188/vetheder.713559

* *Sorumlu Yazar e-posta adresi / Corresponding Author e-mail address:* ulasacaroz@aku.edu.tr

1. Introduction

Sideritis genus of *Labiateae* family possesses more than 150 different species and they are located at the Northern Hemisphere from the Western China to Bahamas. Many of them are largely distributed in the Mediterranean area. In particular, Turkey and Spain have a lot of different species. While predominate areas of Spain are Canary Islands and the Southeast of Iberian Peninsula, most species in Turkey found to be in Marmara and Aegean regions (1, 6, 10). 25 *Sideritis* species are endemic to Turkey and some of them have been exported (15). This genus mainly grows in mountainous areas (over 800-1.000 m) (8). The origin of *Sideritis* genus comes from the Greek word "sideros". Its meaning is iron because these plants are used to treat wounds caused by metal weapons in the ancient times (4).

Sideritis species are traditionally employed as flavoring agents, teas, and for medicinal purposes in the Mediterranean and Balkan regions and also in Turkey (13). They recently became quite popular and found in several of shops. They are called and marketed as malotira, té de Puerto, mountain tea, ada çayı, dağ çayı. This herb is found to be whole or in the cut form (8, 15).

Traditional medicine uses the species of this genus for centuries due to their anti-inflammatory, anticonvulsant, antiulcerative, antispasmodic, analgesic, antioxidant, and antimicrobial properties (5, 8). These effects are related to various compounds of this genus such as terpenes, flavonoids, essential oil, lignanes, iridoids, coumarins and sterols. In addition, almost every species contain essential oils, flavonoids, and diterpenes (4).

The present study investigated antibacterial and antifungal activities of Turkish endemic *Sideritis galatica* plant by evaluating its water, methanol and acetone extracts against eight bacteria and yeasts including *L. monocytogenes, B. cereus, S. aureus, E. faecalis, E. coli, E. coli O157, S. typhimurium, C. albicans.*

2. Material and Methods

Plant Material:

Sideritis galatica samples were collected from Şuhut districts of Afyonkarahisar of Turkey in June 2017. This plant is registered at the Herbarium of Biology Department of Afyon Kocatepe University with Herbarium number of AKU-8315.

Extraction:

Firstly, a drying process of the plant was performed at shadow for its aerial parts. Then, water, methanol and aceton extracts of *Sideritis galatica* were prepared as previously described by Knörle et al. (8) with some modifications. Briefly, dried herb samples were ground to powder for 2 min by a grinder. Powdered samples were dissolved in different solvents including water, acetone and methanol as extracting agent. A filtration step was performed to the obtained extracts and they were dried by rotary evaporation. The resulted extracts were expressed as water, aceton, methanol extracts.

Microorganism strains and determination of antimicrobial activity:

The microorganism strains employed in the study were presented in Table 1. Antimicrobial activity of the tested microorganisms was determined by disc diffusion method. For that aim, a suspension containing tested microorganisms with a concentration of 10^6-10^7 cfu/ml was prepared and then the related microorganism spread on the solid media plates. Then 20 µL of the *Sideritis galatica* extracts were impregnated to the 6-mm diameter paper discs and they were placed on the inoculated agar. An incubation period followed this step for bacterial strains (37°C, 18-24 h) and yeast (27ºC, 24-48 h).

Table 1: The microorganism strains employed in the study

Tablo 1: *Çalışmada kullanılan mikroorganizma suşları*

Microorganism Strains	ATCC Strain Number
B. cereus	ATCC 11778
C. albicans	ATCC 90028
E. faecalis	ATCC 29212
E. coli	ATCC 25292
E. Coli O157	ATCC 43894
L. monocytogenes	ATCC 7644
S. aureus	ATCC 25923
S. typhimurium	ATCC 14028

3. Results

The activities of water, methanol and aceton extracts of *Sideritis galatica* at the concentrations of 2.5 %, 5 %, 10 %, 20 % against the tested gram-positive bacteria strains and *C. albicans* were given in Table 2 and gram-negative bacteria were given in Table 3. The study results showed that water extract of *Sideritis galatica* showed activity against *C. albicans* at all tested concentrations. In addition, water extract did not exhibit any activity against other tested seven bacteria strain at the 2.5 %, and 5 % concentrations whereas higher concentration of water extract (20%) showed antibacterial activity against all tested microorganism except *E. coli* and *E. coli O157*. When the methanol extract evaluated, the highest concentration (20%) of this extract possessed antibacterial and antifungal activity against all tested microorganisms. However, the lower concentrations of methanol extract were not effective except *L. moncocytogenes* (5% and 10%) and *S. aureus* (10%). Similarly, highest concentration of acetone extract was effective against all tested microorganisms except *E. fecalis*. Only 10 % of acetone extract showed activity against *B. cereus* and *C. albicans* while the other concentrations were ineffective.

Table 2: Antimicrobial activities of water, methanol and acetone extracts of *Sideritis galatica* (2.5, 5, 10, and 20%) against Gram-positive bacteria and *C. albicans*.

Tablo 2: *Sideritis galatica'nın (% 2,5, 5, 10 ve 20) su, metanol ve aseton ekstraktlarının Gram pozitif bakterilere ve C. albicans'a karşı antimikrobiyal aktiviteleri.*

	Water				Methanol				Acetone			
	2.5	5	10	20	2.5	5	10	20	2.5	5	10	20
L. monocytogenes	-	-	-	15	-	10	13	14	-	-	-	14
B. cereus	-	-	-	14	-	-	-	15	-	-	10	14
S. aureus	-	-	10	11	-	-	12	15	-	-	-	12
C. albicans	10	11	12	13	-	-	-	14	-	-	10	13

The values express zone of inhibition (mm), -: no inhibition

Table 3: Antimicrobial activities of water, methanol and acetone extracts of *Sideritis galatica* (2.5, 5, 10, and 20 %) against Gram-negative bacteria.

Tablo 3: *Sideritis galatica'nın (% 2,5, 5, 10 ve 20) su, metanol ve aseton ekstraktlarının Gram pozitif bakterilere ve C. albicans'a karşı antimikrobiyal aktiviteleri.*

	Water				Methanol				Acetone			
	2.5	5	10	20	2.5	5	10	20	2.5	5	10	20
E. faecalis	-	-	-	11	-	-	-	12	-	-	-	-
E. coli	-	-	-	-	-	-	-	16	-	-	-	13
E. coli O157	-	-	-	-	-	-	-	15	-	-	-	12
S. typhimurium	-	-	-	10	-	-	-	11	-	-	-	10

The values express zone of inhibition (mm), -: no inhibition

4. Discussion and Conclusion

Many researchers have investigated the antimicrobial activities of different *Sideritis* species. Sagdic et al. (12) determined the antimicrobial activities of the methanol extracts of *S. ozturkii* and *S. caesarea* which are endemic plants for Turkey. They employed agar diffusion method against fifteen microorganisms for four different concentrations (1, 2.5, 5, 10 %) of these extracts. They reported that the extracts of both plants were least effective at lower concentrations (1 % and 2.5 %) and generally yeast strains were found to be more resistant than tested bacterial strains. Their antimicrobial activities changed related to the tested microorganisms and the highest concentration (10 %) was reported as the most active of the concentrations against all of the tested microorganisms. In another study, *Sideritis* essential oils were reported to show both antifungal and antibacterial activities (11). The extent of the antibacterial activity of the extracts of *Sideritis* genus could be attributed to their phenolic content such as diterpenoids and flavonoids (2). Also, Iscan et al. (7) obtained essential oils from *Sideritis cilicica* and *Sideritis bilgerana* and performed their analysis by GC and GC/MS. Their results showed that major constituents in the *S. cilicica* oil were found to be as β-phellandrene (20%), α-pinene (28%), and β-Pinene (39%) whereas the main components in the *S. bilgerana* oil were determined as α-pinene (32%), and β-pinene (48%). To evaluate antimicrobial activities of these oils, microdilution broth method was used and both oils were reported to show good inhibitory effects on *C. albicans*. Dulger et al. (3) investigated the activity of the methanol extracts of seven endemic *Sideritis* species for Turkey were (30 mg/ml) on clotrimazole-resistant *C. albicans*. *S. trojana* and *S. bilgerana* were reported to be most active plants against *C. albicans.* Temel et al. (15) reported that different levels of water extracts of *Sideritis akmanii* showed significant acitivity on several bacteria including *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas aeruginosa, Aeromonas hydrophila*. However, it did not exhibit any antimicrobial activity against yeasts. Koutsaviti et al (9) evaluated the antibacterial activity of five taxa of Greek *Sideritis* by broth microdilution method against five gram-positive (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Micrococcus luteus*, *Enterococcus faecalis*, *Bacillus subtilis)* and three gram bacteria strains (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*), and two different strains of *Candida albicans*. They used hydrodistillation to obtain extracts of the related *Sideritis* species. Considerable antibacterial and antifungal activity against certain microorganism strains were reported. Even, the MIC values of *S. lanata* against *M. luteus* and *S. aureus* were comparable to reference antibiotics. Tadić et al. (13) reported about the antimicrobial activity of *Sideritis romana L. subsp. purpurea* in a recent study. In their study, *Candida albicans*, methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*, carbapenem-resistant and carbapenem-susceptible *Klebsiella pneumoniae*, and

Escherichia coli were employed as test microorganisms. The obtained essential oil was highly potent against both methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* strains. In addition, 1,2-dichloroethane and n-hexane extracts of the plant was reported to show a potent fungicidal activity. In another study, antimicrobial effects of *Sideritis scardica* extracts were determined by Tadić et al. (14). According to the results of their study, ethanolic extract of the plant showed various level of antimicrobial activity all tested strains which were *Micrococcus luteus*, *Candida albicans*, *Staphylococcus epidermidis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. Also, they have stated that maximum activity was seen against *M. luteus*, *S. epidermidis*, *P. aeruginosa* and *E. coli* while moderate activity was observed against *K. pneumoniae*.

Different *Sideritis* species were reported to show various level of antimicrobial activity against tested bacteria and yeast strains. This may be caused by the different active ingredients of the respective *sideritis* species which could be affected by several factors such as harvesting time of the herb and extraction procedure.

In conclusion, three different extracts of *Sideritis galatica* including water, methanol, acetone extracts were evaluated for the antimicrobial activity. Although lower extract concentrations were not so effective against tested microorganisms, higher concentrations especially 20 % concentrations of the extracts were able to show antimicrobial activity. However, water extract did not show activity against *E. coli* and *E. coli O157* while acetone extract was not able to show any activity against *E. faecalis*.

Acknowledgement

The summary of the present study was orally presented at the International Science and Academic Congress'18, 8-9 December 2018, Konya, TURKEY.

Conflict of Interest

The author declared no conflict of interest.

Funding

No funding.

Authors' Contributions

Idea / concept: Ulaş ACARÖZ, Sinan İNCE
Experiment design: Ulaş ACARÖZ, Sinan İNCE, Recep KARA
Supervision / Consultancy: Ulaş ACARÖZ, Sinan İNCE
Data collecting: Ulaş ACARÖZ, Sinan İNCE, Recep KARA, Zeki GÜRLER, Ali SOYLU
Data analysis and interpretation: Ulaş ACARÖZ, Sinan İNCE, Recep KARA, Zeki GÜRLER
Literature search: Ulaş ACARÖZ, Sinan İNCE, Zeki GÜRLER, Ali SOYLU
Writing the article: Ulaş ACARÖZ, Sinan İNCE
Critical review: Ulaş ACARÖZ, Sinan İNCE, Recep KARA

Ethical Approval

An ethical statement was received from the authors that the data, information and documents presented in this article were obtained within the framework of academic and ethical rules and that all information, documents, evaluations and results were presented in accordance with scientific ethics and moral rules.

References

1. Aslan I, Kiliç T, Gören AC, Topçu G (2006): *Toxicity of acetone extract of Sideritis trojana and 7-epicandicandiol, 7-epicandicandiol diacetate and 18-acetylsideroxol against stored pests Acanthoscelides obtectus (Say), Sitophilus granarius (L.) and Ephestia kuehniella (Zell.)*. Ind. Crops Prod, **23**, 171-176.

2. Basim E, Basim H, Özkan G, Sağdiç O (2012): *Antibacterial activity of the methanol extracts of two endemic Sideritis species of Turkey against plant pathogenic bacteria*. Sci. Res. Essays, **7**, 4146–50.
3. Dulger B, Gonuz A, Aysel V (2006): *Inhibition of clotrimazole-resistant Candida albicans by some endemic Sideritis species from Turkey*. Fitoterapia, **77**, 404-405.
4. González-Burgos E, Carretero ME, Gómez-Serranillos MP (2011): *Sideritis spp.: Uses, chemical composition and pharmacological activities - A review*. J. Ethnopharmacol, 135(2), 209-225.
5. González-Burgos E, Gómez-Serranillos, MP Palomino O, Carretero M (2009): *Aspectos botánicos y farmacológicos del género Sideritis*. Rev. Fitoter, **9**, 133-145.
6. Güvenç A, Houghton PJ, Duman H, Coşkun M, Şahin P (2005): *Antioxidant activity studies on selected Sideritis species native to Turkey*. Pharm. Biol, **43**, 173-177.
7. Iscan G, Kirimer N, Kurkcuoglu M, Baser KHC (2005): *Composition of the essential oils of two Sideritis species from Turkey and antimicrobial activity*. Chem. Nat. Compd, **41**, 679-682.
8. Knörle R (2012): *Extracts of Sideritis scardica as triple monoamine reuptake inhibitors*. J. Neural Transm, **119**, 1477-1482.
9. Koutsaviti A, Bazos I, Milenkovic M, Pavlović-Drobac M, Tzakou O (2013): *Antimicrobial activity and essential oil composition of five sideritis taxa of empedoclia and hesiodia sect. from Greece*. Rec. Nat. Prod, **7**, 6-14.
10. Loğoğlu E, Arslan S, Öktemer A, Şakiyan I (2006): *Biological activities of some natural compounds from Sideritis sipylea boiss*. Phyther. Res, **20**, 294-297.
11. Rodríguez-Linde ME, Díaz RM, García-Granados A, Quevedo-Sarmiento J, Moreno E, Onorato MR, Parra A, Ramos-Cormenzana A (1994): *Antimicrobial activity of natural and semisynthetic diterpenoids from Sideritis spp*. Microbios, **77**, 7-13.
12. Sagdic O, Aksoy A, Ozkan G, Ekici L, Albayrak S (2008): *Biological activities of the extracts of two endemic Sideritis species in Turkey*. Innov. Food Sci. Emerg. Technol, **9**, 80-84.
13. Tadić V, Oliva A, Božović M, Cipolla A, De Angelis M, Vullo V, Garzoli S, Ragno R (2017): *Chemical and antimicrobial analyses of Sideritis romana L. subsp. purpurea (Tal. ex Benth.) Heywood, an endemic of the Western Balkan*. Molecules, **22**, 1-15.
14. Tadić V, Djordjević S, Arsić I, Dobrić S, Milenković M, Antić-Stanković J (2007): *Anti-inflammatory and antimicrobial activity of Sideritis scardica extracts.* Planta Med, **73**, 98.
15. Temel M, Kara R, Müdüroğlu R, Akkaya L (2012): *Antibacterial Activity of Turkish Endemic Sideritis Akmanii (Lamiaceae)*. Glob. J. Res. Anal, **3**, 83-84.

VETERİNER HEKİMLER DERNEĞİ DERGİSİ
JOURNAL OF THE TURKISH VETERINARY MEDICAL SOCIETY
ISSN: 0377-6395
e-ISSN: 2651-4214
Dergi ana sayfası:
Journal homepage:
http://dergipark.org.tr/vetheder

DOI: 10.33188/vetheder.678481

Araştırma Makalesi / Research Article

Assessment of cattle and sheep Brucellosis in Turkey

Anıl DEMELİ [1, a*], ***Murat FINDIK*** [2, b]

[1] *The Ministry of Agriculture and Forestry, The General Directorate of Food and Control, Department of Animal Health and Quarantine, Ankara, Turkey*
[2] *Ondokuz Mayıs University, Faculty of Veterinary Medicine, Department of Gynaecology and Obstetrics, Samsun, Turkey*
ORCID: 0000-0001-8901-713X [a]*; ORCID: 0000-0003-1408-2548* [b]

MAKALE BİLGİSİ / ARTICLE INFORMATION:

Geliş / Received:
22 Ocak 20
22 January 20

Kabul / Accepted:
18 Ağustos 20
18 August 20

Keywords:
Brucellosis
Abortion in ruminants
Infertility
Epidemiology
Register-based study

Anahtar Sözcükler:
Brusellozis
Ruminantlarda abort
İnfertilite
Epidemiyoloji
Kayıt-tabanlı çalışma

ABSTRACT:

The objective of this study is to epidemiologically describe large and small ruminant Brucellosis in Turkey by conducting a register-based study. For this purpose, the data concerning Brucellosis which is a notifiable disease in Turkey, were obtained from anonymous disease reports of the World Organization for Animal Health. Descriptive and analytical statistics used in that study, in the logistic regression analysis, odds ratios were calculated within a 95 % confidence interval. In cattle, 7,889 outbreaks of Brucellosis were registered in all 81 provinces of Turkey between 2005 and 2015 were 15,059 animals contracted the disease. In sheep and goats, 2,277 outbreaks of Brucellosis occurred in the same time period, where 21,241 animals also got sick. There was a decreasing trend in Brucellosis both in humans and in ruminants after the new combating strategy in 2012. The disease in cattle was mostly seen in winter and the highest prevalence was in the East Anatolian Region. Furthermore, Brucellosis in small ruminants was mostly seen in the Central Anatolian region during the winter months. Both species (large and small ruminants) have the lowest rate of outbreaks in the summer months.

Türkiye'deki sığır ve koyun brusellozunun değerlendirilmesi

ÖZET:

Sunulan bu çalışmada Türkiye'de büyük ve küçük ruminantlarda yavru atımına neden olan enfeksiyöz hastalıkların kayıt tabanlı epidemiyolojik bir çalışma ile ortaya konulması amaçlandı. Bu amaçla ihbarı mecburi Brusellozis hastalığında ulusal sistemlere kaydedilen ve Dünya Hayvan Sağlığı veri tabanında herkese açık olarak yayınlanan kayıtlardan yararlanıldı. Bu çalışmada betimsel ve analitik istatistik kullanılmıştır, lojistik regresyon analizinde %95 güven aralığında hesaplanmıştır. 2005-2015 yılları arasında ülkemizde 81 il genelinde 7.889 büyükbaş Brusellozis mihrakı görülmüştür, hastalığa yakalanan hayvan sayısı 15.059 adettir. Aynı tarihler arasında 2.277 küçükbaş Brusellozis mihrakı görülmüş ve 21.241 adet küçükbaş hayvan hastalığa yakalanmıştır. Brusella hastalığı, 2012 yılındaki yeni mücadele stratejisi sonrasında insanda ve gevişgetiren hayvanlarda azalma eğilimindedir. En yüksek prevalans büyükbaş hayvanlarda Doğu Anadolu illerinde, küçükbaş hayvanlarda ise İç Anadolu'da ve kış aylarında en yüksek düzeydedir. Her iki türde de yazın salgınlar en düşük seviyededir.

How to cite this article: **Demeli A, Fındık M:** Assessment of cattle and sheep Brucellosis in Turkey. *Veteriner Hekimler Dernegi Dergisi,* 92(1): 7-15, 2021, DOI: 10.33188/vetheder.678481

* *Sorumlu Yazar e-posta adresi / Corresponding Author e-mail address:* mfindik@omu.edu.tr

1. Introduction

Abortions cause serious reproduction problems in small and large ruminants. The increasing meat demand in Turkey and around the world seen diseases, new disease-causing agents and animal welfare issues making abortion problems a major priority. Moreover, some diseases are zoonotic and threaten human health. The most common infectious agent for ruminant abortion in Turkey is Brucellosis, which has been monitored by the Turkish Veterinary Service since the 1930s.

Infectious agents take a leading role in small ruminant infertility. *B. melitensis*, which causes Brucellosis in these species, is the most dangerous and prevalent bacteria in human Brucellosis, because sheep and goat farming are mostly practised in rural areas of developing countries by indigent farmers the eradication programmes of the disease in these animals are often neglected (2).

The aetiological agent of Brucellosis was identified as the cause of deadly Malta Fever by Dr. David Bruce for the first time in 1886. Danish Veterinary Surgeon L. F. Benhard Bang isolated *B. abortus* from a cattle foetus in 1895 (12). Brucellosis in Turkey was first seen during the importation of high yield cattle in the 1930s, and the first isolation occurred in 1931 (4).

Brucella agents are non-spore-forming, nonmotile, gram-negative, intracellular pathogens that live in the reticuloendothelial system in the reproductive cells of the organism. They usually cause chronic infections that persist for life. Brucella pathogens can live for a long-time outside of the host in optimal conditions. They live for 6 months in a carcase at 0 ºC, 125 days on the land, and 1 year in faeces. They are susceptible to most disinfectants (12). Brucellosis in cattle is mainly caused by *B. abortus* however *B. melitensis*, which is the cause of Brucellosis in sheep and goats, can spread to cattle. *B. abortus* in cattle is widespread and it causes production losses in dairy and beef farms, therefore, there are eradication schemes in most countries for this disease (11).

Infected cattle usually abort only once, or rarely more than once, however, the placenta is colonised by Brucella agents in each pregnancy. Subsequent calves might be born weak or healthy in appearance. They can take the agent during the intrauterine life or by drinking the contaminated mother's milk, so these animals become carriers (12).

B. melitensis, which seen in small ruminants, is the most invasive and pathogenic species amongst the Brucella agents, is the most common in humans. Besides protecting both human and animal health, the biggest benefits of the disease combatting schemes are to increase animal production, to eliminate costs of treatment and hospitalization of people due to the illness, as well as to prevent from labour losses.

Brucellosis is prevalent in Mediterranean States such as Turkey; Greece; Italy; Portugal; Spain; North African countries, sub-Saharan Africa; the Arabian Peninsula; India; China and South America. On the other hand, the disease has been eradicated in Northern European countries such as Sweden, Denmark and the United Kingdom (UK). The first eradication occurred in Norway; Australia; New Zealand and Canada which are now free from Brucellosis. Russia has been combating the disease for a long-time and they use their vaccine called SR82 which was developed in 1970. Some parts of the country have an officially free status from the disease. Brucella eradication began in the United States of America (USA) in 1934, and the country is now mostly Brucella free. While France was previously free, the disease reappeared in that country again in 2010. Brucellosis is widespread among our neighbours: Armenia, Iran, Azerbaijan, Iraq, and Syria (3, 6, 8, 11).

Many combating strategies are used in both national and international levels. The approaches to diagnosing and preventing Brucellosis (for example vaccination of all animals, culling of positive ones, and the vaccination of calves etc.) have been accepted and standardised internationally. What should be taken into consideration while deciding on the strategy is the organisational quality of the veterinary service, prevalence of the disease and their economic resources (2). Consideration should be given to reducing the prevalence first, after eradication takes place. It would create great economic losses, if an epidemic country culled all positive cases and paid compensation. After keeping Brucellosis under control, it will be easier to eradicate it. Then, new outbreaks and reoccurrence of the disease could be prevented by early warning systems. Furthermore, Brucellosis in wild-life always exists as a potential threat (2).

2. Material and Methods

This study contains register-based epidemiological analyses. For Brucellosis, which is a notifiable disease in Turkey, the World Organisation for Animal Health (OIE), and the World Animal Health Information System (WAHIS) data, based on the national database, are used. Animal population data were obtained from the Turkish Statistical Institute, and the large ruminant figures. Small ruminant populations were not used in the project.

According to national regulations, all aborted animals must be tested for Brucellosis. Aborted materials (e.g. foetus, discharge, placenta) are sent to the National Veterinary Institute of each Turkish province by an official veterinarian, who works in the district or province directory of the Ministry. The material is examined to detect Brucellosis. Positive cases are registered on Turkish Veterinary System (Turkvet) by the official veterinarian in the district or province. That data is sent to the OIE by the contact person from the headquarters of the Veterinary Service, every 6 months. These results are published publicly on the organization's website.

The study unit of this research project is the Brucella diagnosed and registered female cattle, sheep and goats. Brucella positive male animals are not monitored and there is no record in the system, therefore it is not included in the study. The target population is the 81 provinces of Turkey, and the sub-population is exclusively large and small female ruminants. There were 14 million cattle, 31 million sheep, and 10 million goats in Turkey in the year 2015. According to the animal statistics of the Turkish Statistical Institute, there were approximately 9 million cows over the age of 12 months.

The study period was between 2005 and 2015. All positive female cattle were used for this project, and cattle and buffalo discrimination was not made in the large ruminant data. Considering the biological age of reproduction, cattle under the age of 12 months were excluded from the population data.

There are descriptive and analytical statistics in that study. Chi-square and Fisher's Exact Test were used to analyse the statistical significance of the contingency tables. Median, mean and standard deviation was shown. In the logistic regression analysis, odds ratios were calculated within a 95 % confidence interval. The p-value threshold between the groups was 0.05.

Statistical calculations and graphics were made by R software and its packages. Outbreak maps were made by QGIS. Since this research used data collected by other organisations and individuals already based on records and animal experiment, biological samples and genetic material were not made. Therefore, Ethics Committee approval is not required.

3. Results

According to the World Organisation for Animal Health (OIE) registers, which were based on Turkish Brusellozis data, there were 7,889 large ruminant outbreaks around Turkey. During this period, 15,059 animals (cases) caught the disease. Three of a total 81 Turkish provinces (Kilis, Yalova, and Zonguldak) had no outbreaks during those dates.

There was an increase in cattle Brucellosis outbreaks in autumn and winter. The number of outbreaks rose from 2005 to 2012, and then showed a decreasing trend. The highest outbreaks were seen in 2012, and the lowest was in 2005. The peak month of Brucellosis was in January, the lowest month was July, increasing slightly in August and September.

Cattle outbreaks were concentrated in the Eastern Anatolia region with a total of 3,382 outbreaks in 2005-2015, and the lowest figures were in the Marmara region with 205. Considering the number of female animals over 12 months in all the regions, only the Aegean and the Mediterranean regions had changed in outbreak numbers according to the animal population, however, the ranking of other regions had not changed (Table 1-2).

Table 1: The monthly distribution of Large and Small Ruminant Brucellosis Outbreaks
Tablo 1: *Büyük ve küçük ruminant Brusellozis mihraklarının aylara göre dağılımı*

Months	2005-2011		2012		2013		2014		2015		Total	
	L.Rum	S.Rum	L.Rum	S.Rum	L.Rum	S.Rum	L.Rum	S.Rum	L.Rum	S.Rum	L.Rum	S.Rum
January	465	233	132	45	392	232	204	28	68	13	1,261	551
February	471	234	220	54	247	185	128	22	120	29	1,186	524
March	392	107	219	17	173	48	73	5	140	34	997	211
April	295	66	148	9	91	3	29	1	132	24	695	103
May	230	66	132	10	60	0	16	2	110	18	548	96
June	184	54	67	4	13	0	13	0	83	11	360	69
July	165	38	33	2	17	2	7	0	45	1	267	43
August	169	39	46	5	11	0	9	1	37	1	272	46
September	175	61	54	3	21	3	12	1	30	3	292	71
October	166	68	114	2	41	4	3	1	32	9	356	84
November	287	130	211	16	96	13	49	7	65	21	708	187
December	292	175	319	55	157	22	58	10	121	30	947	292
Total	3291	1271	1,695	222	1,319	512	601	78	983	194	7,889	2,277

Table 2: The Large and small ruminant Brucellosis outbreaks by regions
Tablo 2: *Büyük ve küçük ruminant Brusellozis mihraklarının bölgelere göre dağılımı*

Regions	2005-2011		2012		2013		2014		2015		Total	
	L.Rum	S.Rum	L.Rum	S.Rum	L.Rum	S.Rum	L.Rum	S.Rum	L.Rum	S.Rum	L.Rum	S.Rum
Aegean	201	175	57	19	38	32	8	9	10	5	314	240
Black Sea	744	161	579	74	440	151	149	16	190	66	2,102	468
East. Anatolia	1093	163	715	24	611	27	336	13	627	28	3,382	255
Inner Anatolia	784	293	175	42	127	118	75	15	65	45	1,226	513
Marmara	152	291	16	35	16	26	5	15	16	37	205	404
Mediterranean	120	131	81	20	27	132	6	9	12	11	246	303
S. East Anat.	197	57	72	8	60	26	22	1	63	2	414	94
Total	3,291	1,271	1,695	222	1,319	512	601	78	983	194	7,889	2,277

The difference in the distribution of outbreaks according to the seasons was statistically significant ($p < 0.05$). The logistic regression analysis showed that winter had the highest value followed by autumn and summer, and spring was chosen as a reference category (Table 3).

Table 3: Seasonal distribution of Brucellosis in Turkey
Tablo 3: *Türkiye'de Brusellozisin mevsimsel dağılımı*

Category	Estimate	Std. Error	OR (95% CI)	P value
Spring(Intercept)	0	0	ref	P<0.001
Winter	1.0077	0.4019	2.73 (1.24-6.02)	
Autumn	-0.9407	0.4482	0.39 (0.16-0.93)	
Summer	-1.5607	0.4736	0.20 (0.082-0.053)	

As reported by the registers, 412 of the 15,059 bovine cases with Brucellosis had died, and 2,201 were destroyed because they were not suitable for consumption. The remaining 12,446 cattle were slaughtered to consume with heat treatment. The lethality rate, or the case fatality rate, (the mortality rate of infected animals) was 3.9 % between the dates mentioned (Table 4).

Table 4: The number of dead, destroyed and slaughtered animals due to Brucellosis
Tablo 4: *Brusellozisten dolayı ölen, imha edilen ve kesilen hayvan sayıları*

Years	Died	Destroyed	Slaughtered	Cases	Case-Fatality Rate (%)
2005-2011	285	201	8490	8974	4.95
2012	57	1,517	760	2,336	2.44
2013	42	316	1,979	2,337	1.8
2014	18	108	733	859	2.1
2015	10	59	484	553	1.81
Total	412	2,201	12,446	15,059	(mean 3.9)

Considering the animal population of the provinces, there was no significant change in the ranking between regions. Also, there was a positive correlation between the number of animals and the number of Brucellosis outbreaks (Figure 1).

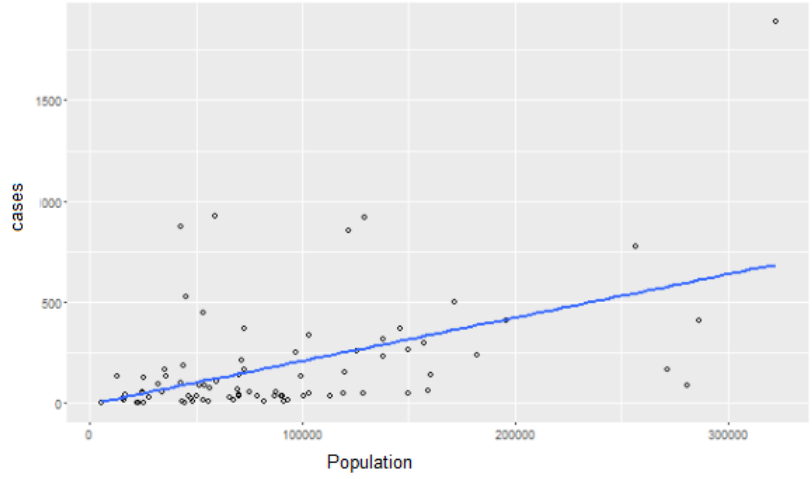


Figure 1: Correlation between population and cases

Şekil 1: *Popülasyon ve olgular arasında korelasyon*

According to The World Organisation for Animal Health (OIE) registers, which were based on Turkish Brucellosis data, there were 2,277 small ruminant outbreaks around Turkey (Table 1), and total 21,241 animals caught the disease. In Bartın and Batman provinces, B. melitensis had not seen between 2005 and 2015.

Outbreaks in autumn and winter peaked with 512 in 2013 and went back to 78 in 2014, which was the lowest number. January was the most intensive month for sheep and goat Brucellosis, and July was the lowest.

Small ruminant Brucellosis were concentrated in the Marmara region with a total of 404 outbreaks in 2005-2015, and the least in the South East Anatolia region with 94 outbreaks. The cases (number of sick animals) were seen mostly in the Central Anatolia and the least number was in the Eastern Anatolia region and the least numbers were in the Eastern Anatolia region over the period (Table 1, Figure 2-3).

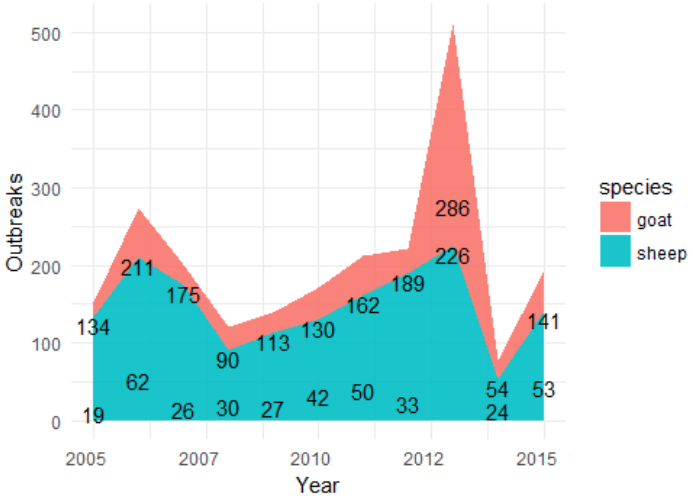


Figure 2: Sheep and goat Brucellosis in Turkey, 2005-2015

Şekil 2: *Türkiye'de koyun ve keçi Brusellozisi, 2005-2015*

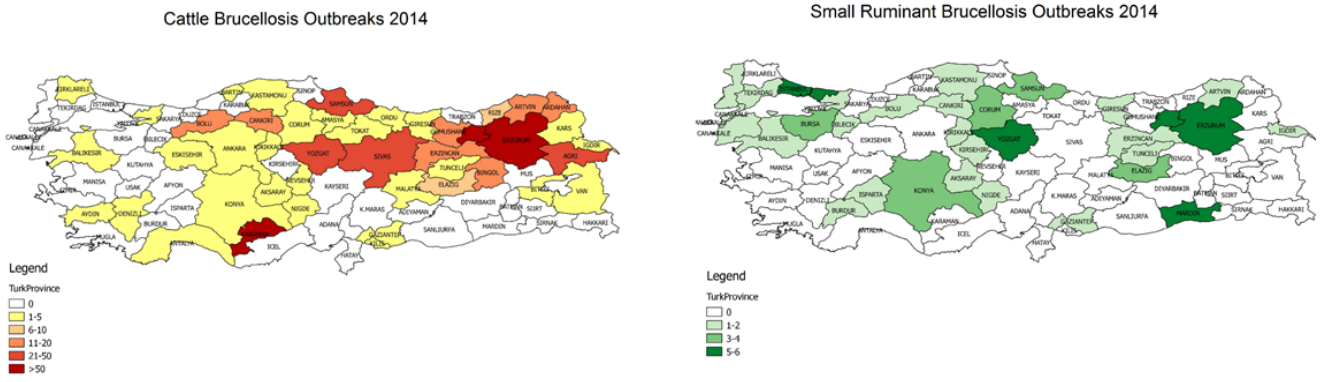


Figure 3: Brucellosis maps of large and small ruminants in Turkey

Şekil 3: *Türkiye'de büyük ve küçük ruminantlarda Brusellosiz haritaları*

It can be seen that there was a decline in human Brucellosis from 2005 to 2015 (Table-5). Two deaths, due to Brucellosis was recorded over a two-year period in 2005 and 2008.

Table 5: Human Brucellosis Cases (OIE, Wahis)

Tablo 5: *İnsan Bruselloz Vakaları (DHÖ, Wahis)*

Year	Human cases	Died
2005	14644	1
2006	10790	0
2007	11803	0
2008	9818	1
2009	9324	0
2010	7658	0
2011	7177	0
2012	6759	0
2013	7225	0
2014	4475	0
2015	4173	0

4. Discussion and Conclusion

This study was conducted to determine the distribution of Brucellosis in Turkey based on national data in recent years. It covered the 11-year period, from 2005 to 2015.

According to the distribution of the Brucellosis outbreaks during the year, the highest numbers were observed in December, January and February months. Considering the periods in which cows give birth in our country; the abortion cases are considered to be in the 5-7th month of pregnancy.

Another important finding is the decrease in human Brucellosis cases and deaths. This may be related to disease control in ruminants, or to the widespread use of pasteurized milk and dairy products instead of raw milk. In Italy, for example, the Northern regions are officially free from Brucellosis and human cases are sporadic. However, in the Southern regions, Brucellosis is high in animals and seroprevalence in humans is about 3%. Also, it is common to sell raw milk and milk products directly in local markets in the Southern regions of Italy (9). In a study conducted in the El Behira region in Egypt, it was reported that human cases and animal Brucellosis progressed in parallel (5).

The case/fatality rate (the lethality) due to Brucellosis was 3.9 % between the dates 2005 and 2015. It is reported that the mortality rate in animals aborted from Brucellosis is around 1% (1). The 16% recorded in 2005 may have been a registration error, and when this data is removed, the mortality rate drops to 2.6%.

Due to the geographical distribution of the disease, large and small ruminant outbreaks accumulated in the Eastern and Central Anatolia regions respectively. The least number of outbreaks were observed in the Marmara region for large ruminants and the South East Anatolia for small ruminants.

A two-fold increase in outbreaks was observed after 2012 when the vaccination strategy was changed and vaccination of all small ruminants was implemented (Figure 2). This increase was thought to be due to vaccine-induced false positivity or false infection.

It is debatable that eradication of a disease is beneficial and even possible to eradicate. A systematic approach is required before, during and after the eradication program. Moreover, eradication approaches varied from each other (7). Thanks to epidemiological studies, the existence and prevalence of the disease in our country will be able to be understood better, and the competent authorities will be able to take measures to protect and control the disease in accordance with that knowledge.

The studies showed that the outbreaks are concentrated during the winter period, when animals are usually kept in barns to keep warm; hence, the spread of the disease between animals inside barns can be prevented by strict biosecurity measures during that time. The seasonality of the disease must be considered as a combat strategy. It was observed that the positive correlation between animal population and the disease. Personal and equipment requirement of eradication schemes should be planned due to these findings. Especially in the rural areas where people keep livestock animals intensively, the sufficient number of personnel should be charged to monitor the disease, take the control measures and train the farmers. The medical and technical experts should be well-educated and experienced; it should be close collaboration with universities and international institutes.

Under-reporting of animal diseases is a common problem around the world, including developed countries (3, 10). To minimise this issue, private and government veterinarians and farmers should be educated on abortions, biosecurity and legal requirements; brochures and public service announcements should be produced and circulated to increase disease awareness. Farmers should cooperate with the component authority while combating the disease to gain their confidence. It should also be mentioned that the benefits of the eradication of the disease in the awareness programs. Active surveillance should be conducted as much as possible as well passive surveillance. The information collected from the farmers should be accurately collected using better-designed questionnaires/surveys and the collected and summarised information should be more accurately analysed.

Conflict of Interest

The author declared no conflict of interest.

Funding

No funding.

Authors' Contributions

Idea / concept: Anıl Demeli, Murat Fındık
Experiment design: Anıl Demeli
Supervision / Consultancy: Murat Fındık
Data collecting: Anıl Demeli
Data analysis and interpretation: Anıl Demeli, Murat Fındık
Literature search: Anıl Demeli
Writing the article: Anıl Demeli
Critical review: Murat Fındık

Ethical Approval

An ethical statement was received from the authors that the data, information and documents presented in this article were obtained within the framework of academic and ethical rules and that all information, documents, evaluations and results were presented in accordance with scientific ethics and moral rules. A permission was granted for publishing Brucella data by Republic of Turkey, Ministry of Agriculture and Forestry (Date: 13.10.2017; number: 604.01.01 / E.2556063).

References

1. **Bernues A, Manrique E, and Maza M** (1997): *Economic evaluation of bovine brucellosis and tuberculosis eradication programmes in a mountain area of Spain*. Preventive veterinary medicine, **30**, 2, 137-149.
2. **Blasco JM, Molina-Flores B** (2011): *Control and eradication of Brucella melitensis infection in sheep and goats*. Veterinary Clinics: Food Animal Practice, **27**, 1, 95-104.
3. **Bronner A, Hénaux V, Fortané N, Hendrikx P, Calavas D** (2014): *Why do farmers and veterinarians not report all bovine abortions, as requested by the clinical brucellosis surveillance system in France?* BMC veterinary research, **10**, 1, 93.

4. **Can M** (2010): *Türkiye'de Brusella abortus ve Brusella melitensis enfeksiyonlarından kaynaklanan finansal kayıplar ve alternatif brusella kontrol stratejilerinin maliyet-fayda analizleri*. Ankara Üniversitesi, Ankara.
5. **El-Ghitany E, Omar S, Abaza A, Hassan EM** (2014)*: Trends in the Epidemiology of Brucellosis in a Highly Afflicted Region in Egypt: A 16 Year Experience (1997-2012).* Int J Trop Dis Health, **4**, 2, 250-271.
6. **Erganiş O** (2010): *Dünya'da Brusellosis Eradikasyon Programları ve Ülke Modelleri.* Türkiye Zoonotik Hastalıklar Sempozyumu. p. 30-53.
7. **Houe H, Nielsen LR, and Nielsen SS** (2014): *Control and eradication of endemic infectious diseases in cattle.* College publications, Copenhagen 117-125.
8. **Ivanov AV, Salmakov KM, Olsen SC, Plumb GE** (2011): *A live vaccine from Brucella abortus strain 82 for control of cattle brucellosis in the Russian Federation.* Animal Health Research Reviews, **12**, 1, 113-121.
9. **Mancini F, Bella A, Graziani C, Marianelli C, Mughini-Gras L, Pasquali P, Pompa MG, Rizzo C, Rizzuto E, Busani L** (2014): *Trends of human brucellosis in Italy, 1998–2010.* Epidemiology & Infection, 142, **6**, 1188-1195.
10. **Martins G, Lilenbaum W** (2014): *Leptospirosis in sheep and goats under tropical conditions.* Tropical animal health and production, **46**, 1, 11-17.
11. **Noakes DE PT, England GCW, Arthur GH** (2001): *Arthur's Veterinary Reproduction and Obstetrics.* Saunders, Toronto.
12. **Post K, Songer G** (2005): *Microbiology Bacterial and Fungal Agent of Animal Disease.* Els Saunders Microbiol, 19, 134-44.

VETERİNER HEKİMLER DERNEĞİ DERGİSİ
JOURNAL OF THE TURKISH VETERINARY MEDICAL SOCIETY

ISSN: 0377-6395
e-ISSN: 2651-4214
Dergi ana sayfası:
Journal homepage:
http://dergipark.org.tr/vetheder

DOI: 10.33188/vetheder.714491

Araştırma Makalesi / Research Article

Determination of the presence and antibiotic resistance of *Listeria* species and aerobic mesophilic bacteria count of cow milks

Orkun BABACAN [1,a*]

[1] *Balıkesir University Kepsut Vocational School Department of Veterinary,Kepsut Balıkesir, 10660 Turkey*
ORCID: 0000-0003-0258-1825 [a]

MAKALE BİLGİSİ /

ARTICLE INFORMATION:

Geliş / Received:
04 Nisan 20
04 April 20

Kabul / Accepted:
01 Ekim 20
01 October 20

Keywords:
Aerobic mesophilic bacteria
Antibiotic resistance
Cow
Listeria
Milk

Anahtar Sözcükler:
Aerobik mezofilik bakteri
Antibiyotik direnci
İnek
Listeria
Süt

ABSTRACT:

Listeria species lead to mastitis infection in cows. The aerobic mesophilic bacteria count (total bacteria count) is one of the most important factors affecting udder health and determining the milk quality. The aim of this study was to determine the aerobic mesophilic bacteria count, one of the most important factors affecting cow's milk quality, and presence and the antibiotic resistance profiles of *Listeria* spp., one of the factors causing mastitis in cows. As a result of isolation and identification for *Listeria* spp., totally 3 *L. monocytogenes* (n: 68, 4.41%), 7 *L. innocua* (n: 68, 10.29%) and 3 *L. ivanovii* (n: 68, 4.41%) were isolated from cow milk samples. According to results of the disc diffusion method performed to determine antibiotic susceptibility, it was found that *L. monocytogenes*, *L. innocua*, and *L. ivanovii* isolates were susceptive against sulfamethoxazole/ trimethoprim, meropenem, vancomycin, streptomycin, oxacillin and erythromycin. The aerobic mesophilic bacteria in the cow milk samples were detected 1.1x107 cfu/ml as the highest and 2.3x102 cfu/ml as the lowest. The average aerobic mesophilic bacteria count of milk samples was calculated 256623.971 cfu/ml. The total bacteria (aerobic mesophilic bacteria) count (cfu/ml) of milk samples in the study was found to be high based on the criteria stated in the national and international standards. Also, *Listeria* species were isolated from these samples. Since intermediate and resistant *Listeria* species were determined against the antibiotics used as a treatment option in these isolates, it is thought that Listeria species should also be considered in mastitis infections in terms of etiology and treatment. It is considered that a national mastitis control program is needed for preventing the mastitis infections and antibiotic resistance development causing economic losses in dairy cattle enterprises in order to provide milking hygiene completely.

İnek sütlerinde Listeria türlerinin varlığı ve antibiyotik direnci ile aerobik mezofilik bakteri sayısının belirlenmesi

ÖZET:

Listeria türleri ineklerde mastitis infeksiyonuna da neden olmaktadır. Aerob mezofilik bakteri sayısı (toplam bakteri sayısı), meme sağlığını etkileyen ve süt kalitesini belirleyen en önemli parametrelerden biridir. Bu çalışmada inek sütü kalitesini etkileyen en önemli parametlerden olan aerob mezofilik bakteri sayısı ve ineklerde mastitise neden olan etkenlerden olan *Listeria* spp. varlığının ve antibiyotik direnç profillerinin belirlenmesi amaçlandı. *Listeria* spp. için izolasyon ve identifikasyon sonucunda toplam 3 *L. monocytogenes* (n: 68,% 4.41), 7 *L. innocua* (n: 68,% 10.29) ve 3 *L. ivanovii* (n: 68,% 4.41) inek sütü örneklerinden izole edildi. Antibiyotik duyarlılığını belirlemek için yapılan disk difüzyon yönteminin sonuçlarına göre *L.monocytogenes, L.innocua* ve *L.ivanovii* izolatlarının sülfametoksazol/ trimetoprim, meropenem, vankomisin, streptomisin, oksasilin ve eritromisine karşı duyarlı oldukları bulundu. İnek sütü örneklerinde aerob mezofilik bakteri sayısı en yüksek 1.1x107 cfu/ml; en düşük ise 2.3x102 cfu/ml bulundu. Süt örneklerinin ortalama aerob mezofilik bakteri sayısı 256623,971 cfu/ml olarak bulundu. Çalışmada süt örneklerinin toplam bakteri (aerob mezofilik bakteri) sayısının (cfu/ml) ulusal ve uluslararası standardlarda belirtilen kriterlere göre yüksek bulundu, aynı zamanda bu örneklerden *Listeria* türlerinin izole edildi. Ayrıca, bu izolatlarda tedavi seçeneği olarak kullanlan antibiyotiklere karşı orta (ilaçla artmış temeasta duyarlı-I-intermediate) ve dirençli *Listeria* türleri tespit edildiğinden, mastitis infeksiyonlarında etiyolojik ve tedavi açısından *Listeria* türlerinin de göz önünde bulundurulması gerektiği düşünüldü. Sağım hijyeni tam anlamıyla sağlanması için, süt ineği işletmelerinde ekonomik kayıplara neden olan mastitis infeksiyonlarınnı ve antibiyotik direnci gelişiminin önlenmesi amacıyla ulusal düzeyde mastitis kontrol programına ihtiyaç duyulduğu düşünüldü.

How to cite this article: Babacan O: Determination of the presence and antibiotic resistance of Listeria species and aerobic mesophilic bacteria count of cow milks. *Veteriner Hekimler Dernegi Dergisi*, 92(1): 16-23, 2021, DOI: 10.33188/vetheder.714491

* *Sorumlu Yazar e-posta adresi / Corresponding Author e-mail address:* orkun_babacan@hotmail.com

1. Introduction

Mastitis is defined as an inflammatory response in udder glands leading to economic losses due to non-use of milk in dairy cattle enterprises and treatment expenses, impairing milk quality, and being caused by the bacterial, chemical, and traumatic reasons or heat (6,7,15,24,38). Mastitis are classified as clinical and sub-clinical mastitis. The ones not causing clinical symptoms and visible change in udder tissue and milk are classified as "subclinical" and those causing more or less swelling, pain, temperature increase and color change in sick udders by causing visible symptoms are classified as "clinical". Subclinical mastitis infects easily among cows and these kinds of mastitis cause economic loss in the enterprises. In the clinical mastitis, disorders such as decrease in milk yield, agalactia, presence of watery secretions and clotting may develop (6,11).

The aerobic mesophilic bacteria count (total bacteria count) is one of the most important parameters determining the udder health and milk quality (16,24,28). The total bacteria count in raw cow milk must be <100.000 (piece) in milliliter (ml) at 30°C according to raw and heat-treated drinking milk communique (2) in the Turkish Food Codex numbered 2000/6 published in the Official Gazette dated 14/02/2000 and numbered 23964 and the EU regulation dated 30/04/2004 and numbered 853/2004 (3,4).

There are National Mastitis Councils in the developed countries in order to eliminate mastitis. Nationwide struggle with mastitis is performed with the National Mastitis Control Programs prepared by these councils (6,7,38) The targets in the mastitis control programs are stated to be the elimination of the current infections, protecting from new infections and following udders constantly in terms of mastitis. It has been reported that all cows in a herd is enabled to be protected simultaneously by the udder health control program. The protection from the udder infections has been achieved at the rate of 80-90% in the herds in which the udder health control programs are applied for a long time (6,31).

Listeria genus is composed of totally 17 species, identified within the 2 groups, which are gram-positive, small rod-shaped bacteria surviving in the environments containing low pH and temperature and high salt (5,29,36). One of these groups is *Listeria* sensu stricto including *Listeria monocytogenes*, *Listeria seeligeri*, *Listeria marthii*, *Listeria ivanovii*, *Listeria welshimeri*, and *Listeria innocua* and the other is *Listeria* sensu lato including *Listeria grayi* and 10 *Listeria* species recent identified as of 2009 (29). Among these species, only *L. monocytogenes* and *L. ivanovii* identified to be pathogenic species (29,36). The species in *Listeria* sensu stricto group exhibit high adaption in soil and water (26). *Listeria* species are also observed in farms. Especially, they are frequently found in fertilizer and fermented silage (8,36). For this reason, animals may be infected through bad quality silage, pathogenic inhalation, direct contact, water, forage components, litter mat, soil and dung (5). *Listeria* species (12), which are ubiquiter and intracellular pathogen, cause abortion, stillbirth, encephalitis and neonatal septicemia in human beings and animals (5,23,30). Also, *Listeria* species cause mastitis infection in cows (5,23). *L. monocytogenes* causes encephalitis, abortion, septicemia, ocular form and mastitis infections in cows (27). Also, *L. monocytogenes* is an opportunistic pathogen and causes especially important food-borne infections in human beings as well as the infections it causes in animals (10). Particularly elderly people, pregnant women, newborns and the people with a suppressed immune system are quite susceptible to these infections (25,36). *L. ivanovii* is a predominant pathogenic species in animals (8) and causes clinical infections in cows and sheep causing abortion and stillbirth (26,27). In their study, Alexander et al. (2) declared *L. ivanovii* is the potential abortion agent in cows. Rocha et al. (34) isolated *L. innocua* from fatal meningoencephalitis case in fattening bull and this was the first. *Listeria* species, mainly *L. monocytogenes*, have been isolated from cow milk and identified to be clinical mastitis cause in cows in numerous studies. Haggag et al. (19) was isolated and identified *L. monocytogenes* (2.4%), *L. ivanovii* (2%), *L. innocua* (1.2%), and *L. grayi* (2.8%) in totally 250 milk samples including 150 cows, 50 buffaloes, and 50 sheep. Jamali et al. (24) was isolated 17 L. monocytogenes, *3 L. innocua*, and 1 *L. ivanovii* from 201 milk samples obtained from clinical mastitis of cows.

Penicillin and/or its derivatives are an effective treatment option in the infections in animals caused by *Listeria*. Gentamicin provides an effective treatment in the genital system listeriosis infections of cows. Additionally, erythromycin and trimethoprim/sulfamethoxazole may be useful in septicemic form treatment (27). Also, ampicillin, chloramphenicol, rifampicin, tetracycline and aminoglycoside antibiotics may be used in treatment (5).

In this study looked at the aerobic mesophilic bacteria count, one of the most important parameters affecting cow milk quality, and presence and the antibiotic resistance profiles of *Listeria* spp., one of the factors causing mastitis in cows.

2. Material and Methods

In the study, 68 cow milk samples, which were taken from Holstein and Simental cows and sent to laboratory for analysis by veterinarians, were examined in terms of the prevalence of *Listeria* spp. and the aerobic mesophilic bacteria count. The milk samples were collected aseptically from each cow to sterile container during milking and sent them to laboratory under aseptic and cold chain conditions (+2-+8°C) and their analyses were performed immediately.

Detection of *Listeria* species:

The cow milk samples were analyzed for *Listeria* spp. isolation based on ISO 11290-1 method (20).

25 milliliters from each sample were put into the sterile sample bags and homogenized in stomacher (Bigmixer, Intersience) by 225 ml sterile Half-Fraser Broth (Merck, Germany) for 1 minute. The homogenized samples were incubated at 30 °C for 24±2 hours for pre-enrichment. And then, 0.1 ml of the samples was taken and these samples were inoculated to Fraser Broth (Merck, Germany), a pre-enrichment, and incubated at 37 °C for 24-48 hours. And then, Fraser Broth's of each sample was inoculated in selective agars Ottoviani and Agosti (Merck, Germany), PALCAM (Oxoid, UK) and Rapıd' L. mono agars (Bio-Rad, USA) and incubated at 37 °C for 24-48 hours (20). In Rapid' L. mono agar (Bio-Rad, USA), *L. monocytogenes* colonies in green color, *L. ivanovii* colonies reproduce in blue color by forming a yellow zone around them. *L. innocua* colonies reproduce in white color without forming a zone around them (12).

After incubation, the colonies were confirmed by gram staining, catalase, oxidase and mobility tests based on Bergey's Manuel of Systematic Bacteriology (35). Afterwards, he isolates were verified in automatic identification system (Vitek 2 Compact). *Listeria* spp. suspected colonies were arranged in tubes with 3 ml sterile salty water based on McFarland 0.5-0.63 turbidity and identified with Gram positive identification card in Vitek 2 Compact (Biomerieux, France) device. Afterwards, verified strains were kept by being taken into bead storage tubes and kept at (-20) °C.

Aerobic mesophilic bacteria count:

In order to determine the aerobic mesophilic bacteria count, 10 ml milk sample was diluted with 90 ml Maximum Recovery Diluent (MRD, Merck, Germany). Then, each milk sample was diluted 10 times up to 10^9. One ml was taken from each dilution and they were inoculated in two petri dishes by pour plate inoculation technique in Plate Count Skimmilk Agar (Merck, Germany) and incubated at 30°C for 72 hours. And then, the aerobic mesophilic bacteria count of the milk samples was calculated in colony forming unit (cfu)/ml (21,22).

Investigation of antibiotic susceptibility of isolated *Listeria* isolates:

Antibiotic susceptibility was investigated according to disc diffusion method based on European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) (9,13,14,18). For this aim, in first step, the isolates were taken from the bead storage tubes and inoculated to Nutrient Broth (Merck, Germany) and incubated at 37 °C for 24 hours. And then, they were inoculated in Rapıd' L. mono (Bio-Rad, UK) agar and incubated at 37 °C for 24-48 hours. All pure *Listeria* spp. isolates were diluted in tubes containing 3 ml sterile salty water with McFarland 0.5 turbidity. The sterile swabs were immersed to the bottom of the tubes. Then, the remaining liquid was removed by applying pressure inside tubes and it was inverted into the tubes. The inoculum was spread evenly over the entire surface by swabbing in three directions to Mueller-Hinton F agar (Merck, Germany). It was incubated at 35+/-1 °C for 16-20 hours. Gentamicin (Oxoid, 10µg), meropenem (Oxoid, 10µg), amoxicillin clavulanic acid (Oxoid, 30µg), streptomycin (Oxoid, 10µg), chloramphenicol (Oxoid, 30µg), ciprofloxacin (Oxoid, 5µg), amikacin (Oxoid, 30µg), ampicillin (Oxoid, 10µg), erythromycin (Oxoid, 15µg), trimethoprim-

sulfamethoxazole (Oxoid, 25µg), penicillin G (Oxoid, 10U), rifampicin (Liofilchem, 5µg) oxacillin (Oxoid, 1µg) and tetracycline (Oxoid, 30µg), vancomycin (Oxoid, 30µg) were used.

After incubation, the inhibition zones were measured on Mueller-Hingon F agar plates and evaluated as resistant (R), intermediate (I) and susceptible (S) based on the breakpoints suggested by EUCAST (18) for *L. monocytogenes* on ampicillin, penicillin G, oxacillin, erythromycin, trimethoprim-sulfamethoxazole, meropenem and for the other antibiotic discs commented based on the breakpoints suggested by CLSI (14) for other Gram-positive bacteria (1,13,14,18). *Escherichia coli* (ATCC ® 25922™) were used as reference on antibitoic susceptability tests.

3. Results

Three *L. monocytogenes* (n:68, 4.41%), 7 *L. innocua* (n:68, 10.29%) and 3 *L. ivanovii* (n:68, 4.41%) were isolated from cow milk samples (Table 1).

Table 1: Isolation and identification results of *Listeria* spp. from cow milk samples

Tablo 1: *İnek sütlerinden Listeria spp. izolasyon ve identifikasyon sonuçları*

***Listeria* species**	**Number of positive samples**
L. monocytogenes	3 (4.41%)
L. innocua	7 (10.29%)
L. ivanovii	3 (4.41%)
Total	13 (19.11%)

In antibiogram tests, *L. monocytogenes*, *L. innocua,* and *L. ivanovii* isolates were susceptible against sulfamethoxazole/trimethoprim, meropenem, vancomycin, streptomycin, oxacillin, and erythromycin. Table 2 shows the disc diffusion test results of *Listeria* spp. isolates.

Table 2: Disc diffusion test results of *Listeria* spp. isolates

Tablo 2: *Listeria spp.'lerin disk difüzyon test sonuçları*

Antibitotic discs	***Listeria monocytogenes strains* (n:3)**			***Listeria innocua* strains (n:7)**			***Listeria ivanovii strains (n:3)***		
	S	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
Amikacin (Oxoid, 30µg)	1	1	1	7	-	-	2	1	-
Amoxicillin-clavulanic acid (Oxoid, 30µg)	3	-	-	7	-	-	3	-	-
Ampicillin (Oxoid, 10µg)	2	1	-	6	1	1	2	-	1
Erithromycin (Oxoid, 15µg)	3	-	-	7	-	-	3	-	-
Gentamicin (Oxoid, 10µg)	2	1	-	4	2	1	2	1	-
Chloramphenicol (Oxoid, 30µg)	3	-	-	6	-	1	3	-	-
Meropenem (Oxoid, 10µg)	3	-	-	7	-	-	3	-	-
Penicillin G (Oxoid, 10U)	2	1	-	4	-	3	1	1	1
Ciprofloxacin (Oxoid, 5µg)	3	-	-	5	2	-	3	-	-
Oxacillin (Oxoid, 1 µg)	3	-	-	7	-	-	3	-	-
Rifampicin (Liofilchem, 5µg)	2	1	-	6	1	-	2	1	-
Streptomycin (Oxoid, 10µg)	3	-	-	7	-	-	3	-	-
Tetracyclin (Oxoid, 30µg)	2	1	-	4	2	1	1	1	1
Trimethoprim-Sulfametoxazole (Oxoid, 25µg)	3	-	-	7	-	-	3	-	-
Vancomycin (Oxoid, 30 µg)	3	-	-	7	-	-	3	-	-

Table 3 shows the average aerobic mesophilic bacteria count of each milk sample. The aerobic mesophilic bacteria count in the cow milk samples were found to be 1.1x107 cfu/ml as the highest and 2.3x102 cfu/ml as the lowest. Average aerobic mesophilic bacteria count of the milk samples was calculated 256623.971 cfu/ml.

Table 3: Avarage Aerob mesophilic bacteria count results of cow milk samples

Tablo 3: *İnek sütü örneklerinin ortalam aerob mezofilik bakteri sayımı sonuçları*

Cow milks	Avarage aerob mesophilic bacteria count (cfu/ml)
68	256623.971

4. Discussion and Conclusion

For quality milk, the total bacteria count is quite important. The total bacteria count in the milk obtained in dairy cow enterprises is requested to be low. The most important factor affecting the bacteria count in milk is mastitis infection. Mastitis infections cause inflammation in udder and injury in udder tissue and increases in the total bacteria count and, thus, in the number of somatic cells and give harm to both cows and milk in dairy cow enterprises and therefore cause economic losses. Throughout the world, national mastitis control programs are formed in many countries. Within the frame of these programs, the applications on barn and environment planning, milking management and hygiene, the maintenance and cleaning of milking equipment, teat cleaning and applying antiseptic (teat dipping) on teats before and after milking, monitoring the udders of dairy cows in terms of clinical and subclinical mastitis infections in farms, and dry period management are determined as the standards (6,10).

Dhuol and Osman (17) said total bacteria count in the 30-morning milking and 30 evening milking milk samples was 650.000 cfu/ml on average. Tosun and Baki Acar (6) in Tekirdağ province, they determined that the total bacteria count was 1.796.718.36±156.573.31 cfu/mL in bulk tank samples. Darbaz et al. (16) on cow milk in the Turkish Republic of Northern Cyprus, they reported that the total bacteria count in bulk tank changed based on seasons and the highest average total bacteria count was 182.000 cfu/ml in fall. The aerobic mesophilic bacteria count in this study was found to be higher than limit values determined for raw cow milk in national and international standards. In the studies of the other researchers, this data was higher compared to the criteria. But, the average values of the aerobic mesophilic bacteria count were calculated lower compared to the results of the other researchers. This was considered to be associated with that fact that the milk samples taken from animals were individuals assessed instead of the bulk tank.

There is a limited number of studies on *Listeria* spp. isolated from mastitis cases and antibiotic resistance profiles. Rahimi et al. (32) was examined 85 milk samples isolated 3 (3.5%) *L. monocytogenes*, 5 (5.9%) *L. innocua* and 1 (1.2%) *L. ivanovii* as a result of the identification of cultures. Rawool et al. (33) isolated *L. monocytogenes* from the milk of 1 of the 3 cows with mastitis and from the dung of the other 2 cows and *L. ivanovii* from the dung of 1 buffalo with mastitis, among the milk of 650 cows and buffaloes. Vilar et al. (37) stated that they isolated 6 (6.1%) *L. monocytogenes* and 7 (7.1%) *L. innocua* in 98 bulk tank samples they obtained from cow farms. Konosonoka et al. (25) isolated 3 (1.4 %) *L. monocytogenes* in 221 bulk tank cow milk samples. Yadav et al. (39) stated that they isolated 3 *L. monocytogenes* in 85 milk samples of the cows and buffaloes with clinical mastitis infection. Aksoy et al. (1) stated that they isolated 8 *L. monocytogenes*, 4 *L. seeligeri*, 5 *L. ivanovii*, and 1 *L. welshimeri* in 100 raw cow milk samples after isolation, identification, and polymerase chain reaction (PCR). The isolation findings and isolates rates obtained in this study had shown similarity with the results of other studies. For this reason, it was thought that the *Listeria* species causing mastitis in cows. So, these bacteria should also be considered in the causes of mastitis and treatment stages in mastitis infections.

Today, antibiotic resistance is a wide spreading issue addressed by the World Health Organization (WHO). Aksoy et al. (1) stated that they found intermediate and resistant to amikacin, meropenem, vancomycin and penicillin G, and Trimethoprim-Sulfamethoxazole in 15 *L. monocytogenes* isolates they isolated from raw cow milk, cheese and

butter. Jamali and Redmehr (23) stated resistance against penicillin G, amoxicillin-clavulanic acid, chloramphenicol, erythromycin, and tetracycline at the rates of 66.7%, 23.8%, 19%, 4.8% and 52.4%, respectively, in 17 *L. monocytogenes* isolates they have isolated from the clinical mastitis infections of cows. Also, they reported that the resistance against 1,2 and more than 2 antibiotic active substances was 33.3%, 38.1%, and 14.3%, respectively. They stated resistance against Penicillin G and tetracycline at the rate of 33.3% in 3 *L. innocua* isolates and identified that there were 2 isolates against 1 antibiotic active ingredient. They reported that 1 *L. ivanovii* strain they isolated was resistant against Penicillin G. As there were intermediate and resistant isolates in *Listeria* species isolated in this study against amoxicillin-clavulanic acid, gentamicin, chloramphenicol, rifampicin, tetracycline and Penicillin G, which are used in treatment option especially in animals, it was considered that the antibiotic resistance developed in *Listeria* species, may become common and, therefore, there may be decrease in the number of antibiotics, which are used in treatment option in the future.

The total bacteria (aerobic mesophilic bacteria) count (cfu/ml) was calculated high based on the criteria stated in the national and international standards in milk samples. Also, *Listeria* species were isolated from these samples. Since intermediate and resistant *Listeria* species were determined against the antibiotics used as a treatment option in these isolates, it is thought that *Listeria* species should also be considered in mastitis infections in terms of etiology and treatment. It is considered that a national mastitis control program is needed for preventing the mastitis infections and antibiotic resistance development causing economic losses in dairy cattle enterprises in order to provide milking hygiene completely.

Conflict of Interest

The author declared no conflict of interest.

Funding

During this study, any pharmaceutical company that has a direct connection with the subject of the research, a company that provides and / or produces medical tools, equipment and materials, or any commercial company, during the evaluation process of the study, no financial and / or moral support was received.

Authors' Contributions

Idea / concept: Orkun BABACAN
Experiment design: Orkun BABACAN
Supervision / Consultancy: Orkun BABACAN
Data collecting: Orkun BABACAN
Data analysis and interpretation: Orkun BABACAN
Literature search: Orkun BABACAN
Writing the article: Orkun BABACAN
Critical review: Orkun BABACAN

Ethical Approval

An ethical statement was received from the authors that the data, information and documents presented in this article were obtained within the framework of academic and ethical rules, and that all information, documents, evaluations and results were presented in accordance with scientific ethics and moral rules.

References

1. Aksoy A, Sezer Ç, Vatansever L, Gülbaz G (2018): *Presence and antibiotic resistance of Listeria monocytogenes in raw milk and dairy products.* Kafkas Univ Vet Fak Derg, **24(3),** 415–421. https://doi.org/10.9775/kvfd.2017.19081
2. Alexander AV, Walker RL, Johnson BJ, Charlton BR, Woods LW (1992): *Bovine abortions attributable to Listeria ivanovii: four cases (1988-1990).* J Am Vet Med Assoc, **200 (5),** 711-4.

3. Anonyms. (2000): *Türk Gıda Kodeksinde çiğ ve ısıl işlem görmüş içme sütleri tebliği.* Resmi Gazete No: **23964.** Erişim adresi: https://www.resmigazete.gov.tr/arsiv/23964.pdf. Erişim tarihi: 15/03/2020

4. Anonymus. (2004): *Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin.* Erişim adresi: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:139:0055:0205:en:PDF#:~:text=This%20Regulation%20defines%20specific%20hygiene,origin%20imported%20into%20the%20Community.&text=28%20January%202002%20laying%20down,1). Erişim tarihi: 15/03/2020

5. Amene Y, Firesbhat A (2016): *Listeriosis in Large Ruminants A Review.* Acad J Anim Diseases, **5 (1),** 16–21. https://doi.org/10.5829/idosi.ajad.2016.16.21

6. Baki Acar D, Tosun S (2019): *The Comparison of Milking Hygiene with Bulk Tank Somatic Cell Count and Total Bacterial Count in Dairy Herds in Tekirdag Province.* Kocatepe Vet J, **12,** 1–1. https://doi.org/10.30607/kvj.582212

7. Baştan A (2010): *İneklerde Meme Sağlığı ve Sorunları.* Kardelen Ofset Matbaacılık, Ankara.

8. Bangiev DR, Rusev VN (2017): *Prevalence of Listeria Monocytogenes in Raw Cow Milk – a Review.* Bulg J Vet Med, **20 (1),** 430-436.

9. Bauer AW, Kirby WM, Sherris JC, Turck M (1996): *Antibiotic susceptibility testing by a standardized single disk method.* Am J Clin Pathol, **45,** 493- 496.

10. Benhalima L, Merad T, Bensouilah M, Ouzrout R (2019): *Listeria monocytogenes and other Listeria species in raw milk and sausage in East Algeria.* Asian J Dairy & Food Res, **38 (1),** 7-11. https://doi.org/10.18805/ajdfr.dr-128

11. Blowey R, Edmondson P (2010): *Mastitis Control in Dairy Herds.* CAB International, 2010, Oxfordshire,UK.

12. Chen JQ, Healey S, Regan P, Laksanalamai P, Hu Z (2017): *PCR-based methodologies for detection and characterization of Listeria monocytogenes and Listeria ivanovii in foods and environmental sources.* Food Sci Hum Wellness, **6 (2),** 39–59. https://doi.org/10.1016/j.fshw.2017.03.001

13. Clinical and Laboratory Standards Institute (CLSI) (2012): *Performance standards for antimicrobial susceptibility testing; twenty second informational supplement (Vol. 32, No. 3)* Wayne, PA, USA: M100-S22.

14. Clinical and Laboratory Standards Institute (CLSI) (2014): *Performance standards for antimicrobial susceptibility testing; twenty forth informational supplement (Vol. 34, No. 1).* Wayne, PA, USA: M100-S24.

15. Darbaz İ, Baştan D (2018): *Investigation of udder health and milk quality parameters of dairy farms in Northern Cyprus. Part I: SCC and bacteriologic examination.* Ank Univ Vet Fak Derg **65 (2),** 145–154. https://doi.org/10.1501/vetfak_0000002841

16. Darbaz İ, Baştan D (2018) : *Investigation of udder health and milk quality parameters of dairy farms in Northern Cyprus. Part II: Milk quality.* Ank Univ Vet Fak Derg 2018, **65 (2),** 155–161.

17. Dhuol KRR, Osman AM (2014): *Study on total bacteria count of raw milk produced in the farm of the college of Veterinary Medicine and Animal Production - Sudan University of Science and Technology.* Int J Curr Res Aca Rev, **2 (1),** 1-06.

18. European Committee on Antimicrobial Susceptability Testing (EUCAST) (2019): Breakpoint tables for interpretation of MICs and zone diameters. Version 9.0. Erişim adresi: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_9.0_Breakpoint_Tables.pdf. Erişim tarihi: 10/12/2019

19. Haggag, YN, Nossair MA, Shehab SA (2019): Is raw milk still vehicle for transmitting *Listeria* species to pregnant women? Alex J Vet Sci, **61 (1),** 67-73.

20. International Standards Organisation (2017): *Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 1: Detection method (ISO 11290-1:2017).*

21. International Standards Organisation (2013): *Microbiology of the food chain - Horizontal method for the enumeration of microorganisms - Part 1: Colony count at 30 degrees C by the pour plate technique.(ISO 4833).*

22. International Standards Organisation (2013): *Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations.(ISO 7218).*

23. Jamali H, Radmehr B (2013): *Frequency, virulence genes and antimicrobial resistance of Listeria spp. isolated from bovine clinical mastitis.* Veterinary J, **198 (2),** 541–542. https://doi.org/10.1016/j.tvjl.2013.06.012

24. Kaygısız A, Kaynak İ (2012): *Evaluation of Somatic Cell Count in Raw Milk Samples Collected from Dairy Farms in Kahramanmaras Province for EU Norms and Subclinical Mastitis.* KSÜ Doğa Bil Derg, **15 (3),** 9-15

25. Konosonoka IH, Jemeljanovs A, Osmane B, Ikauniece D, Gulbe G (2012): Incidence of *Listeria* spp. in Dairy Cows Feed and Raw Milk in Latvia. ISRN Vet Sci, 1–5. https://doi.org/10.5402/2012/435187

26. Linke K, Rückerl I, Brugger K, Karpiskova R, Walland J, Muri-Klinger S, Tichy A, Wagner M, Stessl B (2014): *Reservoirs of Listeria species in three environmental ecosystems.* App Environ Microbiol, **80 (18),** 5583–5592. https://doi.org/10.1128/AEM.01018-14

27. Luque-Sastre L, Arroyo C, Fox EM, McMahon BJ, Bai L, Li F, Fanning S (2018): *Antimicrobial Resistance in Listeria Species.* Microbiol Spectr, **6 (4),** 1–23. https://doi.org/10.1128/microbiolspec.arba-0031-2017

28. Mundan D, Meral BA, Demir A, Doğaner M (2015): *Evaluation an Economic of Total Bacteria and Somatic Cell Count in Dairy Cattle Farms.* Harran Üniv Vet Fak Derg, **4 (2),** 84–89.

29. Orsi RH, Wiedmann M (2016): *Characteristics and distribution of Listeria spp., including Listeria species newly described since 2009.* Appl Microbiol and Biotechnol, **100 (12),** 5273–5287. https://doi.org/10.1007/s00253-016-7552-2

30. Osman KM, Zolnikov TR, Samir A, Orabi A (2014): *Prevalence, pathogenic capability, virulence genes, Biofilm formation, and antibiotic resistance of Listeria in goat and sheep milk confirms need of hygienic milking conditions.* Pathog Glob Health, **108 (1),** 21–29. https://doi.org/10.1179/2047773213Y.0000000115

31. Petersson-Wolfe CS, Tholen AR, Currin J, Leslie KE (2013): *Practical methods for mastitis control.* WCDS Adv Dairy Technol, **25,** 341-358.

32. Rahimi E, Momtaz H, Behzadnia A, Zeinab Torki Baghbadorani (2014): *Incidence of Listeria species in bovine, ovine, caprine, camel and water buffalo milk using cultural method and the PCR assay.* Asian Pac, **4 (1),** 50–53. https://doi.org/10.1016/S2222-1808(14)60313-3

33. Rawool DB, Malik SV, Shakuntala I, Sahare AM, Barbuddhe SB (2007): *Detection of multiple virulence-associated genes in Listeria monocytogenes isolated from bovine mastitis cases.* Int J Food Microbiol, **113 (2),** 201–207. https://doi.org/10.1016/j.ijfoodmicro.2006.06.029

34. Rocha PRDA, Dalmasso A, Grattarola C, Casalone C, Del Piero F, Bottero MT, Capucchio MT (2013): *Atypical cerebral listeriosis associated with Listeria innocua in a beef bull.* Res Vet Sci, **94 (1),** 111–114. https://doi.org/10.1016/j.rvsc.2012.07.017

35. Seeliger HPR, Jones D (1987): *Listeria.* In: Holt JG (Ed.), Bergey's Manual of Systematic Bacteriology, 9th Edition, Williams & Wilkins, Baltimore MD.

36. Sarfraz M, Ashraf Y, Ashraf S (2017): *A Review : Prevalence and antimicrobial susceptibility profile of Listeria species in milk products.* Mat Sc Med, **1 (1),** 3–9.

37. Vilar MJ, Yus E, Sanjuán ML, Diéguez FJ, Rodríguez-Otero JL (2007): *Prevalence of and risk factors for Listeria species on dairy farms.* J Dairy Sci, **90 (11),** 5083–5088. https://doi.org/10.3168/jds.2007-0213

38. Vural R, Ergün Y, Özenç E (2010): *Büyük Ruminantlarda Mastitis.* 149-259. In: Kaymaz M, Fındık M, Rişvanlı A, Köker A (Eds.), Evcil Hayvanlarda Meme Hastalıkları, Medipres Matbaacılık, Ankara.

39. Yadav MM, Roy A, Bhanderi B, Joshi C (2010): *Pheno-genotypic characterization of Listeria monocytogenes from bovine clinical mastitis.* Buffalo Bull, **29 (1),** 39–48.

VETERİNER HEKİMLER DERNEĞİ DERGİSİ
JOURNAL OF THE TURKISH VETERINARY MEDICAL SOCIETY

ISSN: 0377-6395
e-ISSN: 2651-4214
Dergi ana sayfası:
Journal homepage:
http://dergipark.org.tr/vetheder

DOI: 10.33188/vetheder.771696

Araştırma Makalesi / Research Article

The effect of heat stress on total oxidant capacity in hair goats

Serdal KURT [1,a*], Funda EŞKİ [2,b], Seçkin SALAR [3,c], Tevhide SEL [4,d], Ayhan BAŞTAN [3,e]

[1] Dicle University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, 21280, Diyarbakır, Turkey
[2] Cukurova University, Faculty of Ceyhan Veterinary Medicine, Department of Obstetrics and Gynecology, 01330 Adana, Turkey
[3] Ankara University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, 06110, Ankara, Turkey
[4] Ankara University, Faculty of Veterinary Medicine, Department of Biochemistry, 06110, Ankara, Turkey
ORCID: 0000-0002-0191-3245 [a]; 0000-0002-9242-927 1[b]; 0000-0001-9303-6253 [c]; 0000-0002-9753-779X [d]; 0000-0001-8291-114 7 [e]

MAKALE BİLGİSİ / ARTICLE INFORMATION:

Geliş / Received:
20 Temmuz 20
20 July 20

Kabul / Accepted:
09 Kasım 20
09 November 20

Keywords:
Altitude
Hair Goat
Heat Stress
Total oxidant capacity

Anahtar Sözcükler:
Isı stresi
Kıl keçisi
Rakım
Toplam oksidan kapasitesi

ABSTRACT:

The aim of this study was to investigate the effect of heat stress on total oxidant capacity (TOC) in hair goats of different ages grown at different altitudes in the same season. The study was carried out on 208 hair goats (25 male and 183 female) in 11 different districts of Adana province (Turkey) in the summer season. The districts were grouped as 0-99 m (Group 1), 100-500 m (Group 2) and higher than 500 m (Group 3) according to altitude characteristics, and goats were classified into two groups as under 3 years and 4 years or older. Blood samples were collected, serums harvested and stored at -20°C until TOC analysis. Heat stress was determined according to the temperature humidity index (THI). There were severe and moderate heat stress in groups 1 and 2, respectively (P<0.001). TOC was significantly lower in group 2 compared to the other groups (P<0.001). The highest TOC was measured in group 3 (P<0.001). And TOC was significantly higher in animals aged 4 and older compared to under 3 years of age (P<0.05). Although male goats were exposed to significantly higher THI than female goats (P<0.01), TOC levels were found similar in both groups (P>0.05). In conclusion, altitude, THI, age and gender had a complex effect on TOC.

Kıl keçilerinde ısı stresinin toplam oksidan kapasitesine etkisi

ÖZET:

Bu çalışmanın amacı, aynı sezonda farklı rakımlarda yetiştirilen farklı yaştaki kıl keçilerinde ısı stresinin toplam oksidan kapasite (TOC) üzerindeki etkisini araştırmaktı. Çalışmada Türkiye, Adana'nın 11 farklı ilçesinden 25 erkek 183 dişi olmak üzere 208 keçi kullanıldı. İlçeler rakım özelliklerine göre 0-99 m (Grup 1), 100-500 m (Grup 2) ve 500 m'den daha yüksek (Grup 3) olmak üzere gruplandırıldı. Keçiler ise 3 yaşından küçük ve 4 yaş ya da daha yaşlı olarak iki gruba ayrıldı. Kan örnekleri alınarak serumları çıkarıldı ve TOC analizine kadar -20°C'de saklandı. Isı stresi sıcaklık nem indeksine (THI) göre belirlendi. Grup 1 ve 2'de sırasıyla şiddetli ve orta dereceli ısı stresi vardı (P <0.001). TOC diğer gruplara göre grup 2'de anlamlı olarak düşük bulundu (P <0.001). En yüksek TOC grup 3'te ölçüldü (P <0.001) ve TOC değeri 4 yaş ve üzerindeki hayvanlarda 3 yaşın altındakilere göre anlamlı derecede yüksekti (P <0.05). Erkek keçiler dişi keçilerden anlamlı derecede daha yüksek THI'ye maruz kalmalarına rağmen (P <0.01), TOC seviyeleri benzer bulundu (P >0.05). Sonuç olarak, rakım, THI, yaş ve cinsiyet TOC üzerinde kompleks bir etkiye sahipti.

How to cite this article: **Kurt S, Eşki F, Salar S, Sel T, Baştan A:** The effect of heat stress on total oxidant capacity in hair goats. *Veteriner Hekimler Dernegi Dergisi*, 92(1): 24-30, 2021, DOI: 10.33188/vetheder.771696

* *Sorumlu Yazar e-posta adresi / Corresponding Author e-mail address:* serdal.kurt@dicle.edu.tr

1. Introduction

Farm animals are exposed to stress which is affecting their production and welfare, depending on environmental factors and management conditions (1, 2). The environmental factors such as high ambient temperature and relative humidity can cause heat stress in farm animals (2, 3). The heat stress occurs, when the effective temperature of the environment exceeds the animal's comfort temperature (4, 5). Temperature humidity index (THI) values of 75 or higher are considered stressful. The values greater than 78 cause excessive stress, and animals cannot maintain thermoregulatory mechanisms (3). It has a multifactorial effect on health, so it is an important source of economic loss, including fertility problems in farm animals (6). Heat stress primarily causes dehydration (7). It also causes biological reactions in the physiological, biochemical, hormonal and haematological functions of animals. Thus, animals try to eliminate or minimize the harmful effects of stress (8). At the same time, these changes lead to disruptions in the homeostasis balance of important metabolites such as antioxidants, prooxidants, insulin, lipids, proteins, cholesterol and glucose (2). Most importantly, it is known that heat stress increases reactive oxygen species (ROS) production (9) which are the most abundant oxidant substances in biological systems (10). Already, oxidant substances are normally produced at moderate levels during the conversion of glucose into the form of adenosine triphosphate (ATP) using oxygen (O2) in mitochondria (11). And under normal conditions, antioxidants substances complete the missing electron of ROS and repair them enzymatically or reduce them into new molecules such as H2O that is harmless to the body (12). However, heat stress results in the excessive production of free radicals (13). Since it also causes a decrease in the antioxidant defense system, it results in oxidative stress, which can cause tissue pathologies by damaging macromolecules of healthy cells including deoxyribonucleic acid (DNA), lipids and proteins (2, 14). Moreover, altitude can affect the antioxidant defense system and lead to oxidative stress. And it is known that high altitude is associated with increased production of free radicals due to low oxygen pressure (15). So, it is understood that heat stress and excessive oxidant production are critical for farm animals. The aim of this study was to investigate the effect of heat stress on total oxidant capacity (TOC) in hair goats of different ages grown at different altitudes in the same season.

2. Material and Methods

The study was carried out on 208 hair goats (25 male and 183 female) in 11 different districts (Table 1) of Adana province (Turkey) in the summer season (June, July and August months). All of the goats used in the study were mature and cyclically active animals that reached puberty. Power analysis was taken into account in determining the sample size. The districts were grouped as 0-99 m (Group 1), 100-500 m (Group 2) and higher than 500 m (Group 3) according to altitude characteristics. Goats were classified into two groups as under 3 years and 4 years or older. Since the sample distribution was chosen randomly, age groups could not be classified according to altitude within themselves. All goats had similar properties, were managed in extensive systems under the same conditions and were clinically healthy including rectal temperature, respiratory, appetite,physical posture and anamnesis. Blood samples (10 ml) were collected from the jugular vein into sterile vacutainer tubes (Hema & Tube®, Italy) containing a clot activator using 18-G needles at the same periods. The blood samples were centrifuged at 3000 xg for 10 minutes, then harvested serums were stored at -20°C until TOC analysis.

Calculation of temperature humidity index (THI):

THI is a combination of environmental temperature and relative humidity. It is used to assess the risk of heat stress (3). The daily temperature and humidity data were obtained from the meteorological station with the official application. Daily measurements (temperature, humidity) were not made in the farms where the study was conducted because all goats were managed under extensive systems. The THI data of each region were obtained using temperature and relative humidity according to the formula described below (Table 1) (16). And THI values of the time the study was performed in each zone was taken into account. THI data calculated according to the regions are presented in the results section (Table 2). THI levels are considered normal if they are 70 or less, moderate stress if between 75 and 78, and severe heat stress if they are greater than 78 (3).

Table 1: Temperature humidity index formula

Tablo 1: *Sıcaklık nem indeksi formülü*

THI	$[(1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26.8)]$

T is the air temperature in ° C. RH is the relative humidity in %.

Table 2: The districts where the study was conducted and the calculated THE data

Tablo 2: *Çalışmanın yürütüldüğü ilçeler ve hesaplanan THI değerleri*

Altitude (m)	Districts	THI
0-99	Ceyhan	80
	Yumurtalık	80
	Yüreğir	79
	İmamoğlu	78
	Karataş	81
	Kozan	78
	Çukurova	77
100-500	Sariçam	78
≥501	Tufanbeyli	66
	Pozantı	75
	Aladağ	78

Total oxidant capacity (TOC) and test principle:

Serum TOC levels were measured using an ELISA device (TECAN, Sunrise® Swiss) and commercial kits (LOT: OK18104O, Rel Assay Diagnostics, Clinical Chemistry Solutions, Gaziantep) based on the method developed by Erel (17, 18). The test principle is a colorimetric method, which can be measured spectrophotometrically. It indicates the total amount of oxidant substances in the samples. Hydrogen peroxide is used as a calibrator in this test method (17). The results were expressed as micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Equiv. /L).

Statistical analysis

All data were analyzed using SPSS 21.0 Windows statistical package program evaluation version. The TOC analyzes of goats in different districts were compared using one-way Anova test. The age and gender groups were evaluated by independent-T test. A P-value less than 0.05 was considered statistically significant. Obtained results were presented as Mean ± Standard deviation (Mean ± Std deviation).

3. Results

In the present study, it was found that THI levels decreased significantly as altitude increases ($P<0.001$). There were severe and moderate heat stress in groups 1 and 2, respectively ($P<0.001$). But, there was no heat stress in group 3 ($P<0.001$). Serum TOC levels were significantly lower in group 2 compared to the other two groups ($P<0.001$). And, the highest TOC value was measured in group 3 compared to other groups ($P<0.001$). It was determined that the

distribution of age was similar in different THI values according to regions (p>0.05). Serum TOC levels were significantly higher in animals 4 years and older compared to under 3 years old (P <0.05). It also was observed that male goats were exposed to significantly higher THI than females goats (P<0.01). TOC levels were similar in male goats compared to female goats (P>0.05). TOC levels, THI values and other results in groups are shown in Table 3.

Table 3: Effect of THI on serum TOC levels in hair goats. (Mean ± Std. deviation).

Tablo 3: *Kıl keçilerinde THI'nın serum TOC seviyesine etkisi. (Aritmetik Ort. ± Std. sapma).*

Variables		Number of animals (n)	TOC/µmol/L	P Value	THI	P Value
Altitude (m)	0-99 (G 1)	89	8.733±4.112	<0.001[a]	79.40±1.105	<0.001[a]
	100-500 (G 2)	50	6.954±2.815		77.86±0.351	
	≥501 (G 3)	69	9.733±3.526		72.91±5.075	
Age	<3	69	7.762±2.831	0.018[b]	76.91±4.385	0.935
	≥4	139	9.072±4.103		76.86±4.045	
Gender	Female	183	8.687±3.832	0.610	76.57±4.302	0.003[c]
	Male	25	8.276±3.335		79.16±1.434	

TOC: Total oxidant capacity, G 1: Group 1, G 2: Group 2, G 3: Group 3. [a] P <0,001; [b] P <0,05; [c] P <0,01

4. Discussion and Conclusion

In the present study, the effects of heat stress, altitude, gender and age on TOC were evaluated in hair goats. It is reported that goats have good tolerance to heat stress due to their thermoregulation ability, and long-haired goats more resistant to heat stress compared to short-haired goats (16). No matter how resistant the farm animals to heat stress, this is considered a major problem for livestock. Because it negatively affects the health and production of farm animals in many ways (19, 20). Considering the increasing global temperature (21), the issue becomes more important. It has been reported that environmental temperature and humidity should be evaluated together and heat stress should be monitored according to THI values. Because the effect of ambient temperature on farm animals changes according to the moisture content (21, 22). THI levels were used as a heat stress index in this study.

In the present study, the altitude had an effect on THI levels. This was due to the decrease in air temperature and humidity as altitude increases. Teama (14) reported that THI has an effect on oxidative stress. Kumar et al. (23) noticed that oxidative stress increases in goats during the summer season. Similarly, several researchers reported that heat stress has increased the production of oxidant substances (14, 24). The overall oxidation status of the body is determined by measuring the TOC level. It represents the sum of all the oxidants substances present in blood serum. It is also defined as reactive oxygen metabolites (17). In our study, it was observed that the THI increased serum TOC levels in group 1 when compared to group 2. However, there was no positive correlation between THI and TOC in group 3 compared to other groups. This situation showed that THI did not have a similar effect on TOC in all groups. Although THI value was also lower in group 3 according to the other groups, the TOC value was measured higher. This situation can be associated with the altitude. Because it was reported that exposure to high altitude results in the increase of ROS production by the mitochondrial metabolism of cells due to differences in oxygen pressure (25). Similarly, Dosek et al. (26) reported that at high altitude, oxygen pressure decreases and production of reactive nitrogen and oxygen species increases, causing oxidative stress. It has also been declared that the degree of oxidative stress is associate with the level of altitude. Moreover, it was revealed that exposure to high altitude could reduce the activity of antioxidant systems (26). So, it is thought that the increase of the TOC levels of group 3 was caused by the high altitude and low oxygen pressure of the districts. It is thought that these results are very important for goat breeding because goat farming is common at high altitudes. Furthermore, antioxidant activity should be investigated in goats

raised at different altitudes. But, antioxidant levels were not detected in this study. Because our main aim was to determine the effect of THI and altitude on TOC levels. The major findings of this study are that the altitude and THI are significant determinants for TOC levels.There is no specific study on the effect of gender and age on TOC levels in hair goats under heat stress. Chaturvedi and Kataria (27) reported that age and gender have affected concentrations of some antioxidant substances at different ambient temperatures in Marwari goats. Also, it was revealed that levels of antioxidant substances were higher in male goats than female goats and antioxidant levels increased with age in all groups (27). On the other hand, it has been reported that there is a positive relationship between biological aging and oxidant production in some animal models (28, 29). Similarly, in our study, aging was linked to an increase in oxidant generation in hair goats. This is thought to result from an increased ROS accumulation in aging organs. Balci et al. (30) reported that gender difference has an effect on adaptation process to changes in antioxidant capacity. Oxidative stress risk is higher in males compared to females (31). And Razmara et al. (32) informed that estrogen increases the antioxidant activity in the mitochondria. According to this information, TOC level is expected to be lower in female goats than in male goats. In this study, male animals were exposed to higher ambient THI levels than females, but this did not affect the TOC level. Therefore, it was thought that male hair goats were more resistant to heat stress in terms of TOC levels.

In conclusion, THI levels had an effect on TOC in hair goats. However, at high altitude TOC increased despite low THI. Age and gender also affected the TOC level. So, studies investigating the effect of THI on oxidant capacity in hair goats should also take into account age, gender and altitude.

Conflict of Interest

The author declared no conflict of interest.

Funding

No funding.

Authors' Contributions

Idea/Concept: Serdal KURT, Funda EŞKİ
Experiment design:Serdal KURT, Funda EŞKİ, Ayhan BAŞTAN, Seçkin SALAR
Supervision/Consultancy: Funda EŞKİ, Ayhan BAŞTAN, Seçkin SALAR
Data collecting: Funda EŞKİ, Serdal KURT
Data analysis and interpretation: Tevhide SEL
Literature research: Serdal KURT, Funda EŞKİ
Writing the article: Serdal KURT, Funda EŞKİ
Critical review: Funda EŞKİ, Ayhan BAŞTAN, Seçkin SALAR

Ethical Approval

This study was carried out with the permission of the Cukurova University, Local Ethics Committee of Ceyhan Faculty of Veterinary Medicine (Decision Date and Number: 12.12.2018 and 1/11).

References

1. Blokhuis HJ, Hopster H, Geverink NA, Korte SM, Van Reenen CG (1988): *Studies of stress in farm animals*. Comp Haematol Int, **8(2),** 94-101.

2. Belhadj Slimen I, Najar T, Ghram A, Abdrrabba M (2016): *Heat stress effects on livestock: molecular, cellular and metabolic aspects: a review*. J Anim Physiol An N, **100(3)**, 401-412.

3. Silanikove N (2000): *Effects of heat stress on the welfare of extensively managed domestic ruminants*. Livest Prod Sci, **67(1-2),** 1-18.

4. Al-Tamimi HJ (2007): *Thermoregulatory response of goat kids subjected to heat stress*. Small Ruminant Res, **71(1-3),** 280-285.
5. Srikandakumar A, Johnson EH, Mahgoub O (2003): *Effect of heat stress on respiratory rate, rectal temperature and blood chemistry in Omani and Australian Merino sheep*. Small Ruminant Res, **49(2),** 193-198.
6. Das R, Sailo L, Verma N, Bharti P, Saikia J (2016): *Impact of heat stress on health and performance of dairy animals.* Vet world, **9(3),** 260-268.
7. Maloiy GMO, Kanui TI, Towett PK, Wambugu SN, Miaron JO, Wanyoike MM (2008): *Effects of dehydration and heat stress on food intake and dry matter digestibility in East African ruminants.* Comp Biochem Phys A, **151(2),** 185-190.
8. Ribeiro MN, Ribeiro NL, Bozzi R, Costa RG (2018): *Physiological and biochemical blood variables of goats subjected to heat stress: a review.* J Appl Anim, **46(1),** 1036-1041.
9. Del Vesco AP, Gasparino E (2013): *Production of reactive oxygen species, gene expression, and enzymatic activity in quail subjected to acute heat stress*. JAS, **91(2)**, 582-587.
10. Abuelo A, Hernaandez J, Benedito JL, Castillo C (2015): T*he importance of the oxidative status of dairy cattle in the periparturient period: revisiting antioxidant supplementation*. J Anim Physiol An N, **99(6),** 1003-1016.
11. Mavangira V, Sordillo LM (2018): *Role of lipid mediators in the regulation of oxidative stress and inflammatory responses in dairy cattle.* Res Vet Sci, **116(1),** 4-14.
12. Puppel K, Kapusta A, Kuczyńska B (2015): *The etiology of oxidative stress in the various species of animals: a review*. J Sci Food Agric, **95(11)**, 2179-2184.
13. Kumar S, Ajeet K, Meena K (2011): *Effect of heat stress in tropical livestock and different strategies for its amelioration.* J Stress Physiol Biochem, **7(1),** 45-54.
14. Teama FEI (2018) (2004): *Evaluation of some oxidative-stress and antioxidant markers in goats during estrous cycle under Egyptian environmental conditions*. Rev bras zootecn, **47,** 1-8.
15. **Bakonyi T, Radak Z (2004):** *High altitude and free radicals.* JJSM, **3(2),** 64.
16. Salama AAK, Caja G, Hamzaoui S, Badaoui B, Castro-Costa A, Facanha DAE, Bozzi R (2014): *Different levels of response to heat stress in dairy goats.* Small Ruminant Res, **121(1),** 73-79.
17. Erel O (2005): *A new automated colorimetric method for measuring total oxidant status*. Clin Biochem, **38(12),** 1103-1111.
18. Karapehlivan M, Kaya I, Sag A, Akin S, Ozcan A (2013): *Effects of early and late lactation period on plasma oxidant/antioxidant balance of goats*. Kafkas Univ Vet Fak Derg, **19(3),** 529-533.
19. Alam MM, Hashem MA, Rahman MM, Hossain MM, Haque MR, Sobhan Z, Islam MS (2011): *Effect of heat stress on behavior, physiological and blood parameters of goat*. Progress Agric, **22(1-2),** 37-45.
20. Habeeb AGA, EL-Tarabany A, Atta M (2018): *Negative effects of heat stress on growth and milk production of farm animals.* Res j Animal Hus Dairy Sci, **2(1),** 1-12.
21. Avendano-Reyes L (2012): *Heat stress management for milk production in arid zones*. 165-184. In: Chaiyabutr N (Ed), Milk production-An up-to-date overview of animal nutritio, management and health. IntechOpen, London.
22. De Rensis F, Garcia-Ispierto I, López-Gatius F (2015): *Seasonal heat stress: Clinical implications and hormone treatments for the fertility of dairy cows*. Theriogenology, **84(5),** 659-666.
23. Kumar M, Jindal R, Nayyar S (2011): *Influence of heat stress on antioxidant status in Beetal goats*. IJSR, **17(2),** 178-181.
24. Sivakumar AVN, Singh G, Varshney VP (2010): *Antioxidants supplementation on acid base balance during heat stress in goats.* Asian-Australasian J Anim Sci, **23(11),** 1462-1468.
25. Bakonyi T, Radak Z (2004): *High altitude and free radicals*. J Sport Sci Med, **3(2),** 64-69.
26. Dosek A, Ohno H, Acs Z, Taylor AW, Radak Z (2007): *High altitude and oxidative stress*. Resp Physiol Neurobi, **158(2-3),** 128-131.
27. Chaturvedi M, Kataria N (2013): *Assessment of role of antioxidants in erythrocytes of Marwari goat from arid tracts in India to evaluate oxidative stress.* Elba Bioflux, **5(1),** 1-8.

28. Zhang HJ, Xu L, Drake VJ, Xie L, Oberley L W, Kregel KC (2003): *Heat-induced liver injury in old rats is associated with exaggerated oxidative stress and altered transcription factor activation*. FASEB J, **17(15),** 2293-2295.
29. Kregel KC, Zhang HJ (2007): *An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations.* Am J Physiol Regul Integr Comp Physiol, **292(1),** 18-36.
30. Balci SS, Pepe H, Güney S, Özer Ç, Revan S (2012): *Effects of gender, endurance training and acute exhaustive exercise on oxidative stress in the heart and skeletal muscle of the rat*. Chin J Physiol, **55(4),** 236-44.
31. Wang X, Desai K, Juurlink BHJ, De Champlain J, Wu L (2006): *Gender-related differences in advanced glycation endproducts, oxidative stress markers and nitric oxide synthases in rats.* Kidney Int*,* **69(2),** 281-287.
32. Razmara A, Duckles SP, Krause DN, Procaccio V (2007): *Estrogen suppresses brain mitochondrial oxidative stress in female and male rats.* Brain Res, **1176**, 71-81.

VETERİNER HEKİMLER DERNEĞİ DERGİSİ
JOURNAL OF THE TURKISH VETERINARY MEDICAL SOCIETY

ISSN: 0377-6395
e-ISSN: 2651-4214
Dergi ana sayfası:
Journal homepage:
http://dergipark.org.tr/vetheder

DOI: 10.33188/vetheder.795914

Araştırma Makalesi / Research Article

Evaluation of MMP-9 and iNOS expressions in sheep with encephalitic listeriosis

Emin KARAKURT [1,a*], Fatih BÜYÜK [2,b], Enver BEYTUT [1,c], Serpil DAĞ [1,d],Özgür ÇELEBİ [2,e], Doğan AKÇA [3,f], Hilmi NUHOĞLU [1,g], Elif ÇELİK [2,h], Ayfer YILDIZ [4,i]

[1] Department of Pathology, Faculty of Veterinary Medicine, Kafkas University, Kars, 36100, Turkey
[2] Department of Microbiology, Faculty of Veterinary Medicine, Kafkas University, Kars, 36100, Turkey
[3] Department of Midwifery, Faculty of Health Science, Kafkas University, Kars, 36100, Turkey
[4] Institute Health Sciences, Kafkas University, Kars, 36100, Turkey

ORCID: 0000-0003-2019-3690 [a], 0000-0003-3278-4834 [b], 0000-0003-3360-2940 [c], 0000-0001-7667-689X [d], 0000-0002-3478-008X [e], 0000-0002-3986-8769 [f], 0000-0003-2530-2542 [g], 0000-0003-4531-3863 [h], 0000-0002-6569-5435 [i]

MAKALE BİLGİSİ / ARTICLE INFORMATION:

Geliş / Received:
16 Eylül 20
16 September 20

Kabul / Accepted:
23 Kasım 20
23 November 20

Keywords:
Encephalitic Listeriosis
Histopathology
Immunohistochemistry
iNOS
MMP-9

Anahtar Sözcükler:
Ensefalitik listeriyozis
Histopatoloji
İmmunohistokimya
iNOS
MMP-9

ABSTRACT:

This study aimed to correlate Matrix Metalloproteinase-9 and iNOS expressions with the severity of histopathological findings in tissue samples taken from sheep with encephalitic listeriosis. Thus, the role of these molecules in the pathogenesis of the disease can be elucidated. After systemic necropsy, tissue samples of adult sheeps with meningoencephalitis were investigated by the culture, histopathological and immunohistochemical methods in the presence of *Listeria* spp. isolation from tissues was performed in accordance with the USDA-FSIS method with some modifications. Tissue samples were fixed in a 10% buffered formaldehyde solution. Following routine procedures, tissue sections at 5 µm were stained with Hematoxylin and Eosin, investigated under light microscope and photographed. Immunohistochemical staining was performed on the tissues using the avidin-biotin immune peroxidase complex method. *Listeria* spp. were obtained in 20 (83.3%) of 24 tissue samples with the presence of bright grey-black centred smooth colonies on Listeria Selective Agar and identified as *Listeria monocytogenes* through the phenotypically supportive tests. Liquefaction necrosis, purulent meningoencephalitis, perivascular cuffing, microabscesses and glial nodules were the most important histopathological findings. MMP-9 immunpositive reactions were observed in the cytoplasm of microglial cells and neurons in areas where inflammatory and necrotic areas are concentrated in medulla oblongata and pons. In perivascular cuffing areas, immune reactions in endothelial cells were detected. We detected iNOS positive reactions in the medulla oblangata and pons, especially in inflammatory cells in the microabscesses. Consequently, a positive correlation ($p < 0.05$) was found between MMP-9 expression and the severity of histopathological findings in sheep with encephalitic listeriosis. In addition, we found that iNOS expression increased in parallel with the increase in MMP-9 expression.

Ensefalitik listeriyozisli koyunlarda MMP-9 ve iNOS ekspresyonunun değerlendirilmesi

ÖZET:

Bu çalışmada ensefalitik listeriyozisli koyunlardan alınan doku örneklerinde gözlenen histopatolojik bulguların şiddeti ile Matriks metalloproteinaz-9 ve iNOS ekspresyonlarını korele etmeyi amaçladık.Böylece bu moleküllerin hastalığın patogenezindeki rolü açıklanabilecektir. Sistemik nekropsi sonrası meningoensefalitli erişkin koyunlardan alınan doku örnekleri *Listeria* spp. varlığı için kültür, histopatolojik ve immunohistokimyasal olarak incelendi. Dokulardan *Listeria* spp. izolasyonu bazı modifikasyonlarla USDA-FSIS yöntemine uygun olarak gerçekleştirildi. Doku örnekleri %10'luk tamponlu formaldehit solüsyonunda tespit edildi. Rutin işlemlerden sonra 5 µm kalınlığındaki kesitler Hematoksilen&Eozin ile boyandı, ışık mikroskobu altında incelendi ve fotoğraflandı. Dokulara immunohistokimyasal boya olarak avidin-biotin immunperoksidaz kompleks metodu uygulandı. *Listeria* spp. Listeria Selective Agar üzerinde parlak gri-siyah merkezli pürüzsüz koloniler bulunan 24 doku örneğinden 20'sinde (% 83.3) elde edildi ve fenotipik olarak destekleyici testler yoluyla *Listeria monocytogenes* olarak tanımlandı. Likefaksiyon nekrozu, purulent meningoensefalitis, perivasküler hücre infiltrasyonu, mikroapseler ve glial nodüller en önemli histopatolojik bulgulardı. MMP-9 immunpozitif reaksiyonları yangının ve nekrozun yoğun olduğu alanlardaki mikroglial hücreler ve nöronların sitoplazmasında gözlemledik. Perivasküler hücre infiltrasyonu alanlarında, endotelyal hücrelerde de immun reaksiyonu saptadık. iNOS pozitif reaksiyonları özellikle medulla oblongata ve pons bölgesinde yer alan mikroapselerdeki yangısal hücrelerde tespit ettik. Sonuç olarak ensefalitik listeriyozisli koyunlarda MMP-9 ekspresyonu ile histopatolojik bulguların şiddeti arasında pozitif bir korelasyon tespit ettik ($p<0.05$). Buna ek olarak MMP-9 ekspresyonundaki artışa paralel olarak iNOS ekspresyonun da artış gösterdiğini ortaya koyduk.

How to cite this article: **Karakurt E, Büyük F, Beytut E, Dağ S, Çelebi Ö, Akça D, Nuhoğlu H, Çelik E, Yildiz A:** Evaluation of MMP-9 and iNOS expressions in sheep with encephalitic listeriosis. *Veteriner Hekimler Dernegi Dergisi,* 92(1): 31-41, 2021 DOI: 10.33188/vetheder.795914

* *Sorumlu Yazar e-posta adresi / Corresponding Author e-mail address:* mehmeteminkarakurt@hotmail.com

1. Introduction

Listeriosis caused by members of the genus Listeria, is a ubiquitous, Gram-positive, facultative intracellular bacterium, which is responsible for sporadic and epidemic food-/feed- borne infections in ruminants and humans (12, 13, 26). *Listeria monocytogenes* is the primary pathogen in humans and animals cases, however, *Listeria ivanovii* was reported occasionally (11, 43). The other Listeria species such as *Listeria seeligeri*, *Listeria grayi* and *Listeria innocua* is found rarely in some cases with their unclear pathogenicity (33). Listeriosis causes significant economic losses in ruminants and serious health problems in humans (10, 16). Listeriosis is mostly detected in autumn, winter and early spring in temperate and cold climates and is thought to occur due to consumption of poorly prepared silage (17, 28, 44). This infection can lead to different clinical symptoms such as gastroenteritis with high fever, mastitis, encephalitis, septicemia and abortion (3, 20). Encephalitic listeriosis caused by only species-*L. monocytogenes* is more common especially in small ruminants and has high mortality rates (1, 37). Sheep are more sensitive to listeriosis than cattle (9). Encephalitic listeriosis causes anorexia, depression, excessive salivation, eye infections, keratitis, unilateral facial paralysis, motor incoordination and tremors. It also leads to tilting of the head and permanent circling motions (4, 30). Microabscesses, glial nodules and perivascular cell infiltration observed in the brainstem are diagnostic histopathological findings of encephalitic listeriosis (35).

Matrix metalloproteinases (MMPs) degrade all extracellular matrix (ECM) components and perform important tasks such as tissue remodeling and modulation of the immune system (39, 45). Increased expression of MMPs is known in many central nervous system diseases such as multiple sclerosis, experimental autoimmune encephalomyelitis, alzheimer's disease, stroke and meningitis (38, 42). The increase in MMP-9 expression, especially in samples from meningitis of viral and bacterial origin, suggests that this molecule may be an important factor in the pathogenesis of the disease (23). MMP-9, produced from activated microglia as a result of the effects of cytokines and reactive oxygen species, destroys the extracellular matrix of the brain and causes impaired neuronal function (5).

Nitric oxide (NO) is synthesized from L-arginine via nitric oxide synthase (NOS) (41). NOS enzyme responsible for nitric oxide formation exists in three forms. These are the two constitutive forms (neuronal NOS (nNOS) and endothelial NOS (eNOS)) and inducible NOS (iNOS), respectively (40). NO contributes significantly to the defense against viruses, bacteria, fungi and microbial agents such as protozoan and metazoan parasites. However, NO produced by iNOS-expressing cells contributes to a variety of disease symptoms ranging from immunosuppression to apoptosis and tissue damage. (36). There is a general consensus in the literature that the increase in iNOS expression is important in the pathogenesis of natural listeriosis in the brains of cattle and goats (41).

In this study, it was aimed to correlate MMP-9 and iNOS expressions with the severity of histopathological findings in tissue samples taken from sheep with encephalitic listeriosis.

2. Material and Methods

Animals:

The material of this study was consisted of 24 adult sheep that were brought to Pathology Department for systemic necropsy between 1998 and 2020. Some anamnestic findings were gathered accompanying to the sheep such as various neurological symptoms such as permanent circling movement, head pressing, unable to stand, hypersalivation, paralysis in the eyelid, sagging on the lower lip, blindness and torticollis. We used normal brain tissues of 4 sheep without any histopathological findings as negative controls.

Microbiological Examinations:

In this study, brain (medulla oblongata, pons and cerebellum) tissues from adult sheep were used for the isolation of Listeria agents. Isolation was performed in accordance with the United States Department of Agriculture - The Food Safety and Inspection Service (USDA-FSIS) method reported by McClain and Lee (27) with making some modifications. For this purpose, 2.5 g tissue sample was transferred to 22.5 ml Pre-Enrichment Broth (Trypticase Soy Broth (Merck 1.05459) containing 0.6% yeast extract and incubated at 30 °C for 24 hours under microaerobic

conditions. At the end of this period, 1 ml of this Pre-enrichment Broth was transferred into 9 ml Listeria Enrichment Broth (UVM formulation) (Oxoid CM0863) and incubated at 30 °C under the same atmospheric conditions. At the end of the period, Listeria Selective Agar (LSA) (Oxoid, CM0856) was plated with 25 µl aliquot of Listeria Enrichment Broth and incubated for 24 hours at 30 °C under microaerobic conditions. As a result of cultural process, the bright grey-black centred smooth colonies on the LSA medium were considered as *Listeria* spp.. Within the scope of identification, Gram staining characteristics, mobility at 25 °C, catalase and oxidase activities, carbohydrate (L-rhamnose, D-mannitol, D-xylose and α-methyl-mannosidase) fermentation capabilities and CAMP activities were evaluated. In the CAMP reaction, control strains of *Rhodococcus equi* (ATCC-33701) and *Staphylococcus aureus* (ATCC-25923) were used (2, 15).

Histopathological Examinations:

After systemic necropsy of sheep, tissue samples (cerebrum, cerebellum, etc.) were fixed in a 10% buffered formaldehyde solution. Following routine procedures, tissue sections at 5 µm were stained with Hematoxylin and Eosin (H&E), investigated under light microscope (Olympus Bx53) and photographed with Cell ^P Program (Olympus Soft Imaging Solutions GmbH, 3,4).

Immunohistochemical Examinations:

Immunohistochemical staining was performed on the tissues using the avidin-biotin immune peroxidase complex method. For immunohistochemical staining, the sections of 4 µm in thickness taken to poly-L-lysine coated slides were deparaffinized and rehydrated in graded alcohols. In order to prevent endogenous peroxidase activity, the sections were treated with 3% hydrogen peroxide solution in Phosphate Buffered Saline (PBS) for 15 minutes. For antigen retrieval, the sections were boiled in Citrat Buffer Solution (pH 6) for 25 min in the microwave oven (at 800 watt). In order to prevent nonspecific staining, the sections were incubated for 30 min with non-immune serum (Genemed Biotechnologies REF 54-0003) at room temperature. Diluted antibodies MMP-9 (Santa Cruz, sc-393859, Dilution Ratio: 1/100) and iNOS (Santa Cruz, sc-7271, Dilutio Ratio: 1/100) were incubated for one hour at room temperature. The sections were washed 3 times in PBS solution for 5 minutes, and the biotinylated secondary antibody (Genemed Biotechnologies REF 54-0003) was applied to them at room temperature for 30 minutes. After washing in PBS (3-5 min), all sections were incubated with peroxidase-bound Streptavidin (Genemed Biotechnologies REF 54-0003) for 30 minutes at room temperature. A solution of 3.3-diaminobenzidine tetra hydrochloride (DAB) (Genemed Biotechnologies REF 10-0048) was used as a chromogen for 15 minutes. The sections were treated with Mayer's Hematoxylin for 30 second and washed in running water for 5 min, dehydrated in graded alcohols, cleared in xylene and coated with entellan. Primary antibody was omitted from the negative brain control sections and were treated with diluted normal serum. The slides prepared after the covering were examined under a light microscope and photographed via the Cell^P program. Analyzes of the images were done with Image J Program.

Statistical Analysis:

Histopathological changes (meningitis, perivascular cuffings, microabscesses and necrosis) MMP-9 immunepositive expressions and iNOS scoring were evaluated under a light microscope and scored as absent (-), mild (+), moderate (++) and severe (+++). Correlation tests were used to determine the relationship between the MMP-9 expression and the severity of histopathological changes and between the MMP-9 expression and iNOS variables. In comparing the average of data belonging to the groups where the sample size is less than 20, The Paired Samples T-test was used. The Pearson's Correlation Test was used to calculate the correlation coefficient between the variables. The One-Way Analysis of Variance (ANOVA) was used to test the homogeneity of the variables. Statistical Package for Social Sciences (SPSS) 20 Program was used in statistical tests.

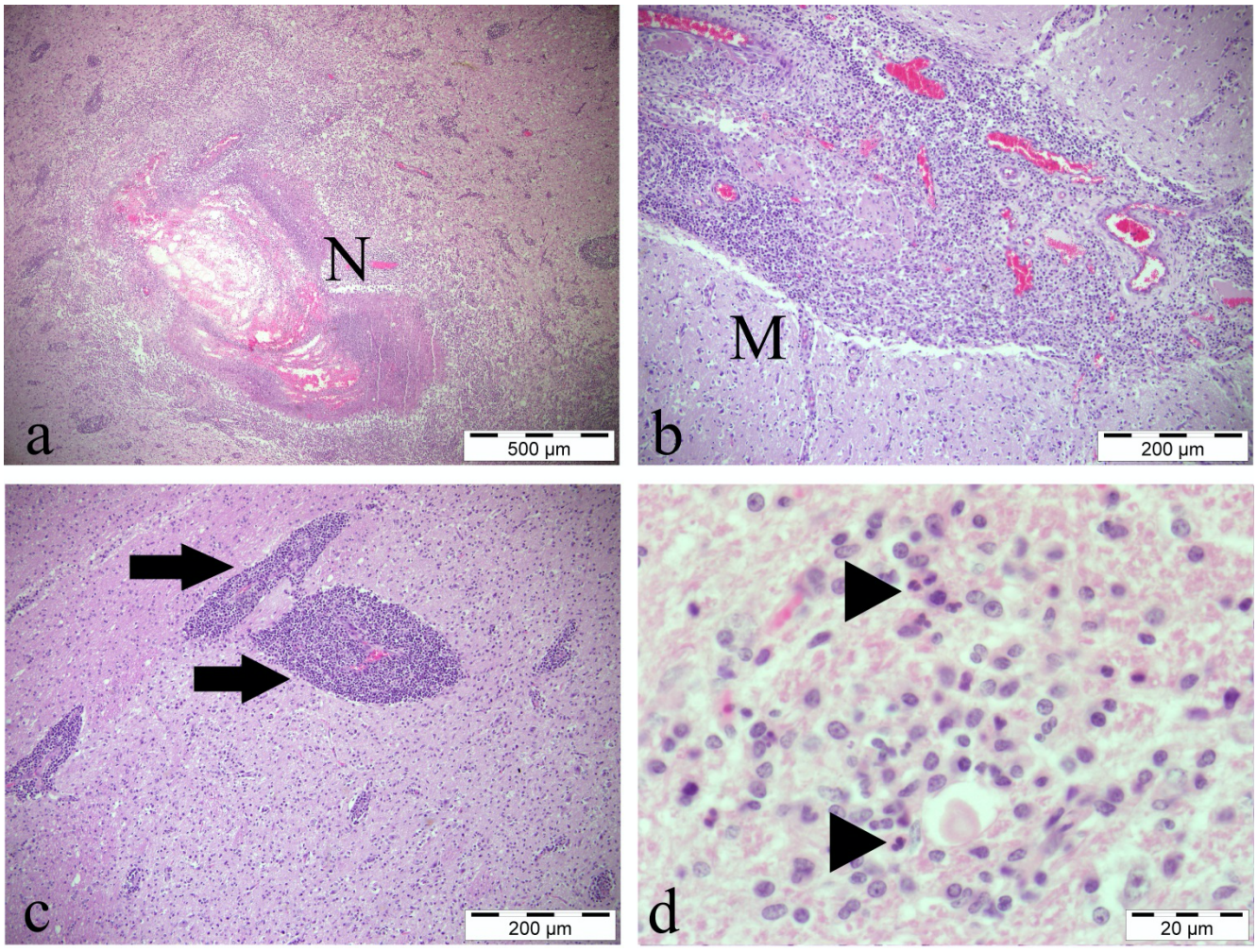


Figure 1: (a) Brainstem, Liquefaction necrosis (N), bar = 500 µm, **(b)** Nonpurulent meningitis (M), bar = 200 µm, **(c)** Pons, Perivascular cuffings (arrows), bar = 200 µm, **(d)** Pons, Microabscess (arrowheads), bar = 20 µm, Hematoxylin & Eosin

Şekil 1: (a) *Beyin kökü, likefaksiyon nekrozu (N), bar = 500 µm,* ***(b)*** *Nonpurulent meningitis (M), bar = 200 µm,* ***(c)*** *Pons, Perivasküler hücre infiltrasyonu (oklar), bar = 200 µm, (d) Pons, Mikroapse (okbaşları), bar = 20 µm, Hematoksilen & Eozin*

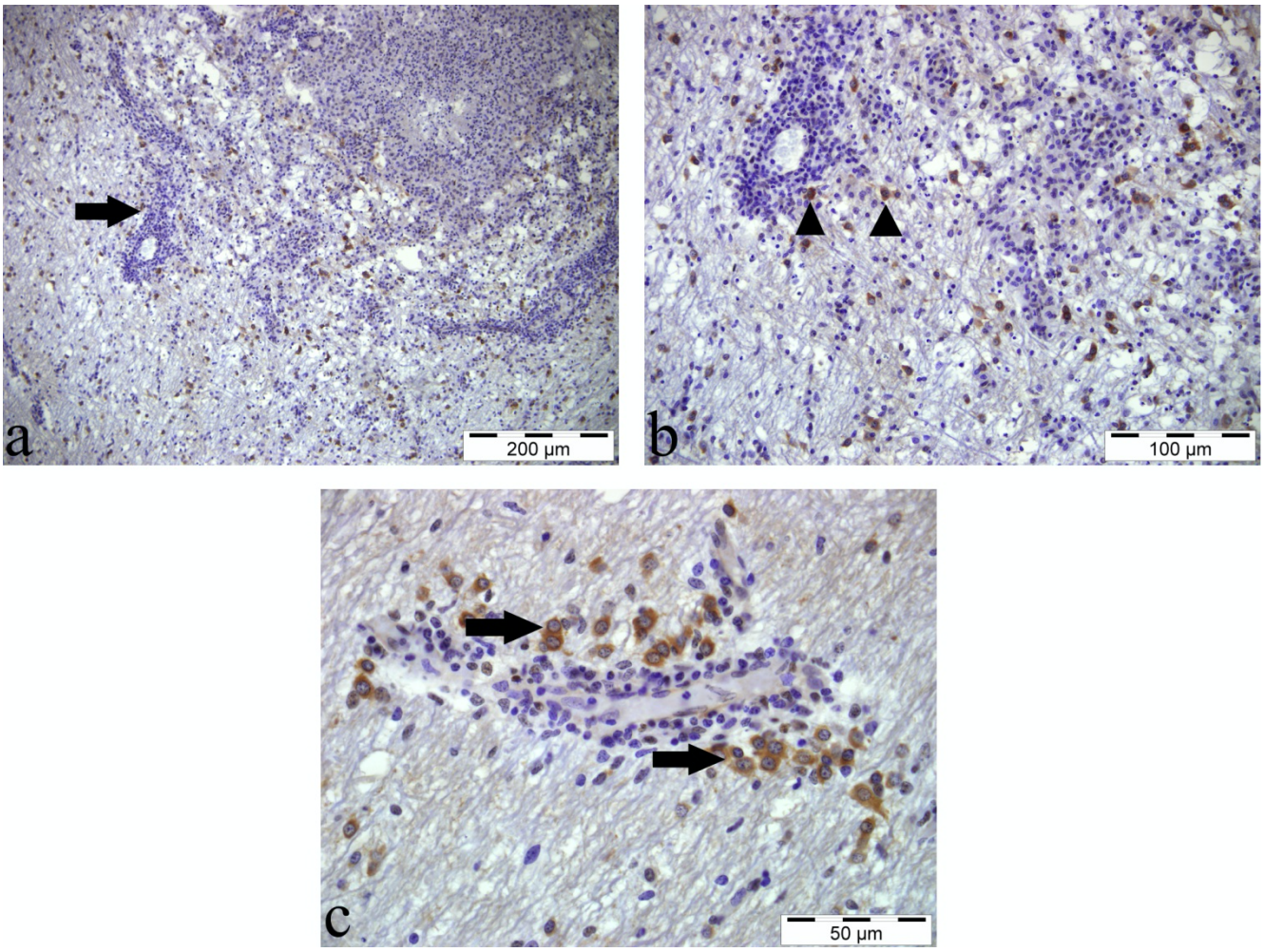


Figure 2: (a) Pons, Intense positive reaction around liquefaction necrosis (N) and perivascular cuffing (arrow), bar = 200 µm, **(b)** Higher magnification, immune positive cells (arrowheads) around perivascular cuffing, bar = 100 µm, **(c)** Immun positive reactions in microglial cells (arrows) around perivascular cuffing and endothelial cells, bar = 50 µm, Immunohistochemistry

Şekil 2: (a) *Pons, Likefaksiyon nekrozu (N) ve perivasküler hücre infiltrasyonu (ok) etrafında yoğun pozitif reaksiyon, bar = 200 µm,* ***(b)*** *Daha yüksek magnifikasyon, perivasküler hücre infiltrasyonu etrafındaki pozitif hücreler (okbaşları), bar = 100 µm,* ***(c)****Perivasküler hücre infiltrasyonu etrafındaki mikroglial hücreler (oklar) ve endotel hücrelerde immun pozitif reaksiyonlar, bar = 50 µm, İmmunohistokimya*

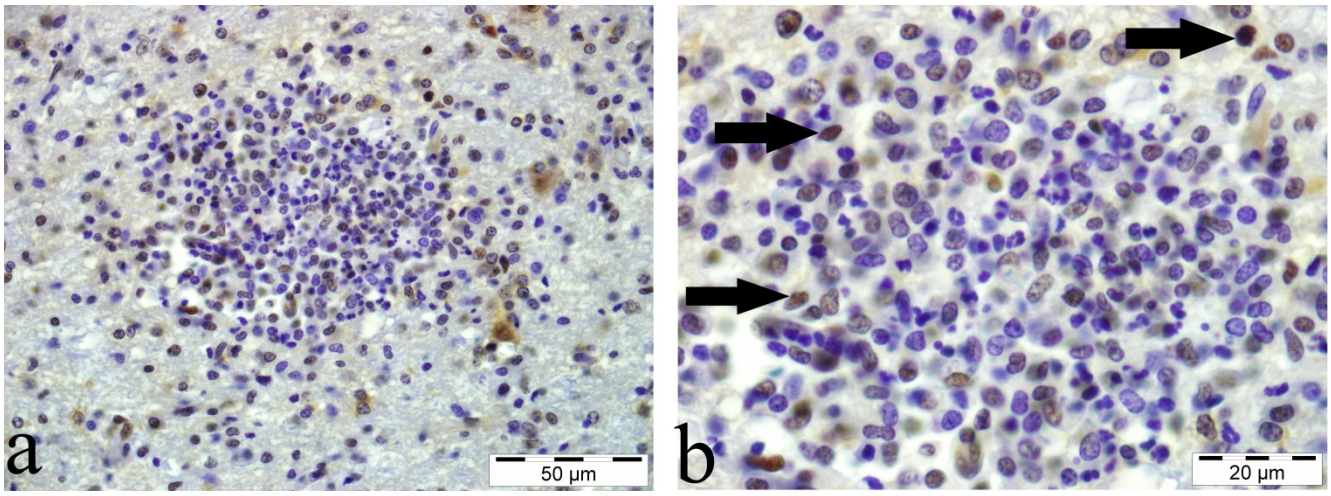


Figure 3: (a) Pons, iNOS immunepositive reactions in inflammatory cells in microabscess, bar = 50 µm,(**b)** Higher magnification, iNOS immunepositive reactions in inflammatory cells (arrows), bar = 20 µm, Immunohistochemistry

Şekil 3: (a) *Pons, mikroapsedeki yangısal hücrelerde iNOS immun pozitif reaksiyonlar, bar = 50 µm, (**b)** Daha yüksek magnifikasyon, yangısal hücrelerde iNOS pozitif reaksiyonlar (oklar), bar = 20 µm, İmmunohistokimya*

3. Results

Microbiological Results:

In this study, *Listeria* spp. were isolated from 20 (%83.3) tissue samples with the presence of specific colonies on LSA, typical microscopic morphologies (0.4-0.5 µm wide and 1-2 µm long, non-spore forming Gram positive bacilli), catalase positive and oxidase negative properties and mobility at 25 °C. All of the isolates were identified as *L. monocytogenes* as the result of L-rhamnose and α-methyl-mannosidase activities and positive CAMP reaction with *S. aureus*.

Hematoxylen & Eosin Results:

Liquefaction necrosis infiltrated by neutrophils (Figure 1a), nonpurulent meningitis (Figure 1b), perivascular cuffing consisted of mostly lymphocytes and fewer histiocytes and plasma cells (Figure 1c), varying sizes and multifocal microabscesses (Figure 1d) (including a small number of neutrophil granulocytes, mostly macrophages, histiocytes and plasma cells), glial nodules, neuronal necrosis and neuronophagia were the most important findings observed in histopathological examination of the medulla oblongata and pons.

MMP-9 and iNOS Results:

In immunohistochemical examinations, we did not detect any MMP-9 expression in normal brain tissues. MMP-9 positive reactions were found in the cytoplasm of microglial cells and neurons in areas where inflammatory and necrotic areas are concentrated (Figure 2-a,b). Immune reactions were detected in endothelial cells in perivascular cuffing areas in the pons and medulla oblongata (Figure 2c). We observed iNOS positive reaction in very few neurons in normal brain tissue. Especially, in cases of encephalitic listeriosis, we detected iNOS immunereactivity in the inflammatory cells in the microabscess foci located in the pons and medulla oblongata (Figure 3-a,b).

Statistical Results:

Initially, the homogeneity test of variances, which is the basic assumption of one-way analysis of variance (ANOVA), was confirmed since p value ($p = 0.016$ for HP/MMP-9 and $p = 0.000$ for MMP-9/iNOS) are greater than 0.01 (Table 1 and Table 2). Due to the sample size is less than 20 and the data of histopathological (HP) analysis, MMP-9 and iNOS groups obtained from the same samples, paired samples t-test was used, and the means of the samples were compared. Additionally, correlation analyzes were conducted between the HP and MMP-9 variables and the MMP-9

and iNOS variables. As the results of paired samples t-test, the average of the HP variables was found as 2.15 and the MMP variables as 1.85. A significance value was detected below 0.05 (p = 0.010 for 2-tailed and p = 0.000 for correlation analysis) with 95% confidence interval (Table 1). Indeed, a statistically high relationship was detected between these two variables and it can be said that the MMP-9 expression has increased in parallel with the increase in HP findings. Although there was no statistically significant value between the averages of MMP-9 and iNOS variables (p = 1.000 for 2-tailed), the correlation value between iNOS and MMP-9 variables was found to be 0.781 (p = 0.000), so a statistically high relationship was found between these two variables at 95% confidence interval. Thus, it can be said that the iNOS expression has increased in parallel with the increase in MMP-9 findings (Table 2).

Table 1: A comparative statistical analysis of HP and MMP-9 findings

Tablo 1: *HP ve MMP-9 bulgularının karşılaştırılmalı istatistiksel analizi*

Case number	HP findings	MMP-9 scores	Correlations				Test of homogeneity of Variances (ANOVA)			
					HP	MMP				
4, 6, 7, 13, 14, 15	+++	+++	HP	Pearson Correlation	1	.867**	HP			
1, 3, 10, 20	+++	++		Sig. (2-tailed)		.000	Levene Statistic	df1	df2	Sig.
17	++	++	MMP	Pearson Correlation	.867**	1				
8, 9	++	+		Sig. (2-tailed)	.000					
2, 5, 11, 12, 16, 18, 19	+	+	** Correlation is significant at the 0.01 level (2-tailed)				5.286	2	17	.016

Table 2: A comparative statistical analysis of MMP-9 and iNOS findings

Tablo 2: *MMP-9 ve iNOS bulgularının karşılaştırmalı istatistiksel analizi*

Case number	MMP-9 scores	iNOS scores	Correlations				Test of homogeneity of Variances (ANOVA)			
					HP	MMP				
4, 6, 15	+++	+++	MMP-9	Pearson Correlation	1	.781**	HP			
7, 13, 14	+++	++								
1, 10	++	+++		Sig. (2-tailed)		.000	Levene Statistic	df1	df2	Sig.
3, 1, 20	++	++	iNOS	Pearson Correlation	.781**	1				
19	+	++		Sig. (2-tailed)	.000					
2, 5, 8, 9, 11, 12, 16, 18	+	+	** Correlation is significant at the 0.01 level (2-tailed)				15.362	2	17	.000

4. Discussion and Conclusion

Clinical findings, bacteriological analysis and histopathological changes in the brain are used in the diagnosis of encephalitic listeriosis (9, 25). Characteristic lesions of listerial encephalitis are microabscesses, focal gliosis and perivascular cuffing (8). Typical lesions of the disease are observed in the brainstem (rhombencephalitis), especially in the pons and the medulla oblongata (6, 16). In this study, it was determined the presence of *L. monocytogenes* in 20 of 24 sheep that showed various neurological symptoms such as permanent circling movement, head pressing, unable to stand, hyper salivation, paralysis in the eyelid, sagging on the lower lip, blindness and torticollis similar to the literature

data (1, 3, 4, 28) by bacteriological methods (4, 10, 44). As reported in previous studies, we observed microscopically large areas of liquefaction necrosis (3, 8, 34), nonpurulent meningitis (7, 19, 31), mostly lymphocyte-containing perivascular cuffing (8, 9, 32) varying sizes of multifocal microabscesses (20, 25, 35) (a small number of neutrophil granulocytes in the middle part) in the brainstem.

Matrix metalloproteinases (MMPs) are a family of 28 zinc-dependent endopeptidases; which are subdivided into collagenases, gelatinases, stromelysins, matrilysin, membrane-type metalloproteinases and metalloelastase (23, 24). MMPs cause to cleavage of ECM and modulate the pathological processes such as inflammation and innate immune defenses (24). MMPs are thought to play an important role in the pathogenesis of meningitis, especially since they perform functions such as the breakdown of the blood-brain barrier (BBB) (typical histopathological feature) and the accumulation of blood-derived immune cells (23, 42). MMP-9 is mainly secreted by monocytes, which are central cells in developing an immune response to infectious diseases. The production of MMP-9 by monocytes is interesting in the context of facilitating leukocyte infiltration into infected areas by breaking down type IV collagen in vascular basement membranes (39). In addition, microglia cells are a remarkable source for the secretion of MMPs (38). Due to the destruction caused by MMP-9 in the extracellular matrix of the brain, a disorder in neuronal functions may occur (5). In many infectious diseases, MMP-9 level has been found to increased (39). MMP-9 activity increases in BBB as a result of bacterial meningitis. This increase in concentration is due to the damage that occurs after meningitis (29). In our study, we found that MMP-9 expressions increased significantly in cases where histopathological findings such as meningitis, microabscesses, necrosis and perivascular cuffings were more severe. Therefore, in line with the data obtained from our study, we concluded that there may be a serious relationship between neuronal dysfunction and MMP-9 expression.

There is only one study in which MMP-9 expression is evaluated immunohistochemically in Listeriosis in sheep (21). In this study conducted by İlhan et al. (21), MMP-9 immunoreactivity was reported in the endothelial cells, microglial cells and neurons especially in inflammatory areas. Sulik and Chyczewski (42) was also reported MMP-9 immunoreactivity in brain endothelial cells, an important factor of the BBB. In the present study, MMP-9 expressions were detected in brainstem (neurons, microglial and endothelial cells in inflammatory and necrotic areas are concentrated) were compared to the previous investigation (5, 21, 23, 42). In this study, it was statistically revealed that there is a positive correlation ($p < 0.05$) between MMP-9 expression and the severity of histopathological findings. The MMP-9 expression has increased in parallel with the increase in HP findings (Table 1). Yamada et al. (45) suggested that MMPs inhibitors increase host resistance in *L. monocytogenes* infection.

The excessive NO production, mainly produced by iNOS, has been indicated as a mediator of cellular damage in inflammatory areas. Under these conditions, nitric oxide reacts with molecular oxygen or superoxide and produces reactive nitrogen species that can modify bioorganic molecules and mediate many biological processes, including ECM proteolysis (18). Oxidants such as superoxide, NO and peroxynitrite are critical to MMP activation (14). MMP-9, produced from activated microglia as a result of the effects of cytokines and reactive oxygen species (especially nitric oxide), destroys the extracellular matrix of the brain and causes impaired neuronal function (5, 14). Similar to the previous studies (22, 36, 40, 41), we observed that iNOS immunereactivity in the inflammatory cells in the microabscess foci located in the pons and medulla oblongata. As a result of our statistical analysis, we revealed that the increase in iNOS and MMP-9 expressions were parallel to each other. We interpreted that NO synthesized by iNOS contributes to the MMP-9 activity and this activation may lead to degradation in the ECM of the brain.

In conclusion, in this study, it was found that there was a positive correlation between MMP-9 expression and histopathological findings in encephalitic listeriosis cases in sheep caused by *L. monocytogenes*. Based on the data obtained from this study, it was believable that MMP-9 plays an important role in the pathogenesis of the disease. In addition, we thought that the activation of iNOS expression increases MMP-9 levels. The given characteristic of MMP-9 can be exploited by the researchers as a marker of prognosis and diagnosis of the disease, or a specific structure targeted by the anti-MMPs for preventing of brain damage. Further investigations focused on the MMP-9 activity in the central nervous system is required.

Conflict of Interest

The author declared no conflict of interest.

Funding

No funding.

Authors' Contributions

Emin KARAKURT contributed to the evaluation of histopathological and immunohistochemical analysis and to write the publication. Fatih BÜYÜK, Özgür ÇELEBİ, Doğan AKÇA and Elif ÇELİK contributed to microbiological analysis. Enver BEYTUT contributed to the evaluation of histopathological and immunohistochemical analysis. Serpil DAĞ contributed to the evaluation of histopathological and immunohistochemical analysis. Hilmi NUHOĞLU contributed to the gross pathology and laboratory procedures of samples taken from animals for histopathological and immunohistochemical analyzes. Ayfer YILDIZ contributed to the gross pathology and laboratory procedures of samples taken from animals for histopathological and immunohistochemical analysis.

Ethical Approval

The ethics committee report of this study was obtained from Kafkas University, Local Ethics Committee of Animal Experiments (Authorization number: KAU-HADYEK-2020/065).

REFERENCES

1. Abdlla OA, Elboshy ME, Reisha EF, Gadlla HA, El-Khodery (2015): *Tumor Necrosis Factor-α, Interleukins-12(p40), 6, and 10 levels in cerebrospinal fluid and outcome prediction in Ossimi sheep with encephalitic listeriosis*. Cytokine, **73(2)**, 283-287.

2. Akça D, Şahin M (2011): *Kars yöresi sığırlarından alınan süt ve vajinal sıvap örneklerinden Listeria türlerinin araştırılması*. Kafkas Univ Vet Fak Derg, **17(6)**, 987-993.

3. al-Dughaym AM, Elmula AF, Mohamed GE, Hegazy AA, Radwan YA, Housawi FM, Gameel AA (2001): *First report of an outbreak of ovine septicaemic listeriosis in Saudi Arabia*. Rev Sci Tech, **20(3)**, 777-783.

4. Barkallah M, Gharbi Y, Hmani M, Mallek Z, Gautier M, Gdoura R, Fendri I (2016): *Locked nucleic acid probe-based real-time PCR for the diagnosis of Listeria monocytogenes in ruminants*. Mol Cell Probes, **30(3)**, 138-145.

5. Berman NE, Marcario JK, Yong C, Raghavan R, Raymond LA, Joag SV, Narayan O, Cheney PD (1999): *Microglial activation and neurological symptoms in the SIV model of NeuroAIDS: association of MHC-II and MMP-9 expression with behavioral deficits and evoked potential changes*. Neurobiol Dis, **6(6)**, 486-498.

6. Braun U, Stehle C, Ehrensperger F (2002): *Clinical findings and treatment of listeriosis in 67 sheep and goats*. Vet Rec, **150(2)**, 38-42.

7. Brugère-Picoux J (2008): *Ovine listeriosis*. Small Rum Res, **76(1-2)**, 12-20.

8. Campero CM, Odeón AC, Cipolla AL, Moore DP, Poso MA, Odriozola E (2002): *Demonstration of Listeria monocytogenes by immunohistochemistry in formalin-fixed brain tissues from natural cases of ovine and bovine encephalitis*. J Vet Med B Infect Dis Vet Public Health, **49(8)**, 379-383.

9. Çeribaşı S, Kızıl Ö, Karahan M (2013): *Listeriyozisli koyunlarda klinik, patolojik ve mikrobiyolojik bulgular*. F Ü Sağ Bil Vet Derg, **27(1)**, 1-5.

10. Dağ S, Akça D, Karaman M, Çelebi Ö, Özen H, Büyük F, Şahin M (2013): *Investigation of immunoperoxidase technique in smears prepared from vaginal secretions in use of early diagnosis of listerial abortions in cattle*. Kafkas Univ Vet Fak Derg, **19(1)**, 1-6.

11. Dhama K, Karthik K, Tiwari R, Shabbir MZ, Barbuddhe S, Malik SVS, Singh RK (2015): *Listeriosis in animals, its public health significance (food-borne zoonosis) and advances in diagnosis and control: A comprehensive review*. Vet Quart, **35(4)**, 211-235.

12. Dreyer M, Aguilar-Bultet L, Rupp S, Guldimann C, Stephan R, Schock A, Otter A, Schüpbach G, Brisse S, Lecuit M, Frey J, Oevermann A (2016): *Listeria monocytogenes sequence type 1 is predominant in ruminant rhombencephalitis*. Sci Rep, **6**, 36419.

13. Dreyer M, Thomann A, Böttcher S, Frey J, Oevermann A (2015): *Outbreak investigation identifies a single Listeria monocytogenes strain in sheep with different clinical manifestations, soil and water*. Vet Microbiol, **179(1-2)**,

69-75.
14. Egi K, Conrad NE, Kwan J, Schulze C, Schulz R, Wildhirt SM (2004): *Inhibition of inducible nitric oxide synthase and superoxide production reduces matrix metalloproteinase-9 activity and restores coronary vasomotor function in rat cardiac allografts*. Eur J Cardiothorac Surg, **26(2)**, 262-269.
15. Food And Drug Administration (FDA) (2015): *Testing methodology for listeria species or L. monocytogenes in environmental samples*. **Version 1**, Pp.2-11.
16. Guldimann C, Bärtschi M, Frey J, Zurbriggen A, Seuberlich T, Oevermann A (2015): *Increased spread and replication efficiency of Listeria monocytogenes in organotypic brain-slices is related to multilocus variable number of tandem repeat analysis (MLVA) complex*. BMC Microbiology, **15(1)**, 134.
17. Haligur M, Aydogan A, Ozmen O, Ipek V (2019): *Immunohistochemical evaluation of natural cases of encephalitic listeriosis in sheep*. Biotech Histochem, **94(5)**, 341-347.
18. Hamada T, Duarte S, Tsuchihashi S, Busuttil RW, Coito AJ (2009): *Inducible nitric oxide synthase deficiency impairs matrix metalloproteinase-9 activity and disrupts leukocyte migration in hepatic ischemia/reperfusion injury.* Am J Pathol, **174(6)**, 2265-2277.
19. Headley SA, Bodnar L, Fritzen JT, Bronkhorst DE, Alfieri AF, Okano W, Alfieri AA (2014): *Histopathological and molecular characterization of encephalitic listeriosis in small ruminants from northern Paraná, Brazil*. Braz J Microbiol, **44(3)**, 889-896.
20. Henke D, Rupp S, Gaschen V, Stoffel MH, Frey J, Vandevelde M, Oevermann A (2015): *Listeria monocytogenes spreads within the brain by actin-based intra-axonal migration*. Infect Immun, **83(6)**, 2409-2419.
21. İlhan F, Ulusoy Y, Halıgür M (2012): *Matrix metalloproteinase expression in sheep with listerial meningoencephalitis*. Res Vet Sci, **92(2)**, 269-272.
22. Jungi TW, Pfister H, Sager H, Fatzer R, Vandevelde M, Zurbriggen A (1997): *Comparison of inducible nitric oxide synthase expression in the brains of Listeria monocytogenes-infected cattle, sheep, and goats and in macrophages stimulated in vitro*. Infect Immun, **65(12)**, 5279-5288.
23. Kieseier BC, Paul R, Koedel U, Seifert T, Clements JM, Gearing AJ, Pfister HW, Hartung HP (1999): *Differential expression of matrix metalloproteinases in bacterial meningitis*. Brain, **122(Pt 8)**, 1579-1587.
24. Liang FR, Wang QQ, Jiang YL, Yue BY, Zhou QZ, Wang JH (2020): *Characterization of matrix metalloprotease-9 gene from Nile tilapia (Oreochromis niloticus) and its high-level expression induced by the Streptococcus agalactiae challenge*. Biomolecules, **10(1)**, 76.
25. Loeb E (2004): *Encephalitic listeriosis in ruminants: Immunohistochemistry as a diagnostic tool*. J Vet Med A Physiol Pathol Clin Med, **51(9-10)**, 453-455.
26. Madarame H, Seuberlich T, Abril C, Zurbriggen A, Vandevelde M, Oevermann A (2011): *The distribution of E-cadherin expression in listeric rhombencephalitis of ruminants indicates its involvement in Listeria monocytogenes neuroinvasion*. Neuropathol Appl Neurobiol, **37(7)**, 753-767.
27. McClain D, Lee WH (1988): *Development of USDA-FSIS method for isolation of Listeria monocytogenes from raw meat and poultry.* J Assoc Off Anal Chem, **71(3)**, 660-664.
28. Morin DE (2004): *Brainstem and cranial nerve abnormalities: Listeriosis, otitis media/interna, and pituitary abscess syndrome*. Vet Clin North Am Food Anim Pract, **20(2)**, 243-273.
29. Nau R, Djukic M, Spreer A, Ribes S, Eiffert H (2015): *Bacterial meningitis: an update of new treatment options*. Expert Rev Anti Infect Ther, **13(11)**, 1401-1423.
30. Nightingale KK, Schukken YH, Nightingale CR, Fortes ED, Ho AJ, Her Z, Grohn YT, McDonough PL, Wiedmann M (2004): *Ecology and transmission of Listeria monocytogenes infecting ruminants and in the farm environment*. Appl Environ Microbiol, **70(8)**, 4458-4467.
31. Oevermann A, Botteron C, Seuberlich T, Nicolier A, Friess M, Doherr MG, Heim D, Hilbe M, Zimmer K, Zurbriggen A, Vandevelde M (2008): *Neuropathological survey of fallen stock: Active surveillance reveals high prevalence of encephalitic listeriosis in small ruminants*. Vet Microbiol, **130(3-4)**, 320-399.
32. Oevermann A, Di Palma S, Doherr MG, Abril C, Zurbriggen A, Vandevelde M (2010): *Neuropathogenesis of naturally occurring encephalitis caused by Listeria monocytogenes in ruminants*. Brain Pathol, **20(2)**, 378-390.
33. Orsi RH, Wiedmann M (2016): *Characteristics and distribution of Listeria spp., including Listeria species newly described since 2009*. Appl Microbiol Biotechnol, **100(12)**, 5273-5287.
34. Ortatatlı M, Çiftçi MK, Tuzcu M (2001): *Bir koyun sürüsünde görülen purulent ensefalitis*. Vet Bil Derg, **17(2)**, 97-102.
35. Özyıldız Z, Dinçel GÇ, Terzi OS, Özsoy ŞY, Kul O (2018): *Immunohistochemical investigation of the damage to and repair of myelin, and astrocyte activity in small ruminants resulting from with natural meningoencephalitic listeriosis*. Ankara Üniv Vet Fak Derg, **65(3)**, 283-290.
36. Pfister H, Remer KA, Brcic M, Fatzer R, Christen S, Leib S, Jungi TW (2002): *Inducible nitric oxide synthase and nitrotyrosine in listeric encephalitis: a cross-species study in ruminants.* Vet Pathol, **39(2)**, 190-199.

37. Precht C, Diserens G, Vermathen M, Oevermann A, Lauper J, Vermathen P (2018): *Metabolic profiling of listeria rhombencephalitis in small ruminants by 1H high-resolution magic angle spinning NMR spectroscopy*. NMR in Biomedicine, **31(12)**, e4023.
38. Rosenberg GA (2002): *Matrix metalloproteinases in neuroinflammation*. Glia, **39(3)**, 279-291.
39. Shihab PK, Al-Roub A, Al-Ghanim M, Al-Mass A, Behbehani K, Ahmad R (2015): *TLR2 and AP-1/NF-kappaB are involved in the regulation of MMP-9 elicited by heat killed Listeria monocytogenes in human monocytic THP-1 cells*. J Inflamm (Lond), **12**, 32.
40. Shin T, Weinstock D, Castro MD, Acland H, Walter M, Kim HY, Ahn M, Purchase HG (2001): *Neuronal constitutive and inducible nitric oxide synthase expression in the brains of Listeria monocytogenes-infected cattle.* Acta Vet Brno, **70(1),** 43-47.
41. Shin T, Weinstock D, Castro MD, Acland H, Walter M, Kim HY, Purchase HG (2000): *Immunohistochemical study of constitutive neuronal and inducible nitric oxide synthase in the central nervous system of goat with natural listeriosis.* J Vet Sci, **1(2)**, 77-80.
42. Sulik A, Chyczewski L (2008): *Immunohistochemical analysis of MMP-9, MMP-2 and TIMP-1, TIMP-2 expression in the central nervous system following infection with viral and bacterial meningitis*. Folia Histochem Cytobiol, **46(4)**, 437-442.
43. Şahin M, Beytut E (2006): *Abortions in sheep due to Listeria ivanovii in the Kars region*. Turk J Vet Anim Sci, **30(5)**, 503-506.
44. Wesley IV, Larson DJ, Harmon KM, Luchansky JB, Schwartz AR (2002): *A case report of sporadic ovine listerial menigoencephalitis in Iowa with an overview of livestock and human cases*. J Vet Diagn Invest, **14(4)**, 314-321.
45. Yamada K, Yoshino K, Sekikawa K, Madarame H, Yagita H, Nakane A (2000): *Effect of a matrix metalloproteinase inhibitor on host resistance against Listeria monocytogenes infection*. FEMS Immunol Med Microbiol, **29(3)**, 187-194.

VETERİNER HEKİMLER DERNEĞİ DERGİSİ
JOURNAL OF THE TURKISH VETERINARY MEDICAL SOCIETY

ISSN: 0377-6395
e-ISSN: 2651-4214
Dergi ana sayfası:
Journal homepage:
http://dergipark.org.tr/vetheder

DOI: 10.33188/vetheder.789268

Araştırma Makalesi / Research Article

Comparison of parthenogenetic oocyte activation in different mouse strains on in vitro development rate and quality

Ali Cihan TAŞKIN [1,a], Ahmet KOCABAY [1,b], Şeref GÜL [2,c], Kübra ÇAĞLAR ERKAL [3,d]*

[1] *Embryo Manipulation Laboratory, Center for Translational Medicine (KUTTAM), Koç University, Sarıyer, Turkey*
[2] *Department of Chemical and Biological Engineering, Koç University, Sarıyer, Istanbul, Turkey,*
[3] *Department of Agricultural Biotechnology, Institute of Natural and Applied Sciences, Tekirdağ Namık Kemal University, Tekirdağ, Turkey*
ORCID: 0000-0003-3196-821X [a]; 0000-0002-2365-7246; 0000-0002-5613-1339 [c]; 0000-0002-0158-9414 [d]

MAKALE BİLGİSİ / ARTICLE INFORMATION:

Geliş / Received:
2 Eylül 20
2 September 20

Kabul / Accepted:
29 Kasım 20
29 November 20

Keywords:
Embryo culture
Mouse
Oocyte
Parthenogenetic activation

Anahtar Sözcükler:
Embriyo kültür
Fare
Oosit
Partenogenetik aktivasyon

ABSTRACT:

The aim of our research is to investigate the effects of parthenogenetic activation on in vitro embryo development rates in different mouse strains. B6CBAF1, C57BL/6j, and B6D2F1 mouse strains were used in this study. Superovulated mice were sacrificed and oocytes were obtained 14 hours after the human chorionic gonadotrophin (hCG) injection and the parthenogenetic activation started 18 hours after hCG injection. The oocytes were activated for 6 hours in 10 mM $SrCl_2$ + 5 µg/mL^{-1} Cytohalasine B (CB) + 5 nM Trichostatin A (TSA) containing Ca^{2+} free Chatot Ziomek Brinster (CZB) activation medium. After this, further incubation was performed for two hours in an incubator at 37 °C and 5% CO_2 in embryo culturing medium + TSA. Finally, embryos were cultured for 120 hours. Parthenogenetic activation success of the B6D2F1 mouse strain was found to be higher than C57BL/6j and B6CBAF1 strains.

Farklı fare ırklarında parthenogenotik oosit aktivasyonun in vitro gelişim oran ve kalitesinin karşılaştırılması

ÖZET:

Çalışmamızın amacı, partenogenetik aktivasyonda farklı fare ırklarında in vitro embriyo gelişimi ve kalitesi üzerindeki etkilerinin araştırılmasıdır. Bu çalışmada, B6CBAF1, C57BL/6j, and B6D2F1 farelerin superovulasyon ile elde edilen oositleri kullanılmıştır. Superovule edilen fareler, insan koryonik gonadotropin (hCG) uygulamasından 14 saat sonra oositler elde edildi ve 18 saat sonra partenogenetik aktivasyona başlandı. Oositler, 10 mM $SrCl_2$ + 5 µg/mL^{-1} sitokalazin B (CB) + 5 nM trikostatin A (TSA) Ca^{2+} içermeyen Chatot Ziomek Brinster (CZB) medyumu içerisinde 6 saat bekletildi. Aktivasyon sonrası, embriyo kültür medyumu + TSA'da inkübatörde 37°C ve %5 CO_2 ortamında 2 saat bekletildi. Son olarak, tüm embriyolar 120 saat süre ile kültüre edildi. Bu çalışmadan elde edilen sonuçlar göre, B6D2F1 ırkının partenogenetik aktivasyon başarısı, C57BL/6j ve B6CBAF1 ırklarına göre daha yüksek bulundu.

How to cite this article: **Taşkın AC, Kocabay A, Gül Ş, Erkal KÇ:** Comparison of parthenogenetic oocyte activation in different mouse strains on in vitro development rate and quality. *Veteriner Hekimler Dernegi Dergisi*, 92(1): 42-48, 2021, DOI: 10.33188/vetheder.789268

* *Sorumlu Yazar e-posta adresi / Corresponding Author e-mail address:* ataskin@ku.edu.tr

1. Introduction

Studies on reproductive biotechnology primarily focus on areas such as obtaining more embryos, cryopreservation, embryo culture, and developmental mechanisms. Parthenogenetic activation, which is used in reproductive biotechnology, provides in vitro embryo development without the presence of sperm (1,2).

Parthenogenetic activation is used in areas like fertilization modeling, somatic cell nuclear transfer (SCNT) as cloning, and research on embryonic stem cell. In cloning, the most important aspect of in vitro development is the activation of the oocyte after somatic cell transfer (3-6). Electrical and chemical methods are widely utilized in mouse embryos to achieve parthenogenetic activation. Among these, the $SrCl_2$ chemical activation method is the most commonly used protocol in mouse embryos (7-9).

Research of ESC purposes is most widely obtained from fertile embryos nowadays. Due to ethical restrictions especially on human embryo studies, parthenogenetic embryos are used as an alternative in stem cell researches. Parthenogenetic embryonic stem cells provide a suitable research model for regenerative medicine studies like cell therapy and tissue repair (10-12).

There are variations among different mouse strains in terms of in vitro development rates (13-14), embryonic stem cell obtaining rates (15), and also cloning success ratios. B6CBAF1 (C57BL/6j×CBA/J), C57BL/6j, and B6D2F1 (C57BL/6j×DBA/J) mouse strains are common used in reproductive biotechnology (ICSI, SCNT and transgenic model) (16-18). No research was found in literature about comparative results of in vitro developments among oocytes belonging to these strains mouse. In this study; therefore, in vitro culture development rates and development qualities of widely used mouse strains through parthenogenetic activation were evaluated.

2. Materials and methods

All animal applications and animal care and maintenance procedure were approved (approval number: 2014 - 05) by the Local Ethics Committee for Animal Experiments of Koç University. The animals were kept in the Koç University, Animal Research Facility (KUARF) of Centre for Translational Medicine (KUTTAM), and the animals were cared on cages (IVC) with HEPA-filtered individual ventilation, 12 hours light - 12 hours dark cycle. Commercial rodent food and water containers were provided ad libitum. In this work, we used 12 female mice. Four mice were used for each group.

Superovulation and oocyte collection:

6-8 weeks old female mice were used in this research. Female mice were chosen from unmated fertile adults. The animals were intraperitoneal injected 10 international unit (IU) pregnant mare serum gonadotropin hormone (SIGMA G4877 - PMSG) at 5:00 pm by intraperitoneal injection for superovulation. 48 hours after, 10 IU human chorionic gonadotropin (SIGMA C8554-hCG) were also applied intraperitoneally at 5:00 pm. Superovulated mice were then sacrificed and then a small incision was cut at ampulla regions of each oviduct in this medium with the help of a sterile toothed forceps. The oocytes were derived from rupturing oviduct ampulla and washed in Human Tubal Fluid + HEPES buffered (HTF, global total w / HEPES) medium + 80 IU/mL hyaluronidase (Sigma H - 3506) + 4 mg/mL Bovine Serum Albumin (BSA, Fraction V. Sigma A3311) and isolated oocyte washed three times in 500 µl HTF medium and selected only high-quality mouse oocytes. Then, oocytes were transferred into four well plates (18-21).

Parthenogenetic oocyte activation and embryo culture:

18 hours after hCG injection, oocytes were incubated for 6 hours in 10 mM $SrCl_2$ + 5 µg/mL $^{-1}$ CB + 5 nM TSA containing Ca^{2+} - free CZB medium. After this, further incubation was performed for two hours in humidified atmosphere of 5% CO_2 at 37°C in embryo culturing medium (LifeGlobal Media, LGGG - 020) + TSA. To assess the embryo development, all embryos were transferred into the embryo medium. Embryo culture drops (10 µl each) were

formed in a petri dish and drops were covered completely with mineral oil (LifeGlobal® Oils, LGOL - 500) to prevent contamination, evaporation, and preserve integrity. At least 2 hours prior, embryo culture media were incubated at 5% CO_2 and 37°C temperature and high humidified in an incubator for gassing. Oocytes were cultured at embryo culture medium (4 mg/mL BSA) for 120 hours until blastocyst stage (18-21).

Determination of cell numbers by differential staining:

Blastocysts were incubated in a solution of 100 µg/mL propidium iodide (for trofectorderm determination) + HTF medium + 1% Triton X 100 for 10-12 seconds and then transferred to 100 µg/mL 100% ethanol (EMPROVE) + 25 µg/mL Hoechst 33258 (H1398, Molecular Probes, Inc.) solution for overnight incubation at 4°C. Next day, blastocysts washed in a 5 µl glycerol droplet were formed on each glass slide for blastocyst stabilization. Blastocysts were transferred to the droplet and covered with a coverslip. These blastocysts preparations were investigated in an inverted microscope with red (propidium iodide) and blue (Hoechst)fluorescence attachment for the determination of trophectoderm (TE) and inner cell mass (ICM) numbers (20,23). For each replication, four blastocysts were used for each group.

Statistical analyses:

Experiments were performed in at least four replications. SPSS Statistics 22.0 program was used for statistical evaluation of the results. One Way ANOVA with Bonferroni post hoc test was used for comparison of the differences among groups.

3. Results

In vitro culture results

According to development evaluations done after in vitro culture, blastocyst development rates were 77.71%, 57.32% and 96.67% in B6CBAF1, C57BL/6j, B6D2F1 groups, respectively. The differences were found significantly important between B6D2F1 and C57BL/6j ($p < 0.05$), B6CBAF1 and B6D2F1 ($p < 0.05$) and B6CBAF1 and C57BL/6j ($p < 0.05$) mouse strains in terms of in vitro development rates (Table 1).

Table 1: In vitro development rates of parthenogenetically activated mouse oocytes (Mean Rate± Std. Error of Mean)

Tablo 1: *Partenogenetik olarak aktive edilmiş fare oositlerinin in vitro gelişim oranları (Aritmetik Ort. ± Std. sapma)*

Group	Number of Embryo (n)	Number of Blastocysts	In Vitro Development Rate (%)
B6CBAF1	88	69	77.71 ± 5.07^{a}
C57BL/6j	65	37	57.32 ± 9.37^{b}
B6D2F1	59	57	96.67 ± 4.08^{c}

Differences between the same columns with different symbols ($^{a, b, c}$) were found to be significant ($p < 0.05$).

Results of cell numbers determined by differential staining

According to differential staining results, mean of total cell numbers in B6CBAF1, C57BL/6j, B6D2F1 groups were determined as 54.8, 44 and 82, respectively. The mean cell number of trophectoderm of each group was calculated as 41, 33.33 and 62, respectively. The mean of ICM of each group were determined as 13.8, 10.67 and 20, respectively. The differences were found significantly important between B6D2F1 and C57BL/6j ($p < 0.05$), B6CBAF1, and B6D2F1 ($p < 0.05$) and, B6CBAF1 and C57BL/6j ($p < 0.05$) mouse strains in terms of total cell numbers, trophectoderm cell numbers and inner cell numbers (Table 2).

Table 2: Cell number of blastocysts according to differential fluorescence labeling (Mean± Std. Error of Mean)

Tablo 2: *Farklı floresan etiketlemeye göre blastosistlerin hücre sayısı (Aritmetik Ort. ± Std. sapma)*

Group	Mean Inner Cell Mass Number	Mean of Trophectoderm Cell Number	Total Cell Number Mean ± St. Dev.
B6CBAF1	13.8 ± 1.47^{a}	41 ± 1.1^{a}	54.8 ± 1.6^{a}
C57BL/6j	10.67 ± 0.94^{b}	33.33 ± 3.4^{b}	44 ± 3.27^{b}
B6D2F1	20 ± 2.16^{c}	62 ± 9.42^{c}	82 ± 11.43^{c}

Differences between the same columns with different symbols ($^{a, b, c}$) were found to be significant ($p < 0.05$).

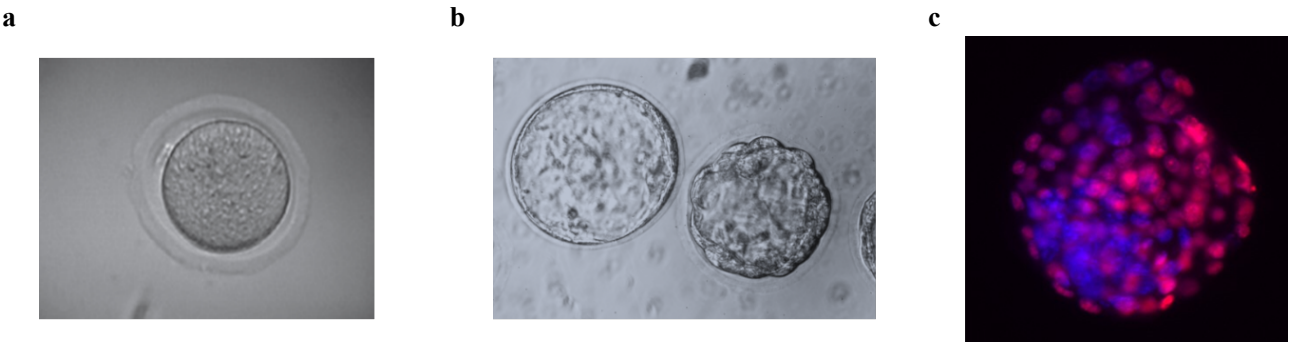


Figure 1: (a) oocyte for activation (20X), **(b)** developed blastocyst at 96 hours (20X), **(c)** differential stained of blastocyst (40X)

4. Discussion and Conclusion

Mice are appropriate in research of parthenogenetic development for improving of cloning technology. In mouse cloning studies, due to the genetic effects of different mouse strain, clone embryo development, as well as the information obtained in this study. However, the effects of different mouse strains are seen in the development effects of parthenogenetic mouse oocyte activations in this study results (24,25). In this study, we showed the importance of genetic influence on parthenogenetic development and embryo quality among different strains.

In their study to identify the optimum conditions for parthenogenetic activation through strontium chloride on mouse oocytes, Ma et al. (2005) achieved a 50.8% blastocyst development ratio 18 hours after hCG injection in Kunming-strain mouse oocytes at 2.5 hours of activation with 10 mM $SrCl_2$ + Ca^{2+}- free +CB 5 µg/mL (8). In the study presented as high as 90% blastocyst development rates were observed with 6 hours of activation in B6D2F1 then, we demonstrated animal strain importance for parthenogenetic activation result.

The activation period has been reported to increase the blastocysts development rate, especially 6 hours of activation in the B6DF1 mouse strain. In the present study, 6 hours of activation of B6DF1 strain were observed significantly higher in vitro embryonic development. Activated B6D2F1 strain mice oocytes for 3 hours at 10 mM

$SrCl_2$ + Ca^{2+}- free + Cytohalasine D 17 hours after hCG injection and observed 65% blastocyst development rate (23). Our study presented also used similar mouse strain mice but obtained blastocyst development rate. Thus, it is possible that our activation period was longer, and Cytohalasine B was used in activation medium instead of Cytohalasine D. The one of the study, 4 hours of activation with 10 mM $SrCl_2$ + Ca^{2+}- free + Cytohalasine D 2 µg/mL to B6D2F1 hybrid mice and obtained 89% blastocyst development rate and mean of total cell numbers, trophectoderm cell numbers, and inner cell mass cell numbers were defined as 75.61, 60.55 ve 15.06 in differential staining to evaluate embryo quality (7). The study we presented also utilized a similar mouse strain but, we used 6 hours of activation with 10 mM $SrCl_2$ + Ca^{2+}- free + Cytohalasine D 5 µg/mL to B6D2F1 hybrid mice and obtained %96 blastocyst development rate and mean of total cell numbers, trophectoderm cell numbers, and inner cell mass cell numbers were also found to 82, 62, 20 cell number. The other study, B6D2F1 mice oocytes were activated for 6 hours with $SrCl_2$ + Ca^{2+} - free + CB 5 µg/mL by Sung et al. (27) and blastocyst development rate was found to be 97.3% Similar B6D2F1 strain mice and same activation period were also used in our presented study and similar in vitro development rate was defined as 96.67%.

The chemical of activation method applied 6-DMAP (2 mmol/l) activation for 4 hours to oocytes of C57BL/6j strain mice obtained a blastocyst development rate of 20.73% (28). C57BL/6j strain mice were also used in our presented study but 6 hours $SrCl_2$ activation was and blastocyst development rate was found to be 57% in our study.Our results of C57BL6j suggest that 6 hours (prolonged activation) can have a positive effect on in vitro development rates, and the longer the duration of strontium treatment, the greater the calcium oscillations in mouse meiotic oocytes. Moreover, mouse oocyte activation was found to increase in aged oocytes more as the oocyte ages, and also as mitogen-activated protein kinase (MAPK) activity decreases (29).

Calcium channels are crucial for oocyte maturation and parthenogenetic activation (30-31). Different mouse strains have different properties in Ca^{2+} storage area and the post-fertilization (32,34). Our results demonstrated that calcium channels might enhance the parthenogenetic embryo development in certain mouse strains consistent with previous reports (30-32,34).

The conclusion of this study allowed the creation of an ideal protocol for chemical activation by comparing in vitro embryo development rates and cell numbers of parthenogenetic mouse oocytes of different strains. These findings are likely to have important applications in reproductive biotechnology. Further studies about molecular mechanisms of parthenogenetic development will be important in research areas such as cloning, ICSI, and stem cell studies.

Acknowledgment

The authors gratefully acknowledge the use of the services and facilities of the Koç University Research Center for Translational Medicine (KUTTAM), funded by the Republic of Turkey Ministry of Development. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Ministry of Development.

Conflict of interest

The author declared no conflict of interest.

Funding

This research was supported by a grant from TUBITAK - The Scientific and Technological Research Council of Turkey (Grant Number: TOVAG 114O638).

Author contribution

Idea / concept: Ali Cihan TAŞKIN
Experiment design: Ali Cihan TAŞKIN
Supervision / Consultancy: Ali Cihan TAŞKIN
Data collecting: Ahmet KOCABAY, Şeref GÜL, Kübra ÇAĞLAR ERKAL
Data analysis and interpretation: Ahmet KOCABAY, Şeref GÜL, Kübra ÇAĞLAR
Literature search: Ali Cihan TAŞKIN, Ahmet KOCABAY, Şeref GÜL
Writing the article: Ahmet KOCABAY, Şeref GÜL, Kübra ÇAĞLAR ERKAL
Critical review: Ali Cihan TAŞKIN

Ethical Approval

All animal applications and animal care and maintenance procedure were approved (Approval number: 2014 - 05) by the Local Ethics Committee for Animal Experiments of Koç University.

References

1. **Cuthbertson KS, Whittingham DG, Cobbold PH** (1981): *Free Ca2+ increases in exponential phases during mouse oocyte activation*. Nature, **294,** 754-757.
2. **Kline D** (1996): *Activation of the mouse egg*. Theriogenology, **45**, 81–90.
3. **Kishikawa H, Wakayama T, Yanagimachi R** (1999): *Comparison of oocyte-activating agents for mouse cloning*. Cloning, **1**, 153–159.
4. **Campbell KHS** (1999): *Nuclear equivalence, nuclear transfer, and the cell cycle*. Cloning, **1**, 3–15.
5. **De Sousa PA, Dobrinsky JR, Zhu J, Archibald AL, Ainslie A, Bosma W, Bowering J, Bracken J, Ferrier PM, Fletcher J, Gasparrini B, Harkness L, Johnston P, Ritchie M, Ritchie WA, Travers A, Albertini D, Dinnyes A, King TJ, Wilmut I** (2002): *Somatic cell nuclear transfer in the pig: control of pronuclear formation and integration with improved methods for activation and maintenance of pregnancy*. Biol Reprod, **66**, 642–650.
6. **Ma SF, Liu XY, Miao DQ, Han ZB, Zhang X, Miao YL, Yanagimachi R, Tan JH** (2005): *Parthenogenetic activation of mouse oocytes by strontium chloride: A search for the best conditions.* Theriogenology, **64**, 1142–1157.
7. **Versieren K, Heindryckx B, Lierman S, Gerris J, De Sutter P** (2010): *Developmental competence of parthenogenetic mouse and human embryos after chemical or electrical activation*. Reprod Biomed Online, **21**, 769-775.
8. **Han BS, Gao JL** (2013): *Effects of chemical combinations on the parthenogenetic activation of mouse oocytes*. Exp Ther Med, **5**, 1281-1288.
9. **Bai GY, Song SH, Wang ZD, Shan ZY, Sun RZ, Liu CJ, Wu YS, Li T, Lei L** (2016): *Embryos aggregation improves development and imprinting gene expression in mouse parthenogenesis*. Dev Growth Differ, **58**, 270-279.
10. **Fulka H, Hirose M, Inoue K, Ogonuki N, Wakisaka N, Matoba S, Ogura A, Mosko T, Kott T, Fulka J** (2011): *Production of mouse embryonic stem cell lines from maturing oocytes by direct conversion of meiosis into mitosis*. Stem Cells, **29**, 517-527.
11. **Didié M, Christalla P, Rubart M, Muppala V, Döker S, Unsöld B, El-Armouche A, Rau T, Eschenhagen T, Alexander P. Schwoerer AP, Ehmke H, Schumacher U, Fuchs S, Lange C, Becker A, Tao W, Scherschel JA, Soonpaa MH, Yang T, Lin Q, Zenke M, Han DW, Schöler HR, Rudolph C, Steinemann D, Schlegelberger B, Kattman S, Witty A, Keller G, Field LJ, Zimmermann WH** (2013): *Parthenogenetic stem cells for tissue-engineered heart repair*. J Clin Invest, **123**, 1285-1298.
12. **Daughtry B, Mitalipov S** (2014): *Concise review: parthenote stem cells for regenerative medicine: genetic, epigenetic, and developmental features*. Stem Cells Transl Med, **3**, 290-298.
13. **Dandekar PV, Glass RH** (1987): *Development of mouse embryos in vitro is affected by strain and culture medium*. Gamete Res, **17**, 279–285.
14. **Herrick JR, Paik T, Strauss KJ, Schoolcraft WB, Krisher RL** (2016): *Building a better mouse embryo assay: effects of mouse strain and in vitro maturation on sensitivity to contaminants of the culture environment*. J Assist Reprod Genet, **33**, 237-245.
15. **Czechanski A, Byers C, Greenstein I, Schrode N, Donahue LR, Hadjantonakis AK, Reinhold LG** (2014): *Derivation and characterization of mouse embryonic stem cells from permissive and nonpermissive strains*. Nat Protoc, **9**, 559-574.
16. **Ogura A, Inoue, K, & Wakayama T** (2013): *Recent advancements in cloning by somatic cell nuclear transfer*. Philos Trans R Soc Lond B Biol Sci, **368**, 20110329.
17. **Bagis H, Aktoprakligil D, Gunes C, Arat S, Akkoc T, Cetinkaya G, Kankavi O, Taskin AC, Arslan K,**

Dundar M, Tsoncheva VL, Ivanov IG (2011): *Expression of biologically active human interferon gamma in the milk of transgenic mice under the control of the murine whey acidic protein gene promoter*. Biochem Genet, **49**, 251-257.

18. **Mallol A, Santaló J, Ibáñez E** (2014): *Psammaplin a improves development and quality of somatic cell nuclear transfer mouse embryos.* Cellular Reprogram, **16**, 392-406.
19. **Taşkın AC, Akkoç T, Sağırkaya H, Bağış H, Arat S** (2016): *Comparison of the development of mouse embryos manipulated with different biopsy techniques*. Turk J Vet Anim Sci, **40**, 157-162.
20. **Taşkın AC, Kocabay A, Ebrahimi A, Karahüseyinoğlu S, Şahin GN, Ozcimen B, Ruacan A, Onder TT** (2019): *Leptin treatment of in vitro cultured embryos increases outgrowth rate of inner cell mass during embryonic stem cell derivation*. In Vitro Cell Dev Biol Anim, **55**, 473-481.
21. **Taşkın AC, Kocabay A** (2019): *Leptin supplementation in embryo culture medium increases in vivo implantation rates in mice*. Turk J Vet Anim Sci, **43**, 359-363.
22. **Mallol A, Santaló J, Ibáñez E** (2014): *Psammaplin a improves development and quality of somatic cell nuclear transfer mouse embryos*. Cellular Reprogram, **16**, 392-406.
23. **Mallol A, Santaló J, Ibáñez E** (2013) *Comparison of three differential mouse blastocyst staining methods*. Syst Biol Reprod Med, **59**, 117-122.
24. **Wakayama T, Perry AC, Zuccotti M, Johnson KR, Yanagimachi R** (1998): *Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei*. Nature, **394**, 369-374.
25. **Beck JA, Lloyd S, Hafezparast M, Lennon Pierce M, Eppig JT, Festing MF, Fisher EM** (2000*): Genealogies of mouse inbred strains*. Nat Genet, **24**, 23–25.
26. **Heytens E, Soleimani R, Lierman S, De Meester S, Gerris J, Dhont M, Van der Elst J, De Sutter P** (2008): *Effect of ionomycin on oocyte activation and embryo development in mouse*. Reprod Biomed Online, **17**, 764-771.
27. **Sung LY, Chang CC, Amano T, Lin CJ, Amano M, Treaster SB, Xu J, Chang WF, Nagy ZP, Yang X, Tian XC** (2010): *Efficient derivation of embryonic stem cells from nuclear transfer and parthenogenetic embryos derived from cryopreserved oocytes*. Cellular Reprogram, **12**, 203-211.
28. **Gao W, Yu X, Hao J, Wang L, Qi M, Han L, Lin C, Wang D** (2019): *Ascorbic acid improves parthenogenetic embryo development through TET proteins in mice*. Biosci Rep, **39**, 1-8.
29. **Alberio R, Zakhartchenko V, Motlik J, Wolf E** (2001): *Mammalianoocyte activation: lessons from the sperm and implications for nuclear transfer*. Int J Dev Biol, **45**, 797-809.
30. **Carvacho I, Lee HC, Fissore RA, Clapham DE** (2013): *TRPV3 channels mediate strontium-induced mouse-egg activation.* Cell Rep, **5**, 1375-1386.
31. **Miao YL, Stein P, Jefferson WN, Padilla-Banks E, Williams CJ** (2012): *Calcium influx-mediated signaling is required for complete mouse egg activation*. Proc Natl Acad Sci U S A, **109**, 4169-4174.
32. **Haverfield J, Nakagawa S, Love D, Tsichlaki E, Nomikos M, Lai FA, Swann K, FitzHarris G** (2016): *Ca 2+ dynamics in oocytes from naturally-aged mice*. Sci Rep, **6**, 19357.
33. **McDonough CE, Bernhardt ML, Williams CJ** (2020): *Mouse strain-dependent egg factors regulate calcium signals at fertilization*. Mol Reprod Dev, **87**, 284-292.
34. **Czajkowska K, Walewska A, Ishikawa T, Szczepańska K, Ajduk A** (2020): Age*-related alterations in fertilization-induced Ca2+ oscillations depend on the genetic background of mouse oocytes*. Biol Reprod, **103**, 986-999.

VETERİNER HEKİMLER DERNEĞİ DERGİSİ
JOURNAL OF THE TURKISH VETERINARY MEDICAL SOCIETY
ISSN: 0377-6395
e-ISSN: 2651-4214
Dergi ana sayfası:
Journal homepage:
http://dergipark.gov.tr/vetheder

DOI:10.33188/vetheder.774408
Araştırma Makalesi / Research Article

İmplantasyon ve desidualizasyon esnasında fare uterus dokusunda PROK1 ve PROKR1'in ekspresyonu

Duygu Mutluay [1, a*], Özlem Özbey [2, b], Leyla Kılınç [2, c], Jale Öner [1, d], Hakan Öner [1, e], İsmail Üstünel [2, f]

[1] *Mehmet Akif Ersoy Üniversitesi, Veteriner Fakültesi, Histoloji ve Embriyoloji Anabilim Dalı, Burdur, Türkiye*
[2] *Akdeniz Üniversitesi, Tıp Fakültesi, Histoloji ve Embriyoloji Anabilim Dalı, Antalya, Türkiye*
ORCID: 0000-0003-3286-130X[a];0000-0002-7239-8762[b];0000-0002-0946-2565 [c];0000-0002-1197-2468 [d],0000-0003-3310-8803[e],0000-0002-3865-6457 [f]

MAKALE BİLGİSİ / ARTICLE INFORMATION:

Geliş / Received:
28 Temmuz 2020
28 July 2020

Kabul / Accepted:
12 Aralık 2020
12 December 2020

Anahtar Sözcükler:
Desidualizasyon,
İmplantasyon,
Preimplantasyon
Prokinetisin 1
Prokinetisin reseptör 1

Keywords:
Desidualization
Implantation
Preimplantation
Prokineticin
Prokineticin receptor 1

ÖZET:

İmplantasyon, embriyonun özel hücreleri olan trofoektoderm ve trophoblast vasıtasıyla uterus dokusuyla bağlantı kurulması ile son bulan bir süreçtir. Başarılı bir implantasyon, plasentasyon ve sonrasında gebeliğin gerçekleşebişmesi için damardan zengin bir endometriyum, koordine olmuş bir damar gelişimi ve plasental villöz damarların genişlemesine gereksinim vardır. Bu bilgiler anjiyogenezin gebeliğin erken dönemleri için önemli fizyolojik bir süreç olduğunu göstermiştir. Prokinetisin ailesinin bir üyesi olan vasküler endoteliyal büyüme faktörü (EG-VEGF) diğer bir adıyla prokinetisin 1 (PROK1) plasentayı da içine alan spesifik endokrin dokular için anjiyogenik bir faktör olarak rapor edilmiştir. Biyolojik aktivitesini iki G protein bağlı reseptör, prokinetisin reseptör 1 (PROKR1) ve prokinetisin reseptör 2 (PROKR2) aracılığı ile gerçekleştirir. Trofoblast invazyonunu kontrol eden PROK1ve PROKR1 plasentada eksprese edilmektedir. Ayrıca, PROK1 plasental anjiyogenezi kontrol eder ve yüksek oranda birinci trimester boyunca eksprese edilmektedir. Çalışmamızda kullanılan dişi fareler, östrus siklusu tayini yapıldıktan sonra, 1 gece erkek fareler ile birlikte bırakılarak gebe kalmaları sağlandı. Vaginal plak (tıkaç) görülen dişiler gebe olarak değerlendirildi. Gebeliğin 1, 2, 3, 4, 5, 6, 7 ve 8. günlerinde alınan uterus doku örneklerinde Western Blot yöntemi kullanılarak PROK1, PROKR1 proteinlerinin ekspresyon analizi yapıldı ve günler arasında bir farklılığın olup olmadığını belirlemek için varyans analizi yöntemi kullanıldı. Çalışmamızda PROK1 ve PROKR1 proteinlerinin gebeliğin ilk 8 günü boyunca eksprese edildiği görüldü. Bu bulgular bize PROK1 ve PROKR1 proteinlerinin erken embriyo gelişimi ve implantasyon sırasında eksprese edildiğini ve bu proteinlerin embriyo gelişiminde önemli roller oynuyor olabileceğini önermiştir.

Expression of PROK-1 and PROKR-1 in mouse uterine tissue during implantation and decidualization

ABSTRACT:

Implantation is a process culminating whereby specialized cells of the embryo, the trophectoderm and trophoblast, establish contact with a specialized tissue of the mother, the uterus. During this process trophoblast invades the maternal endometrium and makes contact with the maternal blood supply, which is critical for establishment of pregnancy. Successful implantation, placentation and subsequent gestation require coordinated vascular development to provide a richly vascularized endometrium for implantation and the development and expansion of the placental villous vasculature to facilitate transport of nutrients and oxygen to the embryo. This knowledge supports that angiogenesis is an essential physiological component of implantation, and placental and embryonic development in early stages of pregnancy. Endocrine gland-derived vascular endothelial growth factor (EG-VEGF), also named prokineticin 1(PROK1), is the member of the prokineticin family which is an angiogenic factor reported to be specific for endocrine tissues, including the placenta. Its biological activity is mediated via two G protein coupled receptors, prokineticin receptor 1 (PROKR1) and prokineticin receptor 2 (PROKR2). PROK1 and PROKR1 are expressed in placenta and control trophoblast invasion. Additionally, PROKR1 controls placental angiogenesis and is highly expressed throughout the first trimester. Mice were mated overnight after determining of estrus. Pregnant mice were determined by vaginal plug control. Expressions of PROK-1 and PROKR1 proteins were determined by Western Blot analysis in uterine tissue samples taken at 1, 2, 3, 4, 5, 6, 7, and 8 days of gestation and the results were compared statistically by using the variance analysis to determine whether there was a significant difference between days. In our study, we showed that PROK1 and PROKR1 proteins were expressed during the first 8 days of gestation. These findings suggested that PROK1 and PROKR1 proteins were expressed during early embryo development and implantation and these proteins may play important roles in embryo development.

How to cite this article: **Mutluay D, Özbey Ö, Kılınç L, Öner J, Öner H, Üstünel İ:** İmplantasyon ve desidualizasyon esnasında fare uterus dokusunda PROK1 ve PROKR1'in ekspresyonu. *Veteriner Hekimler Dernegi Dergisi,* 92(1): 49-59, 2021 DOI: 10.33188/vetheder.774408

* *Sorumlu Yazar e-posta adresi / Corresponding Author e-mail address:* duygumutluay@gmail.com

1. Giriş

Memeli gelişimi fertilizasyon adı verilen sperm ve oositin birleşmesi ile başlar. Fertilizasyonu çeşitli hücre ve yarıklanma bölünmeleri takip eder. Preimplantasyon olarak isimlendirdiğimiz bu dönem farelerde embriyonik gün 3.5 (E 3.5)'de iç hücre kütlesi (ICM) ve trofoektoderm (TE) olarak adlandırılan iki farklı hücre kökeninden oluşan blastosist adı verilen yapının oluşumu ile devam eder. Bu dönem blastositin yaklaşık E 4.5 döneminde zona pellusida'dan kurtularak endometriyuma tutunması ve implantasyonun başlaması ile son bulur (40). E 7.5 güne kadar implantasyon alanı kurulur, ICM ve polar TE hücreleri çoğalmaya devam ederler ve ektoplasental kon'u (EPC) oluşturur. E 6.5'ncı günde EPC'nin dış hücreleri invaziv ikincil trophoblast dev hücreleri içine farklanmaya başlar. Plasentasyon ise uterus stromasının 7.5-10.5 günler arasında bu hücreler tarafından işgal edilmesine bağlıdır (1). Bu sırada doku farklılaşması gerçekleşerek uterus stroması desidual dokuya dönüşür. Desidualizasyon esnasında, foliküler fazda bulunan endometriumun fibronektin, tip I, III, V ve VI kollojen içeren intersitisyel tip ekstraselüler matriksi, laminin, heparan sülfat, perlekan ve tip IV kollojen içeren desidua dokusuna dönüşür. Desidualizasyon esnasında uterusta ekstrasellüler matriks (ECM) yıkımı meydana gelir (3, 20). Bu nedenle ECM'in implantasyon sırasında önemli bir rolü bulunmaktadır (12, 35). Gebeliğin erken dönemlerinde meydana gelen önemli olaylardan bir tanesi de trofoblast invazyonudur. Ekstravillöz trofoblast hücreleri (EVT), desidualize olmuş uterus endometriyumuna doğru istilaya geçerler ve miyometriyuma kadar ulaşırlar. Bu sırada EVT, uterus spiral artelerinin lumenine göç eder. Erken gebelik esnasında uterus spiral arterleri de yeniden yapılanır, muskulo-elastik duvarları kaybolur ve yerini, içinde EVT hücrelerinin gömüldüğü, amorf, fibrinoid bir materyal alır (27, 46, 53). Plasentanın sağlıklı bir şekilde gelişebilmesinin trofoblast hücrelerinin kontrollü büyümesine, invazyonuna, farklılaşmasına ve yeterli bir vasküler gelişime bağlı olduğu belirtilmiştir (4). Oldukça vaskülarize bir organ olan plasenta, gebeliğin sonunda verimli materno-fetal değişim için esas olan ve plasental villöz dallanmanın hazırlanmasında anahtar bir rol oynayan kapiller ağın kurulması için gereklidir (6). Organ spesifik anjiogenik faktörlerin varlığı yıllardır bilinmektedir (45, 49), ancak son yıllarda yapılan çalışmalar çeşitli anjiojenik süreçleri uyardığı belirlenen ve plasental gelişimde anahtar endokrin faktör olarak rol oynadığı düşünülen prokinetisinler üzerine yoğunlaşmıştır.

Prokinetisinler, Prokineticin 1 (PROK1); endokrin bez-vasküler endotelyal büyüme faktörü (EG-VEGF) ve Prokinetisin 2 (PROK2); memeli Bombina variegate 8 (Bv8) olarak bilinen iki salgı proteininden oluşurlar (26, 31, 34, 36). Ancak prokinetisin ailesinin üyeleri farklı ekspresyon örnekleri gösterir. Bv8/PROK2, sinir sistemi ile ilişkili iken, EG-VEGF/PROK1 üreme sistemi ve endokrin organlar ile ilişkilidir (7, 8). Bu aile iki G protein bağlı reseptörlerin, sırasıyla PROKR1 ve PROKR2, aktivasyonu yolu ile biyolojik fonksiyonlarını gerçekleştirir (26). Reseptör aktivasyonunun çeşitli sistemlerde hedef hücrelerin çoğalmasını, anti-apopitozisi, farklılaşma ve göç/hareketini düzenlediği gösterilmiştir (26, 37, 43).

Prokinetisinler orijinal olarak sindirim sisteminde bağırsak motilitesini düzenleyen potent ajanlar olarak tanımlanmıştır, ancak daha sonra steroidojenik bezlerde, kalpte ve üreme organlarda anjiogenezisi teşvik ettiği gösterilmiştir (43). Prokinetisinler nörogenezis, sirkadyan ritm, nosisepsiyon (ağrı hissi), endotel hücre çoğalması, hematopoez (15, 33, 36), inflamasyon ve aynı zamanda immun cevapları düzenler (36, 39). Bunların çeşitli biyolojik fonksiyonları ve fonksiyonel karmaşıklığı reseptör ve ligandlarının farklı ekspresyon modelleri ve birden fazla G-protein bağlamaları ile düzenlenir (43).

Plasentada PROK1'in ekspresyonu Ferrara ve ark (18) tarafından rapor edilmiştir. Sonraki çalışmalar insanda gebelik döneminde desidual ve plasental dokularda ve gebeliğin erken dönemlerinde peptid ekspresyonu üzerine yoğunlaşmıştır (11, 37). PROK1 ve onun G proteinine bağlı reseptörü PROKR1'in yüksek oranda insan plasentasında eksprese edildiği, sinsityotrofoblastlarda (ST), sitotrofoblastlarda (CT), fötal endotelyumda ve Hofbauer hücrelerinde (Ho) immunolokalize olduğu bildirilmiştir (13, 16, 37). İndirekt olarak da PROK-1'in, VEGF ekspresyonunu uyararak anjiogenezisi indüklediği bilinmektedir (37). Ayrıca gebeliğin erken dönemlerinde desiduada PROK-1 ve reseptörü PROKR1 seviyelerinin arttığı bildirilmiştir (16). Plasenta da, endokrin bir unsur olan ST tarafından PROK1'in güçlü bir ekspresyonu, trofoblast farklılaşması için yeni bir plasental büyüme faktörü olarak önerilmesine sebep olmuştur (37).

Yapılan çalışmalarda prokinetisinlerin spesifik anjiogenik bir mitojen olduğu ovaryum (31) ve testiste (32) anjiogenezisi teşvik ettiği ve adrenal bezden köken alan endotelyal hücrelerin çoğalmasını, göçünü ve fenestrasyonunu indüklediği bildirilmiştir (31). Prokinetisinler, ovaryumda (18, 19), tuba uterinada (48), plasentada (14, 22) ve uterusta (5, 16, 17, 42) tanımlanmış rollerinden dolayı dişi üreme fonksiyonlarının önemli bir düzenleyicisi olarak kabul edilmektedir.

Gebeliğin erken döneminde, insanlarda plasental PROK1 ekspresyonu ilk trimester boyunca güçlüdür (22). Gebeliğin 7-8. haftalarında (7-8 h), PROK1ekspresyonu ST, plasental villinin endokrin birimi ve Ho ile sınırlıdır. İleri gebelik haftalarında (9 ile 12 h), PROK1 ekspresyonu ST ve Ho'de daha güçlü, CT de hafiftir (22). PROK1 ekspresyonu ekstravillöz trofoblastlarda bulunmaz. Plasental EG-VEGF ekspresyonu gebeliğin 8-11. haftalarında en yoğundur ve sonra insanlarda gebeliğin ilk trimesterin sonuna doğru tedricen azalır. Bölgesel PROK1'in güçlü ekspresyonu gebeliğin farklı trimesterlerinde gebe kadınlardan alınan serumda da bulunur. Gebe olmayan kadınlarda, plazma PROK-1 düzeyleri 40 pg/ml civarındadır ve ilk trimesterde beş kat artar (yaklaşık 200 pg/ml); bu düzeyler gebeliğin sonuna doğru gebe olmayan kadınlarda gözlemlenen değerlere ulaşarak tedricen azalır (24). Gebelik esnasında yüksek PROK-1 düzeyleri, plasentanın PROK-1'in asıl kaynağı olduğunu göstermektedir; bu daha sonra plasental eksplant kültür ortamlarında PROK-1'in ölçülmesi ile ispatlanmıştır (6, 23). Bununla birlikte, özellikle erken gebelik esnasında dolaşımdaki PROK-1'in tek kaynağının plasenta olmadığı düşünülmektedir. Bununla birlikte PROK-1'in aynı zamanda östrus siklusunun sekresyon (luteal) fazı esnasında korpus luteumun (CL) granuloza kökenli hücreleri tarafından aşırı derecede eksprese edildiği gösterilmiştir (19). Bu durum CL'un şekillenmesinde bir rolü olan PROK-1'in gonadotropin ile uyarılmış bir gen olarak tanımlanmasına sebep olmuştur (28, 29, 30).

Geçtiğimiz son on yılda yapılan çalışmalar PROK1 ve reseptörleri olan PROKR1 ve PROKR2 nin implantasyon sonrası özellikle plasentadaki rolleri ve moleküler mekanizmaları üzerine yoğunlaşmıştır. Yapılan literatür taramalarında, plasental gelişimde önemli ve esansiyel roller üstlenen bu faktörün preimplantasyon sürecinde uterus dokusunda etki mekanizması, ekspresyonu ve rolleri üzerine yapılan bir çalışmaya rastlanmamıştır. Yapılan çalışmanın amacı fare uterus dokusunda preimplantasyon, implantasyon sırasında ve sonrasında, PROK-1 ve PROKR-1'nin ekspresyonu belirlenerek bu dönemlerdeki rolleri hakkında literatüre yeni bilgiler kazandırmaktır.

2. Gereç ve Yöntem

Hayvan materyali:

Bu çalışmada, Burdur Mehmet Akif Ersoy Üniversitesi Deney Hayvanları Laboratuvarından temin edilen her grupta 6 adet olmak üzere toplam 48 adet (yaklaşık 20-24 gr ağırlığında, 8-10 haftalık) yetişkin dişi Swiss albino cinsi fareler kullanılmıştır. Çalışma boyunca farelere ayrı kabinlerde standart yem ve su verilerek, ortamlarının sıcaklık ve nem oranları sabit olacak şekilde, 12 saat karanlık/aydınlık ortamlarda tutuldu. Vajinal smear ile östrus siklusunda oldukları tesbit edilen dişi fareler, 1 gece erkek fareler ile (2 dişi/1 erkek) birlikte bırakıldı. Ertesi sabah, vajinal plug (tıkaç) tespit edilen dişi fareler gebeliğin 1. gününde olarak kabul edildi. Gebeliği takip eden günler için; 1, 2, 3, 4, 5, 6, 7 ve 8. günlerinde, bulunan farelere xylazine (7.5 mg/kg)/ketamin (80 mg/kg) kombinasyonu ile sağlanan genel anestezi altında, servikal dislokasyon ile ötenazi uygulanarak anterior abdominal duvar açılıp uterus dokuları alındı (41). Western blot analizleri için alınan uterus dokuları tüplere konularak -80℃'lik dolaplara kaldırıldı. Çalışmadaki tüm deneysel uygulamalar Burdur Mehmet Akif Ersoy Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu tarafından onaylanmıştır (Karar No: 2016- 173).

Western blot methodu:

Gebeliğin 1, 2, 3, 4, 5, 6, 7 ve 8. günlerinde, elde edilen doku örnekleri 0.2 gr dokuya 600 µl lysis buffer ve 10µl proteaz inhibitör kokteyli olacak şekilde inkübe edilerek sonikatör yardımı ile homojenize edildi. Tüm örnekler, 15000 g'de +4℃ de 10 dakika santrifüj edilerek süpernatant kısımları alındı ve dokuların içerdiği protein miktarlarını ölçmek için BCA kiti kullanıldı. Elektroforezden önce örnekler 100℃'deki 5 dakika kaynatıldı ve jel elektroforezi için (PROK1 için %18'lik, PROKR1için %10'luk) poliakrilamid jel hazırlandı. Her kuyucuğa 20 µl (10'luk tarak için) ve

10 µl (15'lik tarak için) örnek, protein miktarları eşit olacak şekilde konularak Mini Protean Sistem III tankının içine yerleştirildi. Mini Protean Sistem III tankına elektroforez solüsyonu eklenerek güç kaynağına bağlandı. Proteinler güç kaynağı aracılığı ile 100 Volt, 50 miliamperde 80-100 dakika elektroforez edildi.

Proteinler jelde yürürken, PVDF membran üstte ve altta 3'er adet filtre kağıdı olacak şekilde sandviç biçiminde hazırlandı. PVDF membran üzerine proteinleri içeren jel konularak hazırlanan sandviç Mini protean III sistemindeki tank blot içerisine alındı. Mini protean III tankına transfer solüsyonu eklendi ve +4℃'de gece boyu proteinlerin membrana transfer olması sağlandı. Proteinlerin PVDF membrana transferinden sonra Tris tampon solüsyonu (Tris buffered-saline, TBS) ile hazırlanan % 5'lik yağsız kuru süt tozu ile oda ısısında 1 saat çalkalayıcı üzerinde bloklandı. Bloklama solüsyonuna ayrıca % 0.1 Tween-20 ilave edildi. Membran, üreticinin tavsiyesine göre hazırlanmış ve bloklama solüsyonu içinde sulandırılmış olan primer antikor [PROK 1 (1:250; ab72807; abcam) ve PROKR1 (1:200; NBP2-15201; Novus) kullanılarak +4℃ 'de gece boyu karıştırıcı üzerinde inkübe edildi. İnkübasyon sonrasında TBS-T ile 1 saat boyunca 10 dakikada bir TBS-T solüsyonu yenilenerek yıkama yapıldı. Membran, primer antikor için uygun olan ve bloklama solüsyonu ile sulandırılmış horseradish peroksidaz (HRP) konjuge sekonder antikorla [(PROK 1 için; 1/1500; PI-2000; Vector Laboratories ve PROKR1 için; 1/2000; PI-1000 Vector Laboratories)] oda sıcaklığında karıştırıcı üzerinde 1 saat inkübe edildi. İnkübasyon sonrasında TBS-T ile 1 saat boyunca 10 dakikada bir TBS-T solüsyonu yenilenerek yıkama yapıldı. Membran SuperSignal Chemiluminisans (CL)-HRP substrat sistemi ile 5 dakika inkübe edildi ve sonrasında karanlık oda içerisinde membranlardaki sinyaller hiperfilme aktarıldı. Film geliştirici ve tespitten geçirilerek, distile su ile yıkanıp kurutuldu. Böylece, belirlenen günlerde Prokineticin 1 (PROK 1) ve PROKR1 protein ekspresyon miktarları belirlendi ve semikantitatif olarak protein seviyeleri karşılaştırıldı (25). Western blot bantlarının değerlendirilmesi ImageJ analiz sistemi ile gerçekleştirildi. Standart olarak kullanılan beta-aktin ve analizi gerçekleştirilen antikorların bant yoğunlukları karşılaştırılarak protein düzeyleri arasındaki farklar tespit edildi.

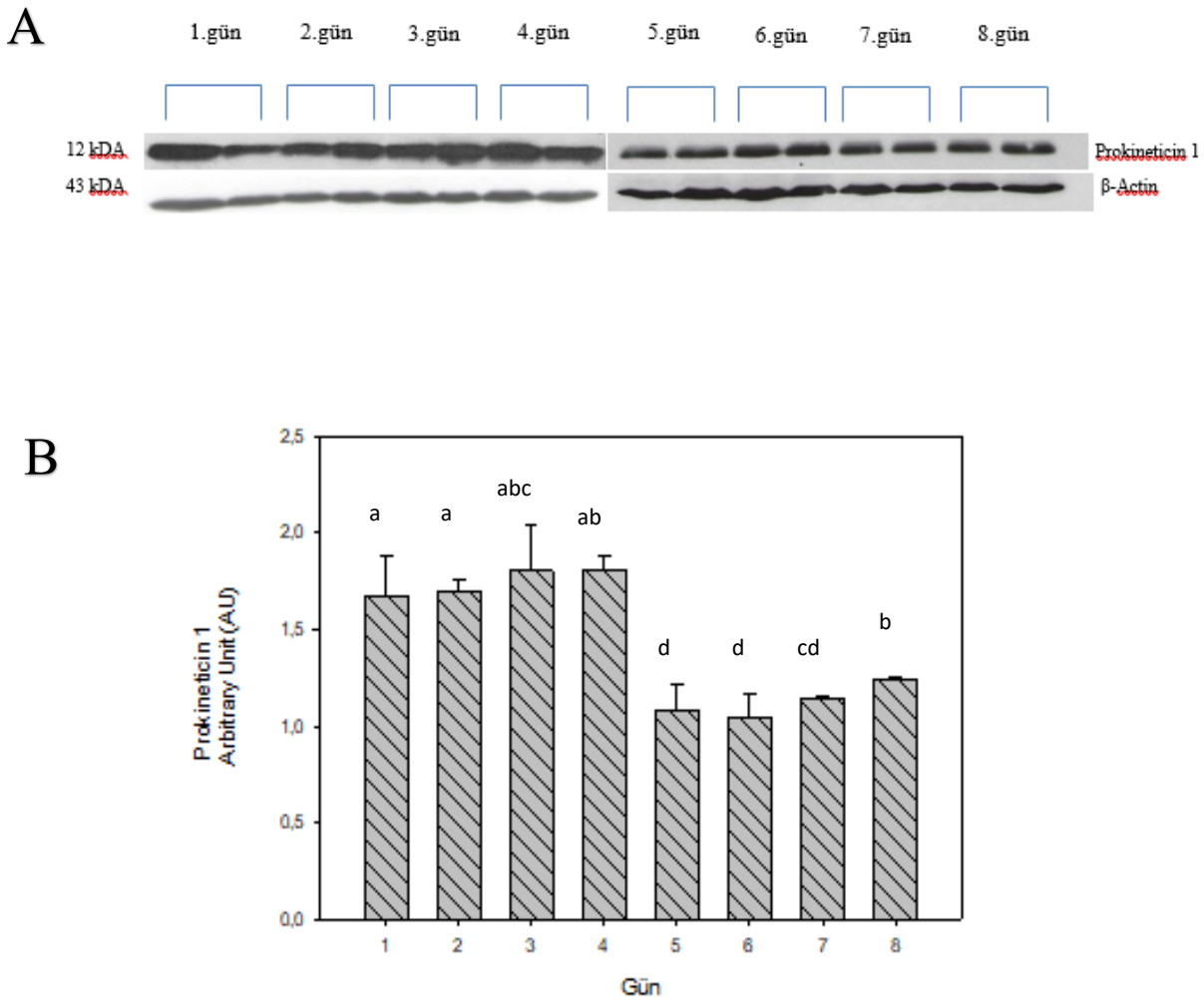
İstatistik analiz:

Deneyler sonucunda elde edilen veriler SPSS (IBM SPSS Statistics version 23) programı ile öncelikle normal dağılım testi için Shapiro-Wilk testi ve varyansların homojenliği için Levene testi uygulanmıştır. Bu sonuçlar doğrultusunda verilerin normal dağılıma uygun ve varyansların homojen olduğu tespit edilmişir. Daha sonra gruplar arası istatistiksel analizler tek yönlü varyans analiz yöntemi ve Tukey post hoc testi ile gerçekleştirilmiştir ($P < 0,05$). Alınan değerler ortalama ± standart hata (SEM) şeklinde belirtilmiştir.

3. Bulgular

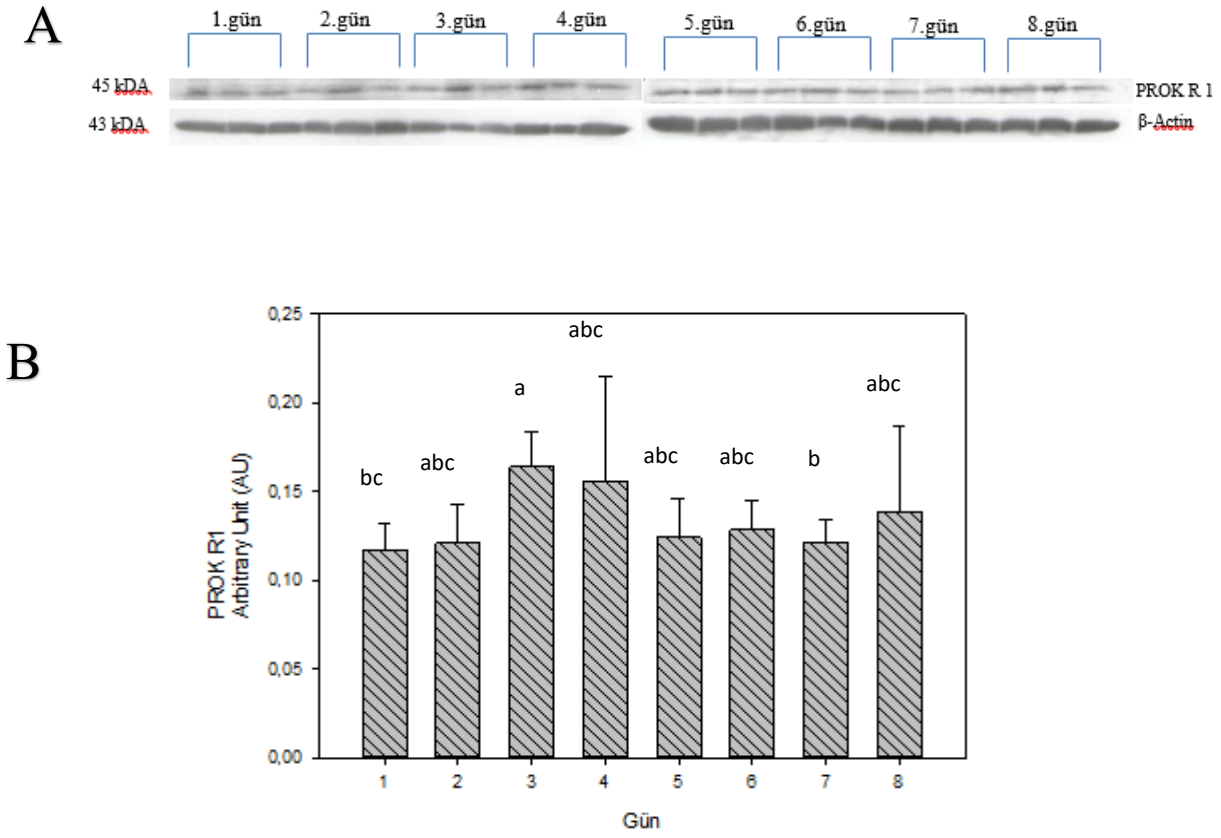
PROK1, PROKR1 protein ekspresyon paternleri 1. 2. 3. 4. 5. 6. 7. 8. günlerde toplanan uterus dokuları üzerine Western Blot analizi uygulanarak belirlenmiştir. Yapılan analizler sonucunda 12 kDa moleküler ağırlığında olan PROK1 proteinin gebeliğin 1. günü ile 8. günü arasında eksprese edildiği tespit edildi. PROK1 ekspresyonunun gebeliğin ilk dört gününde, ilerleyen günler ile karşılaştırıldığında istatistiksel olarak anlamlı şekilde arttığı gözlendi ($P<0,05$). Blastosistin endometriyuma tutunmasının gerçekleştiği E 4.5 aşamasından sonra E 4.5-E 5.5 günleri arasından başlayarak 5.-6. ve 7. günlerde 4. güne oranla istatistiksel olarak anlamlı şekilde PROK1 ekspresyonun azaldığı saptandı ($P<0,05$). 8. günde PROK1 ekspresyon düzeyinin 5., 6. ve 7. günlere kıyasla istatistiksel olarak anlamlı şekilde daha fazla olduğu tespit edildi ($P<0,05$) (Şekil 1).

PROKR1 proteini ise 45 kDa karşılık gelen band üzerinde tespit edilmişdi. PROK1 ile benzer şekilde gebeliğin 1. günü ile 8. günü arasında eksprese edildiği görüldü. PROKR1 proteinin ekspresyonunun gebeliğin 3. gününde en yüksek düzeyde olduğu belirlenmiştir. Yapılan istatistiksel analizler sonucunda PROKR1 ekspresyonun 3. günde 1. ve 2. güne oranla istatistiksel olarak anlamlı şekilde artarken 5., 6. ve 7. günde 3. güne oranla istatistiksel olarak anlamlı şekilde azaldığı saptandı ($P<0,05$). Analiz grafiğinde 4. günde PROKR1 seviyesinde bir artış görülmesine rağmen istatistiksel olarak anlamlılık belirlenemedi ($P>0,05$). PROKR1 ekspresyonun da 7. günden sonra bir artış görülse de istatistiksel olarak anlamlı bir fark bulunamadı ($P>0,05$) (Şekil 2).



Şekil 1: Western blot yöntemiyle gebeliğin ilk 8 günü boyunca PROK1 ekspresyonunun belirlenmesi. **A**: Western blot işlemi sonrası tüm gruplarda 12 kDa değerine karşılık gelen spesifik bandın görünümü. Kontrol amacıyla kullanılan β-aktinin ekspresyonunun görünümü. **B**: Çalışma grupları için Arbitrary Unit (a.u.) cinsinden verilen ekspresyon değişim sonuçlarını göstermektedir. Farklı harfler ile gösterilen indisler arasındaki fark istatistiksel olarak anlamlıdır (P<0,05)

Figure 1: *Determination of Prokineticin 1, expression during the first 8 days of pregnancy with Western blot method.* ***A****: The presence of a specific band corresponding to 12 kDa level is observed in all groups after Western blot analysis. Expression of β-actin used for control purposes.* ***B****: It shows the expression level change results given in Arbitrary Unit (a.u.) for the research groups. The difference between the indices shown with different letters is statistically significant* (P<0.05).



Şekil 2: Western blot yöntemiyle gebeliğin ilk 8 günü boyunca PROKR1, ekspresyonunun belirlenmesi. **A**:Western blot işlemi sonrası tüm gruplarda 45 kDa değerine karşılık gelen spesifik bandın görünümü. Kontrol amacıyla kullanılan β-aktinin ekspresyonunun görünümü. **B**: Çalışma grupları için Arbitrary Unit (a.u.) cinsinden verilen ekspresyon değişim sonuçlarını göstermektedir. Farklı harfler ile gösterilen indisler arasındaki fark istatistiksel olarak anlamlıdır (P<0,05).

Figure 2: *Determination of Prokineticin Receptor 1 expression during the first 8 days of pregnancy with Western blot method.* ***A****: The presence of a specific band corresponding to 45 kDa level is observed in all groups after Expression of β-actin used for control purposes.* ***B****: It shows the expression level change results given in Arbitrary Unit (a.u.) for the study groups. The difference between the indices shown with different letters is statistically significant* (P<0.05).

4. Tartışma ve Sonuç

Son on yıldır yapılan çalışmalar EG-VEGF'nin plasentasyondaki rollerini ve kompleks sinyal yolaklarının bilinmesini belirlemek üzerinedir. Bu çalışmalar, Prokinetisinler ve reseptörlerinin birçok gebelik patolojisini ortadan kaldırabilmek için umut verici tedavi hedefleri olduklarını göstermektedir. Bu amaçla gebeliğin farklı zamanlarında EGF-VEGF ekspresyon çalışmaları yapılmakla beraber, literatürde belirtilen proteinlerin fare uterus dokusunda preimplantasyon dönemi sırasında ve sonrasındaki ekspresyonları ile ilgili bir bilgiye rastlanmamıştır. Bu projenin amacı ise fare uterus dokusunda pre ve peri-implantasyon sürecinde PROK1/PROKR ekspresyonunun tanımlanmasıdır.

Çalışmamızda gebeliğin 1, 2, 3, 4, 5, 6, 7 ve 8. günlerinde, elde edilen doku örneklerinde PROK1 ve PROKR1 protein ekspresyon miktarları belirlenmiştir ve her iki proteinin de belirtilen günler boyunca eksprese edildiği görülmüştür. Gebeliğin ilk dört gününde (1-4. gün), ilerleyen günler (5-8. gün) ile karşılaştırıldığında PROK1 ekspresyonunun istatistiksel olarak anlamlı şekilde yüksek olduğu gözlenmiştir (P<0,05). Özellikle 5., 6. ve 7. güne kıyasla 4. günde gözlenen PROK1 ekspresyonu bize bu proteinin implantasyon sırasında görev yaptığını

göstermektedir. Elde ettiğimiz bulgularla uyumlu olarak, yapılan çalışmalarda da PROK1'in endometriyumun mikrovasküler geçirgenliğini artırarak implantasyonu kolaylaştırabildiği öne sürülmüştür (42). Ayrıca yapılan çalışmalarda PROK1'in implantasyon başarısında etkili olduğu ve implantasyon penceresinin açıldığı dönemde endometriyum-konseptus ağına katıldığını göstermiştir (21). Daha da önemlisi, PROK1'in seksüel siklus döneminde insanlarda endometriyal kabulün (endometriyumun blastosisti başarılı bir şekilde bağlama yeteneği) yeni bir belirteci olarak tanımlanmıştır (16, 17, 36). PROK1'in COX-2 vedickkhopf-1 (DKK1) gibi implantasyon anahtar faktörlerinin ekspresiyonunu da düzenlediği bildirilmiştir (8). Dahası, insanlar da PROK1'in doğrudan endometriyal implantasyon genlerinin ekspresyonunu ve ekstraselüler matriks proteinleri ile trophoblast hücrelerinin yapışmasını arttırdığı, aynı zamanda implantasyon penceresi sırasında embriyo trofektoderm ve endometrial hücreler arasındaki çapraz etkileşimde doğrudan bir rolü olduğu ileri sürülmektedir (2). Preimplantasyon dönemi implantasyon sırası ve sonrasında uterus bezlerinin sayıca arttığı düşünüldüğünde, hücrelerde proliferasyonu destekleyen PROK1 proteininin bu artıştan sorumlu olabileceği ve bezlerde eksprese olabileceği düşünülebilir.

Son yıllarda yapılan çalışmalarla prokinetisinlerin üreme süreçlerinde özellikle endometriyal kabul ve plasental gelişim sırasında anahtar rol oynadıkları gösterilmektedir (8, 10, 37). Hoffman ve ark (24), yaptıkları çalışma ile PROK1'in en yüksek düzeyde birinci trimester döneminde olduğunu,11. haftadan sonra değerlerin düştüğünü sonrasında gebeliğin olmadığı zamandaki düzeylere geldiğini göstermişlerdir. Ayrıca PROK1'in EVT migrasyonunu ve invazyonunu baskıladığını bildirmişlerdir. Yapılan çalışmalar da PROK1 proteininin plasentada esas olarak sinsityotrofoblastlarda lokalize olduğu ve buradan eksprese edildiği bildirilmiştir. Ayrıca PROK1 proteinin trofoblast hücreleri üzerine etki ederek plasenta da proliferasyonu ve büyümeyi tetiklediği (22), EVT, ECM ve başarılı bir gebeliğin gerçekleşmesini sağlayan olayların koordinasyonuna tümden katkıda bulunan villöz trofoblastlar üzerinde de parakrin etkiler sergilediği gösterilmiştir. (8).

Farelerde bilindiği gibi gebeliğin E 4.5 (embriyonik gün 4.5) geç blastosist aşamasında olan embriyo uterusa doğru hareket etmeye başlar ve yaklaşık E 5.0 döneminde hatching olarak adlandırdığımız dönemde blastosist etrafındaki zona pellusida'dan kurtularak endometriyuma tutunur. Blastosist bu sırada uterus lumeninde uterus epiteli ile karşı karşıya gelir. Bu karşılaşma sırasında da blastosist farklılaşmaya devam eder (40). Gelişmenin yaklaşık 5.-6. gününde blastosist uterusun endometriyumuna yapışır. E 5.0 dönemi ile birlikte yapışma embriyoblastın bulunduğu kutuptaki, yapışkan trofoblastlar tarafından gerçekleştirilir. Endometriyum epiteline yapışan trofoblastlar, endometriuma değer değmez hızla çoğalmaya başlarlar ve iki tabakaya farklanırlar. İç tabaka belirgin sınırlı tek çekirdekli hücrelerden meydana gelir ve sitotrofoblast olarak adlandırılır. Dış tabaka ise hücre sınırları belirgin olmayan yalnızca çok çekirdekli sitoplazma kitlesinden oluşan sinsityotrofoblast tabakasıdır (44). Çalışmamız sırasında elde ettiğimiz veriler de 4. günden sonra PROK1 ekspresyonunda bir azalma meydana gelirken (5. ve 6. gün), 7. ve 8. günlerde ise istatistiksel olarak anlamlı artış meydana geldiği görülmüştür. Daha önceden yapılan çalışmalar ile uyumlu olarak elde ettiğimiz bulgular da implantasyon sonrası 5-8 günler arasında eksprese olduğunu tespit ettiğimiz PROK1 proteinin bu süre içinde sinsityotrofoblastlardan sentezlendiğini (22) ve implantasyon sonrası mekanizmalar ile plasenta oluşumunda bir takım roller üstleniyor olabileceğini düşündürmektedir.

PROK1 seviyesi, insan endometriyumunda menstrual siklusun sekresyon fazında artmaktadır (5, 16). PROK1 ve PROKR1 menstrual siklus boyunca uterusta bez epitel hücreleri ve stromada, erken gebelik döneminde de desiduada lokalize olur (5, 16, 36). Çalışmalar PROK1 ekspresyonunun endometriumda progesteron ve insan koryonik gonadotropin (hCG) tarafından düzenlendiğini göstermiştir (5, 17, 42). Ayrıca, endometriyumda PROK1 sinyalinin endometrial kabul ve implantasyona da dahil olan siklooksijenaz 2 (COX2) (16), lösemi inhibitör faktör (LIF) (17), interlökin 8 (IL 8) (38), interlökin 2 (IL 2) (13) ve bağ doku büyüme faktörlerini (CTGF) (52) içeren birkaç gen tarafından düzenlendiği gösterilmiştir. Bu bulgular PROK1'in erken gebeliğin önemli bir düzenleyicisi olduğunu ortaya çıkarmıştır. Prokinetisinlerin, tekrarlayan gebelik kaybı, preeklampsi ve fötal büyüme geriliği (FGR) gibi plasental gelişim patalojilerinde görülen belirgin fonksiyon bozukluklarında, ovaryum, uterus, plasenta ve testisin fizyolojik fonksiyonlarını sürdürebilmelerinde ana düzenleyici olarak görev yaptıkları düşünülmektedir (9, 51). Gerçekten, PROK1 ve reseptörleri ile tekrarlayan gebelik kaybı ve polimorfizim arasında bir ilişki vardır (36, 50). PROK1 ekspresyonundaki anormal artışın bozulmuş desidua ve tekrarlayan gebelik kayıpları ile ilişkili olduğu görülmektedir (47).

Çalışmamızda PROKR1 proteinin ekspresyonunun gebeliğin 3. gününde en yüksek düzeyde olduğu ekspresyonun 3. günde 1. güne oranla istatistiksel olarak anlamlı şekilde artarken, 7. günde 3. güne oranla istatistiksel olarak anlamlı şekilde azaldığı saptanmıştır. Bu veriler bize PROKR1 ekspresyonun 3. günde istatistiksel olarak anlamlı 4. günde ise istatistiksel olarak anlamlı olmayan şekilde bir artış göstererek implantasyon öncesi dönemdeki mekanizmalarda görev aldığını göstermiştir. İmplantasyon sonrasında ise PROK1 ile de uyumlu olarak anlamlı şekilde 7. günde anlamlı 8. günde ise istatistiksel olarak anlamlı olmayan şekilde bir artış gösterdiği tespit edilmiştir.

Bugüne kadar yapılan çalışmalarla, PROK1/PROKR sistemin lokal düzenlenmesi hakkında çok az bilgi elde edilebilmiştir. İnsan gebeliği süresince bu faktörün ve reseptörlerinin güçlü ekspresyon profili, plasentada bu proteinlerin farklı mekanizmalar tarafından kontrol edildiğini göstermektedir. Gebeliğin ilk trimesteri esnasında plasental gelişimin önemli bir parametresi olan hipoksi tarafından PROK1 ve PROKR'in ekspresyon seviyesi artar. (22). Her iki gen, hipoksi ile indüklenebilen faktör 1 (HIF-1) bağlayan düzenleyici bölgelerinde fonksiyonel bir hipoksi yanıt elementi içerir ve düşük oksijen basıncı bu proteinlerin ekpresyonlarının indüksiyonunu düzenler. İnsan gebeliği esnasında, hipoksik çevre ilk trimester boyunca devam eder. Bu yüzden, 8-11. haftada gözlemlenen PROK1 ve PROKR1 ekspresyonundaki yükseliş sadece oksijen basınç düzenlenmesi ile açıklanamamaktadır. Plasentada başka düzenleyicilerin de olduğu ve lokal PROK-1/reseptör ekspresyonunu kontrol ettiği düşünülmektedir (7). Ayrıca gebeliğin 8-10. haftalarında yükselerek pik yapan PROKR1 ekspresyonu PROK1'in ekspresyonu ile paralellik gösterirken, PROKR2 mRNA seviyeleri ilk trimesterin sonuna kadar çok fazla değişiklik göstermez, fakat gebeliğin sonuna doğru giderek azaldığı bildirilmiştir (22, 24).

Sonuç olarak PROKR1 ekspresyonunun özellikle implantasyon öncesi 4. günde artması, implantasyonun meydana geldiği 5. günde azalması ve implantasyon sonrasında 7. günden itibaren tekrar artmaya başlaması bu proteinin gebeliğin sağlıklı şekilde sürdürülebilmesi için gerekli süreçlerde görev aldığını göstermektedir. Ayrıca PROK1 ve PROKR1 proteinlerinin preimplantasyon, implantasyon sırasında ve sonrasında eksprese ediliyor olması bizlere bu proteinlerin bu dönemlerde önemli roller oynadığını akla getirmektedir.

Çıkar Çatışması Beyanı

Çalışma kapsamında herhangi bir kişisel ve finansal çıkar çatışması yoktur.

Finansal Kaynak Beyanı

Bu çalışma Burdur Mehmet Akif Ersoy Üniversitesi Bilimsel Araştırma Projeleri Koordinatörlüğü tarafından 0136-NAP-16 no'lu proje numarası ile desteklenmiştir.

Yazar Katkıları Beyanı

Fikir/kavram: Hakan Öner, Duygu Mutluay
Deney tasarımı: Duygu Mutluay, Hakan Öner
Denetleme/Danışmanlık: İsmail Üstünel, Hakan Öner, Jale Öner
Veri toplama: Duygu Mutluay, Özlem Özbey, Leyla Kılınç
Veri analizi ve yorum: Duygu Mutluay, Özlem Özbey
Kaynak taraması: Duygu Mutluay
Makalenin yazımı: Duygu Mutluay
Eleştirel inceleme: Duygu Mutluay, Özlem Özbey

Etik Onay

Çalışmadaki tüm deneysel uygulamalar Burdur Mehmet Akif Ersoy Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu tarafından onaylanmıştır (Karar No: 2016- 173).

Kaynaklar

1. **Afonso S, Romagnano L, Babiarz B** (1997): *The expression and function of cystatin C and cathepsin B and cathepsin L during mouse embryo implantation and placentation*. Development, **124**, 3415-3425.
2. **Alfaidy N, Hoffmann P, Gillois P, Gueniffey A, Lebayle C, Garçin H, Thomas-Cadi C, Bessonnat J, Coutton C, Villaret L, Quenard N, Bergues U, Feige JJ, Hennebicq S, Brouillet S** (2015): *PROK1 Level in the Follicular Microenvironment: A New Noninvasive Predictive Biomarker of Embryo Implantation*. J Clin Endocrinol Metab, **101(2)**, 435-444.
3. **Aplin JD** (1988): *Cellular biology of the endometrium*. 187-202. In: Wynn RM, Jolie WP, (Ed), Biology of the Uterus. Ed.3 Plenum Press, New York.
4. **Baschat AA** (2004): *Fetal responses to placental insufficiency: an update*. BJOG, **111(10)**, 1031–1041.
5. **Battersby S, Critchley HO, Morgan K, Millar RP, Jabbour HN** (2004): *Expression and regulation of the prokineticins (endocrine gland-derived vascular endothelial growth factor and Bv8) and their receptors in the human endometrium across the menstrual cycle*. J Clin Endocrinol Metab, **89**, 2463–2469.
6. **Brouillet S, Hoffmann P, Benharouga M, Salomon A, Schaal JP, Feige JJ, Alfaidy N** (2010): *Molecular characterization of EG-VEGFmediated angiogenesis: differential effects on microvascular and macrovascular endothelial cells*. Mol Biol Cell, **21**, 2832–2843.
7. **Brouillet S, Hoffmann P, Chauvet S, Salomon A, Chamboredon S, Sergent F, Benharouga M, Feige JJ, Alfaidy N** (2012): *Revisiting the role of hCG: new regulation of the angiogenic factor EG-VEGF and its receptors*. Cell Mol Life Sci, **69(9)**, 1537–1550.
8. **Brouillet S, Hoffmann P, Feige J.J, Alfaidy N** (2012): *EG-VEGF: a key endocrine factor in placental development*. Trends Endocrinol Metab, **23(10)**, 501-508.
9. **Brouillet S, Murthi P, Hoffmann P, Salomon A, Sergent f, De Mazancourt P, Dakouane-Giudicelli M, Dieudonne´ M. N, Rozenberg P, Vaiman D, Barbaux S, Benharouga M, Feige J, Alfaidy N** (2013): *EG-VEGF controls placental growth and survival in normal and pathological pregnancies: case of fetal growth restriction (FGR)*. Cell Mol Life Sci, **70**, 511–525.
10. **Brouillet S, Hoffmann P, Alfaidy N, Feige JJ** (2014): *Prokineticins: new regulatory peptides in human reproduction*. Med Sci (Paris), **30**, 274–279.
11. **Catalano RD, Lannagan TR, Gorowiec M, Denison FC, Norman JE, Jabbour HN** (2010): *Prokineticins: novel mediators of inflammatory and contractile pathways at parturition?* Mol Hum Reprod, **16**, 311–319.
12. **Church HJ, Vicovac LM, Williams JDL, Hey NA, Aplin JD** (1996): *Laminins 2 and 4 are expressed by human decidual cells*. Lab Invest, **74**, 21-32.
13. **Cook IH, Evans J, Maldonado-Perez D, Critchley HO, Sales KJ, Jabbour HN** (2010): *Prokineticin-1 (PROK1) modulates interleukin (IL)-11 expression via prokineticin receptor 1 (PROKR1) and the calcineurin/ NFAT signalling pathway*. Mol Hum Reprod, **16**, 158–169.
14. **Denison FC, Battersby S, King AE, Szuber M, Jabbour HN** (2008): *Prokineticin-1: a novel mediator of the inflammatory response in third-trimester human placenta*. Endocrinology, **149**, 3470–3477.
15. **Dorsch M, Qiu Y, Soler D, Frank N, Duong T, Goodearl A, O'Neil S, Lora J, Fraser CC** (2005): *PK1/EG-VEGF induces monocyte differentiation and activation*. J Leukoc Biol, **78**, 426–434.
16. **Evans J, Catalano RD, Morgan K, Critchley HO, Millar RP, Jabbour HN** (2008): *Prokineticin 1 signaling and gene regulation in early human pregnancy*. Endocrinology, **149**, 2877–2887.
17. **Evans J, Catalano RD, Brown P, Sherwin R, Critchley HO, Fazleabas AT, Jabbour HN** (2009): *Prokineticin 1 mediates fetal-maternal dialogue regulating endometrial leukemia inhibitory factor*. FASEBJ, **23**, 2165–2175.
18. **Ferrara N, Frantz G, LeCouter J, Dillard-Telm L, Pham T, Draksharapu A, Giordano T, Peale F** (2003): *Differential expression of the angiogenic factor genes vascular endothelial growth factor (VEGF) and endocrine gland-derived VEGF in normal and polycystic human ovaries*. Am J Pathol, **162**, 1881–1893.
19. **Fraser HM, Bell J, Wilson H, Taylor PD, Morgan K, Anderson RA, Duncan WC** (2005): *Localization and*

quantification of cyclic changes in the expression of endocrine gland vascular endothelial growth factor in the human corpus luteum. J Clin Endocrinol Metab, **90**, 427–434.

20. **Graham CH, Lala PK** (1992): *Mechanisms of placental invasion of the uterus and their control*. Biochem Cell Biol, **70**, 867-74.
21. **Haouzi D, Mahmoud K, Fourar M, Bendhaou K, Dechaud H, De Vos J, Rème T, Dewailly D, Hamamah S** (2009): *Identification of new biomarkers of human endometrial receptivity in the natural cycle*. Hum Reprod, **24**, 198–205.
22. **Hoffmann P, Feige JJ, Alfaidy N** (2006): *Expression and oxygen regulation of endocrine gland-derived vascular endothelial growth factor/prokineticin-1 and its receptors in human placenta during early pregnancy*. Endocrinology, **147(4)**, 1675–1684.
23. **Hoffmann P, Feige JJ, Alfaidy N** (2007): *Placental expression of EG-VEGF and its receptors PKR1 (prokineticin receptor-1) and PKR2 throughout mouse gestation*. Placenta, **28(10)**, 1049–1058.
24. **Hoffmann P, Saoudi Y, Benharouga M, Graham CH, Schaal JP, Mazouni C, Feige JJ, Alfaidy N** (2009): *Role of EG-VEGF in human placentation: physiological and pathological implications*. J Cell Mol Med, **13(8B)**, 2224–2235.
25. **Izgut-Uysal VN, Acar N, Birsen I, Ozcan F, Ozbey O, Soylu H, Avci S, Tepekoy F, Akkoyunlu G, Yucel G, Ustunel I** (2018): *Apelin-APJ system is responsible for stress-induced increase in atrial natriuretic peptide expression in rat heart***.** Tissue Cell**. 51,** 91-96.
26. **Kaser A, Winklmayr M, Lepperdinger G, Kreil G** (2003): *The AVIT protein family. Secreted cysteine-rich vertebrate proteins with diverse functions*. EMBO Reports, **4**, 469–73.
27. **Kelly BA, Bond BC, Poston L** (2003): *Gestational profile of matrix metalloproteinases in rat uterine artery*. Mol Hum Reprod, **9**, 351-358.
28. **Kisliouk T, Levy N, Hurwitz A, Meidan R** (2003): *Presence and regulation of endocrine gland vascular endothelial growth factor/prokineticin-1 and its receptors in ovarian cells*. J Clin. Endocrinol Metab, **88**, 3700–3707.
29. **Kisliouk T, Podlovni H, Meidan R** (2005): *Unique expression and regulatory mechanisms of EG-VEGF/prokineticin-1 and its receptors in the corpus luteum*. Ann Anat, **187**, 529–537.
30. **Kisliouk T, Podlovni H, Spanel-Borowski K, Ovadia O, Zhou QY, Meidan R** (2005*): Prokineticins (endocrine gland-derived vascular endothelial growth factor and BV8) in the bovine ovary: expression and role as mitogens and survival factors for corpus luteum-derived endothelial cells*. Endocrinology, **146**, 3950–3958.
31. **LeCouter J, Kowalski J, Foster J, Hass P, Zhang ZM, Dillard-Telm L, Frantz G, Rangell L, DeGuzman L, Keller GA, Peale F, Gurney A, Hillan KJ, Ferrara N** (2001): *Identification of an angiogenic mitogen selective for endocrine gland endothelium*. Nature, **412**, 877–84.
32. **LeCouter J, Lin R, Tejada M, Frantz G, Peale F, Hillan KJ, Ferrara N** (2003): *The endocrine-gland-derived VEGF homologue Bv8 promotes angiogenesis in the testis: localization of Bv8 receptors to endothelial cells*. PNAS, **100**, 2685–90.
33. **LeCouter J, Zlot C, Tejada M, Peale F, Ferrara N** (2004): *Bv8 and endocrine gland-derived vascular endothelial growth factor stimulate hematopoiesis and hematopoietic cell mobilization*. PNAS, **101**, 16813–16818.
34. **Li M, Bullock CM, Knauer DJ, Ehlert FJ, Zhou QY** (2001): *Identification of two prokineticin cDNAs: recombinant proteins potently contract gastrointestinal smooth muscle*. Mol Pharmacol, **59**, 692–698.
35. **Lockwood CJ, Krıkun G, Hausknecht VA, Papp C. and Schatz F** (1998): *Matrix metalloproteinase and matrix metalloproteinase inhıbıtor expression in endometrial stromal cells during progestin-inıtiated decidualization and menstruation-related progestin withdrawal*. Endocrinology, **139(11)**, 4607-4613.
36. **Macdonald LJ, Sales KJ, Grant V, Brown P, Jabbour HN, Catalano RD** (2011): *Prokineticin 1 induces Dickkopf 1 expression and regulates cell proliferation and decidualization in the human endometrium*. Mol Hum Reprod, **17**, 626–636.
37. **Maldonado-Perez D, Evans J, Denison F, Millar RP, Jabbour HN** (2007): *Potential roles of the prokineticins*

in reproduction. Trends Endocrin Met, **18**, 66–72.

38. **Maldonado-Perez D, Brown P, Morgan K, Millar RP, Thompson EA, Jabbour HN** (2009): *Prokineticin 1 modulates IL-8 expression via the calcineurin/NFAT signaling pathway*. Biochim Biophys Acta, **1793**, 1315–1324.
39. **Monnier J, Samson M** (2008): *Cytokine properties of prokineticins*. FEBSJ, **275**, 4014–4021.
40. **Mutluay D, Oner H** (2015): *Farelerde preimplantasyon döneminde trofoektoderm ve iç hücre kütlesinin oluşumu*. MAKU J Health Sci Inst, **3**, 1-9.
41. **Mutluay D, Oner H** (2017): *The Abelson tyrosine kinase (c-Abl) localization in preimplantation mouse development*. Rom J Morphol Embryol, **58(4)**, 1385-1391.
42. **Ngan ESW, Lee KY, Yeung WSB, Ngan HYS, Ng EHY, Ho PC** (2006): *Endocrine gland-derived vascular endothelial growth factor is expressed in human peri-implantation endometrium, but not in endometrial carcinoma*. Endocrinology, **147**, 88–95.
43. **Ngan ESW, Tam PKH** (2008): *Prokineticin-signaling pathway*. Int J Biochem Cell B, **40**, 1679–1684.
44. **Piliszek A, Grabarek JB, Frankenberg SR, Plusa B** (2016): *Cell fate in animal and human blastocysts and the determination of viability*. Mol Hum Reprod, **22(10)**, 681-690.
45. **Roberts WG, Delaat J, Nagane M, Huang S, Cavenee WK, Palade GE** (1998): *Host microvasculature influence on tumor vascular morphology and endothelial gene expression*. Am J Pathol, **153**, 1239 –1248.
46. **Salamonsen LA, Woolley DE** (1999): The role of proteinases in implanation. Rev Reprod, **4**, 11-22.
47. **Salker M, Teklenburg G, Molokhia M, Lavery S, Trew G, Aojanepong T, Mardon HJ, Lokugamage AU, Rai R, Landles C** (2010): *Natural selection of human embryos: impaired decidualization of endometrium disables embryo-maternal interactions and causes recurrent pregnancy loss*. PLoS One, 5:e10287.
48. **Shaw JL, Denison FC, Evans J, Durno K, Williams AR, Entrican G, Critchley HO, Jabbour HN, Horne AW** (2010): *Evidence of prokineticin dysregulation in fallopian tube from women with ectopic pregnancy*. Fertil Steril, **94**, 1601–1608.
49. **Stewart PA, Wiley M J** (1981): *Developing nervous tissue induces formation of blood-brain barrier characteristics in invading endothelial cells: a study using quail-chick transplantation chimeras.* Dev Biol, **84**, 183–192.
50. **Su MT, Lin SH, Lee IW, Chen YC, Hsu CC, Pan HA, Kuo PL** (2010): *Polymorphisms of endocrine gland-derived vascular endothelial growth factor gene and its receptor genes are associated with recurrent pregnancy loss.* Hum Reprod, **25**, 2923–2930.
51. **Traboulsi W, Brouillet S, Sergent F, Boufettal H, Samouh N, Aboussaouira T, Hoffmann P, Feige JJ, Benharouga M, Alfaidy N** (2015): *Prokineticins in central and peripheral control of human reproduction*. Horm Mol Biol Clin Investig, **24(2)**, 73-81.
52. **Waddell JM, Evans J, Jabbour HN, Denison FC** (2011): *CTGF expression is up-regulated by PROK1 in early pregnancy and influences HTR-8/ Svneo cell adhesion and network formation*. Hum Reprod, **26(1)**, 67-75.
53. **Wang H, Li Q, Shao L, Zhu C** (2001): *Expression of matrix metalloproteinase -2, -9, -14, and Tissue inhibitors of metalloproteinase -1, -2, -3 in the endometrium and placenta of rhesus monkey (Macaca mulatta) during early pregnancy*. Biol Reprod, **65**, 31-40.

Vet Hekim Der Derg 92 (1): 60-75, 2021

VETERİNER HEKİMLER DERNEĞİ DERGİSİ

JOURNAL OF THE TURKISH VETERINARY MEDICAL SOCIETY

ISSN: 0377-6395
e-ISSN: 2651-4214
Dergi ana sayfası:
Journal homepage:
http://dergipark.org.tr/vetheder

DOI: 10.33188/vetheder.803804

Araştırma Makalesi / Research Article

Structural determination of the relationship between trait anxiety and personal indecisiveness for undergraduates of the faculty of veterinary medicine: The case of Selçuk University

Mert DEMİRSÖZ[1,a*], Zeynep ÖZEL[1,b], Harun YONAR[2,c], Mehmet Emin TEKİN[3,d], Mustafa Agah TEKİNDAL[4,e]

[1] Department of Biostatistics, Selçuk University, Faculty of Veterinary Medicine, Konya, Turkey
[2] Department of Biostatistics, Selçuk University, Faculty of Veterinary Medicine, Konya, Turkey
[3] Department of Biostatistics, Selçuk University, Faculty of Veterinary Medicine, Konya, Turkey
[4] Department of Biostatistics, Izmir Katip Celebi Universitesi, Faculty of Medicine, İzmir, Turkey
ORCID: 0000-0002-4800-2529 [a]; 0000-0002-1077-1250 [b]; 0000-0003-1574-3993 [c]; 0000-0002-3449-9984 [d]; 0000-0002-4060-7048 [e]

MAKALE BİLGİSİ / ARTICLE INFORMATION:

Geliş / Received:
01 Ekim 20
01 October 20

Kabul / Accepted:
27 Aralık 20
27 December 20

Keywords:
Trait Anxiety, Personal Indecision, Explanatory Factor Analysis, Confirmatory Factor Analysis

Anahtar Sözcükler:
Sürekli Kaygı, Kişisel Kararsızlık, Açıklayıcı Faktör Analizi, Doğrulayıcı Faktör Analizi

ABSTRACT:

In this study, it was aimed to determine the relationship between trait anxiety and personal indecisiveness of undergraduate students of the Faculty of Veterinary Medicine. For this purpose, a questionnaire was applied to 244 undergraduate students at Selcuk University Faculty of Veterinary Medicine. Trait anxiety and personal indecisiveness scales were used for this questionnaire. The Cronbach's Alpha (α) coefficient was calculated as 0.798 for the trait anxiety scale and 0.929 for the personal indecisiveness scale. After the factors were determined by explanatory factor analysis, they were tested by confirmatory factor analysis to test the appropriateness of factor structures. Chi-square test, RMSEA, GFI and CFI fit indices were used in confirmatory factor analysis. For the results obtained from indices of fit, the chi-square test was calculated as 1.621, RMSEA 0.051, GFI 0.851, CFI 0.930. The model obtained according to the fit index values was found to fit well. As a result, there was a correlation between students' trait anxiety and personal indecisiveness. As a result of our findings, it was revealed that trait anxiety of students affected the decision-making processes. By carrying out similar studies annually within the universities, positive or negative aspects for the personal development of students can be determined, and it can be ensured that they become healthy physicians in the future. The results can give an idea to the managers about decisions concerning some improvements and developments in universities.

Veteriner fakültesi lisans öğrencileri için sürekli kaygı ve kişisel kararsızlık arasındaki ilişkinin yapısal olarak belirlenmesi: Selçuk Üniversitesi örneği

ÖZET:

Bu çalışmada Veteriner Fakültesi lisans öğrencilerinin sürekli kaygı ile kişisel kararsızlık arasındaki ilişkinin belirlenmesi amaçlanmıştır. Bu amaçla Selçuk Üniversitesi Veteriner Fakültesi'nde 244 lisans öğrencisine anket uygulanmıştır. Bu ankette sürekli kaygı ve kişisel kararsızlık ölçekleri kullanılmıştır. Cronbach Alpha (α) katsayısı sürekli kaygı ölçeği için 0.798, kişisel kararsızlık ölçeği için 0.929 olarak hesaplanmıştır. Faktörler açıklayıcı faktör analizi ile belirlendikten sonra faktör yapılarının uygunluğunu test etmek için doğrulayıcı faktör analizi ile test edilmiştir. Doğrulayıcı faktör analizinde ki-kare testi, RMSEA, GFI ve CFI uyum indeksleri kullanılmıştır. Uyum indekslerinden elde edilen sonuçlar için ki-kare testi 1.621, RMSEA 0.051, GFI 0.851, CFI 0.930 olarak hesaplanmıştır. Uyum indeksi değerlerine göre elde edilen modelin iyi uyum sağladığı görülmüştür. Sonuç olarak, öğrencilerin sürekli kaygısı ile kişisel kararsızlığı arasında bir ilişki vardı. Bulgularımız sonucunda öğrencilerin sürekli kaygılarının karar verme süreçlerini etkilediği ortaya çıkmıştır. Üniversiteler bünyesinde her yıl benzer çalışmalar yapılarak öğrencilerin kişisel gelişimlerine yönelik olumlu veya olumsuz yönleri tespit edilebilir ve ileride sağlıklı hekimler olmaları sağlanabilir. Sonuçlar, üniversitelerdeki bazı iyileştirme ve gelişmelere ilişkin kararlar konusunda yöneticilere fikir verebilir.

How to cite this article: **Demirsöz M, Özel Z, Yonar H, Tekin ME, Tekindal MA:** Structural Determination of the relationship between trait anxiety and personal indecisiveness for undergraduates of the faculty of veterinary medicine: The case of Selcuk University. *Veteriner Hekimler Dernegi Dergisi*, 92(1): 60-75, 2021. DOI: 10.33188/vetheder.803804

* *Sorumlu Yazar e-posta adresi / Corresponding Author e-mail address:* mertdmrsz17@gmail.com

1. Introduction

Anxiety is one of the basic emotions that affect our lives in many social, emotional and mental issues, from early ages when we start making certain decisions to the end of our lives. People may feel anxious when they experience a dangerous situation for various reasons (25). Anxiety is examined in two stages as state anxiety and trait anxiety. State anxiety is anxiety experienced when encountering an unwanted event. Trait anxiety is the type of anxiety that causes us to overreact for any reason. (24). While state anxiety is a temporary state, trait anxiety is a situation that causes a person to misdirect and feel uneasy throughout his life. According to the indications of Arslan (1), Addington (1995) stated that he argued the person did not know how to act in such a situation. This situation may cause the person to make the wrong decisions or to remain indecisive when they need to decide (18).

Indecisiveness is a situation of inability to decide between options or dissatisfaction with the decision made. This situation causes people to have difficulty and anxiety while making decisions. The indecisiveness is examined in two stages. Impetuous indecisiveness is a type of indecisiveness that a person gives to get rid of the options as soon as possible without examining them, and then tries to change them because of uncomfortable with this decision. Exploratory Indecisiveness is a type of indecisiveness that cannot be decided by examining all the options in detail (4).

University life is one of the most critical periods for forming and determining their future. During this period, students make crucial decisions for the rest of their life. Trait anxiety about essential issues such as job choice, friendships, plans and responsibilities related to the place they want to be can cause problems in the decisions of the person and significantly affect their future (11). Family is one of the major factors contributing to this situation. The pressure on the child because of the family's structure, expectations and perfectionist way of thinking play an important role in deciding. These are the most important factors affecting their anxiety level. Also, this pressure created by the family creates various problems for students, and the trait anxiety that occurs seriously affects the situation of the student psychologically (34).

The trait anxiety causes misunderstandings and emotional weariness. Çolak and Doğan (12) stated that controlling one's behaviours would provide control of sadness, and therefore, the condition of trait anxiety would also decrease. This allows the person to make more precise decisions by being less concerned about their decisions.

Anxiety is an emotion that also affects a student's success. In the research of Ergene (14), it was revealed that there is a positive linear relationship between anxiety and success. Aydın and Tiryaki (2) stated as a result of their study that the level of trait anxiety can be reduced by the positive regulation of education policies and education programs. At this point, it is also important to ensure that students can easily access health services and social facilities.

Bozkurt (6) found a positive correlation between depression and anxiety in a study conducted on university students. Accordingly, it is believed that students who experience excessive anxiety affect themselves and their environment negatively.

Urgancı and Güngan (33) stated in their study that for young students, having less future anxiety positively affected their decision-making, and they made more precise decisions. In other words, having future anxiety causes students to make more difficult decisions.

As a result, the study aims to find a meaningful relationship between these two situations by measuring the trait anxiety and personal indecisiveness of the students. Thanks to this interaction, it is aimed to make suggestions for university students to create better educational life and a healthy future.

2. Material and Methods

Research population:

The material of the study was composed of primary data obtained by online and face-to-face questionnaires for students between 1-5 classes at Selcuk University Faculty of Veterinary Medicine. In this study, personal indecisiveness questionnaire (App. A) was used as data collection tool and, trait anxiety questionnaire (App. B) was used to measure trait anxiety. Subsequently, a joint questionnaire was created for the trait anxiety and personal

indecisiveness scale for Veterinary faculty students (App. C). Interviews in the study started after the approval of the Ethics Committee.

The students were selected by stratified random sampling method (classes and gender are defined as layers) and a sufficient number of participants were determined to represent the population (17). The sample size was determined in the 95% confidence range. For this purpose, two new survey forms were created based on survey forms applied in previous studies in similar or close areas.

By using the stratified sampling calculation, the minimum numbers to be taken from departments and classes were determined. A minimum of 126 individuals was projected to be reached. The study was approved by the Local Ethics of Selcuk University, Faculty of Veterinary Medicine (Approval Number: 2020/4-2020/38).

Purpose of research:

The motivation of the study was determined as examining the relationships between trait anxiety and personal indecisiveness and its subscales in university students. For this purpose, the relationship between personal indecisiveness with trait anxiety, hastiness, direct trait anxiety, reversed trait anxiety; the relationship between impetuous indecisiveness with direct trait anxiety and conversely trait anxiety; the relationship between direct trait anxiety and reversed trait anxiety were studied.

Trait anxiety scale:

The trait anxiety scale, which is consists of a total of 20 expressions, was modified in Turkish by Öner and LeCompte (25) There were two types of expressions on this scale. These were direct and reverse expressions. Direct expressions reflect negative emotions, while reverse expressions reflect positive emotions. When scoring these types of expressions, the weight value of 1 turns into 4, and the weight value of 4 turns into 1. The total score ranges from 20 to 80, while the scale consists of 20 expressions. The participation of values that do not change in the scoring process automatically correct the responses to the reversed statements. The answer options on the scale were 4, and they were; 1- almost no time, 2- sometimes, 3 -often, 4- is almost always in the form. Status and trait anxiety scales are independent of each other.

First, measurement models of dimensions were evaluated in the study. Although the compliance values in the measurement models are within the desired limits, the modification indexes were examined due to the fact that the standardized path coefficients of 8 problems of this scale were below 0.5. As a result of these examinations, the relevant items were excluded from the analysis (10). Re-analysis was performed and other substances included in the scale were found to be significant. After this change, the previous and subsequent states were given in Table 3.

Personal indecisiveness scale:

Personal indecisiveness scale is a scale that describes the behaviours adopted by individuals while making decisions and consists of 18 statements. The scale was developed by Bacanlı (3) based on two criteria such as indecision, difficulties in decision making, the cause of indecision or the variables it is associated with. The personal indecisiveness scale has two subscales independent of each other, and it measures personal indecisiveness. These are called exploratory indecisiveness and impetuous indecisiveness. For this reason, it was suggested that the scores obtained from each of the subscales, not the whole scale, should be used in the research. A high score from a subscale indicates a high level of personal indecisiveness measured by that subscale. All items on the scale were arranged in the form of direct statements involving personal indecision, and the total score value ranges from 18 to 90. The impetuous indecisiveness sub-scale was consists of 10 items. These are items 1, 2, 5, 6, 9, 10, 13, 14, 17 and 18. There were 8 items in the exploratory indecisiveness subscale. These items were 3, 4, 78, 11, 12, 15 and 16. Options on the scale with a five-point Likert type; A-it is not appropriate, B-not exactly, C-a bit appropriate, D-appropriate, E-very appropriate in the form. A- 1; B- 2; C- 3; D- 4; E- 5 points were given in the scoring process. Also, the scale was called the ‘personal decision scale’ in order to prevent the responder from being affected (3).

Statistical analysis:

Factor analysis: Factor analysis is a multivariate statistical method used to obtain a small number of identifiable, meaningful variables from a large number of variables that measure the same structure. Factor analysis is divided into two main methods: explanatory factor analysis and confirmatory factor analysis (16).
Explanatory factor analysis: Explanatory factor analysis is a process of finding factors and generating theories based on the relationships between variables. Explanatory factor analysis has three main purposes. The first is to extract dimensions using the correlation or covariance matrix, the second is to decide dimensions, and the third is to determine which rotation technique is used to rotate the obtained dimensions (16).
Confirmatory factor analysis: Confirmatory factor analysis begins with establishing hypotheses that the correlations of variables with factors and factors with each other are defined, and they perform the analysis using a package program such as AMOS (28).

IBM SPSS Statistics for Windows (Version 25.0) and Amos (version 24.0) statistical package were used to evaluate the data. Descriptive statistics (mean, standard deviation, median value, minimum, maximum, number and percentage) were given for categorical and continuous variables in the study. Factor loadings for each question and appropriate sub-dimensions for the two scales were obtained. Reliability analysis was performed for the survey by using Cronbach's Alpha (α) coefficient. In addition, a suitable Structural Equation Model (SEM) was created for confirmatory factor analysis, the accuracy of this model was checked with the fit Index values, and finally, the relationship between the two scales were examined. $P<0.05$ was considered statistically significant.

3. Results

The demographics of 244 students were given as number and percentage in Table 1. In the survey, the student's percentages were determined, 16.8% were first from class, 28.3% were from the second class, 23.8% were from third class, 18% were from fourth class, and 13.1% were from fifth class. Moreover, 36.5% of the students were boys and 63.5% were girls. The average age of the students was 21.73±1.92. 39.8% of the participants stayed in the dormitory. The parents of the participants were mostly graduated from primary or secondary school (60.2% and 46.7%).

Tablo 1: Demografik özellikler, (Aritmetik ort. ± Std. hata)

Table 1: *Demographic informations, (Mean± Std. Error of Mean)*

		n	**$\bar{x}$±SEM - (%)**
Age		244	21.73±1.92
Gender	Female	154	63.1%
	Male	90	36.9%
Class	1	41	16.8%
	2	69	28.3%
	3	58	23.8%
	4	44	18.0%
	5	32	13.1%
Total		**244**	100%

Explanatory and confirmatory factor analysis for trait anxiety scale:

Explanatory factor analysis results for the trait anxiety scale were given in Table 2.

Tablo 2: Sürekli kaygı ölçeği için ortak faktör varyansları ve faktör yükleri

Table 2: *Common factor variances and factor loads for trait anxiety scales*

Items	Factors	
	1st	2nd
9. I worry about trivial things.	**0.747**	
17. No way thoughts bother me.	**0.712**	
11. I take everything seriously and worry.	**0.651**	
18. I take my disappointments so seriously that I will never forget.	**0.620**	
20. The issues that have been on my mind recently make me nervous	**0.618**	
12. I generally lack self-confidence.	**0.573**	
5. I miss opportunities because I cannot make a quick decision.	**0.517**	
8. I feel that the difficulties have accumulated so much that I cannot overcome	**0.514**	
3. I usually cry easily.	**0.500**	
14. I avoid facing difficult and difficult situations.	**0.487**	
15. Usually, I feel sad.	0.471	
2. I usually get tired quickly.	0.462	
4. I want to be as happy as others.	0.264	
10. I am generally happy.		**0.845**
1. I am generally in a good mood.		**0.793**
16. I am generally satisfied with my life.		**0.786**
13. Generally, I feel safe.		**0.655**
6. I feel rested.		**0.638**
19. I am a sane and determined person.		0.375
7. I am generally calm, restrained and cool.		0.241
Self-values	4.235	3.689
Variance description rates %	21.177	18.431
Cronbach's Alpha (α)	0.835	0.776
Total described variance ratio = 39.608 Kaiser Meyer Olkin (KMO) = 0.845 Bartlett test value =1596.700 p=0.001** Total Cronbach's Alpha (α)= 0.857		

**p<0.05* ***p<0.01*

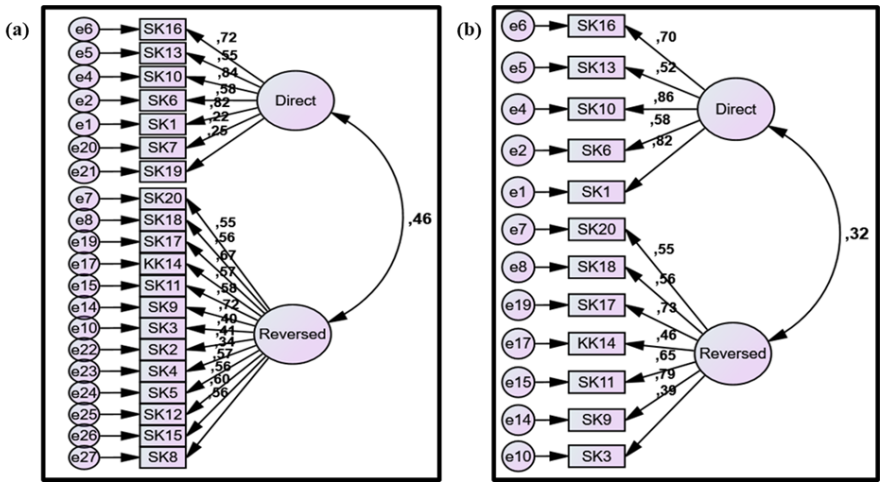
In the first stage, items 4th, 7th and 19th with factor loads below 0.40 items were excluded from the study. Although the 2nd, 5th, 8th, 12th and 15th items were high in the explanatory factor analysis, it was determined that they were not suitable for the model as a result of the confirmatory factor analysis. As a result, the 2nd, 4th, 5th, 7th, 8th, 12th, 15th and 19th questions were removed from the model. The model created for the confirmer factor analysis was given in Figure 1 and the pre-status and post-status compliance values for the model were presented in Table 3. The scale is seen perfect fit after the modification processes (χ^2=90.738, df=53).

Tablo 3: Modifikasyon için uyum indeksi değerleri

Table 3: *Fit index values for modification*

Measure	Before modification	After modification
(χ^2/df)	2.759**	1.712**
RMSEA	0.085	0.054**
SRMR	0.074*	0.049**
IFI	0.799	0.958**
CFI	0.796	0.958*
GFI	0.832	0.943**
TLI	0.771	0.947**

*Acceptable compliance ** *good fit ***



Şekil 1: Sürekli kaygı ölçeği için (a) modifikasyon öncesi, (b) modifikasyon sonrası oluşturulan doğrulayıcı faktör analizi modelleri

Figure 1: *Confirmatory factor analysis models for the trait anxiety scale (a) before modification, (b) after modification.*

The results of the explanatory factor analysis applied after removing the questions were given in Table 4. The factor loads of questions in the first dimension ranged from 0.786 to 0.437 and the factor loads of questions in the second dimension ranged from 0.871 to 0.633. The Cronbach's Alpha (α) coefficient was calculated as 0.798, and it can be assessed as an appropriate level for a reliable measurement tool.

Tablo 4: Sürekli kaygı ölçeği modifikasyon sonrası ortak faktör varyansları ve faktör yükleri

Table 4: *Common factor variances and factor loadings after trait anxiety scale modification.*

Items	Factors	
	1st	2nd
9. I worry about trivial things.	0.786	
17. No way thoughts bother me.	0.759	
11. I take everything seriously and worry.	0.700	
18. I take my disappointments so seriously that I will never forget.	0.651	
20. The issues that have been on my mind recently make me nervous	0.642	
3. I usually cry easily.	0.506	
14. I avoid facing difficult and difficult situations.	0.437	
10. I am generally happy.		0.871
1. I am generally in a good mood.		0.820
16. I am generally satisfied with my life.		0.787
6. I feel rested.		0.680
13. Generally, I feel safe.		0.633
Self-values	3.021	2.996
Variance description rates %	25.172	24.963
Cronbach's Alpha (α)	0.753	0.784
Total Described Variance Ratio = 50.135 Kaiser Meyer Olkin (KMO) = 0.827 Bartlett test value =894.590 p=0.001** Total Cronbach's Alpha (α)= 0.798		

*$p<0.05$ **$p<0.01$

Explanatory and confirmatory factor analysis for personal indecisiveness scale:

Explanatory factor analysis results related to personal indecisiveness scale were given in Table 5. The factor loads of questions in the first dimension ranged from 0.781 to 0.531, and the factor loads of questions in the second dimension ranged from 0.826 to 0.583. Additionally, Cronbach's Alpha (α) was 0.929 and the it was evaluated as a reliable measurement tool.

Tablo 5: Kişisel kararsızlık ölçeği için ortak faktör varyansları ve faktör yükleri

Table 5: *Common factor variances and factor loads for personal indecisiveness scales*

Items	Factors	
	1st	2nd
1. I have great difficulty when I have to make an impetuous indecisiveness.	0.781	
2. I think for hours while making decisions even about simple things.	0.763	
9. I get nervous when I have to make an impetuous indecisiveness.	0.756	
18. I consider myself an indecisive person.	0.720	
14. When I have to make a decision within a certain time frame, I cannot finalize my decision.	0.706	
10. While deciding, I cannot determine which option is the most suitable for me.	0.705	
5. I often cannot finalize my decisions for fear of making mistakes.	0.657	
17. I think for hours, even when making a decision similar to the one I have made before.	0.639	
13. I have difficulty deciding which of the things I should do first.	0.537	
6. When making decisions, I collect information and research about all the options, but I still cannot decide which option is best for me.	0.531	
8. Instead of thinking carefully about my decision, I make a impetuous indecisiveness, then I usually give up my decision.		0.826
7. I make an impetuous indecisiveness for fear of missing opportunities, then I give up my decision.		0.789
11. I make an impetuous indecisiveness because I want to get rid of it as soon as possible, then I usually give up my decision.		0.736
15. As I find it troublesome to research all the options while making a decision, I choose the one that I like best at that moment, then I give up my decision.		0.670
12. I make my decisions quickly and give up quickly.		0.668
3. While making my decision, I make an impetuous indecisiveness because I cannot be patient to exploratory the issue and gather information about it, then I give up my decision.		0.658
4. I consider myself a hasty person.		0.602
16. While making a decision, I choose the option with which I can get quick results, and when I cannot find what I was hoping for, I immediately give up my decision.		0.583
Self-values	5.298	8.870
Variance description rates %	29.437	27.056
Cronbach's Alpha (α)	0.907	0.882
Total described variance ratio = 56.490 Kaiser Meyer Olkin (KMO) = 0.929 Bartlett test value =2533.440 p=0.001** Total Cronbach's Alpha (α)= 0.929		

*$p<0.05$ **$p<0.01$

Confirmatory factor analysis:

Structural equation model (SEM): Since the goodness of fit values for first analysis of the model created were not within the desired limits, necessary corrections and combinations were made by taking the improvement indices into consideration. After improvements were made that theoretically could be installed and made the highest contribution to the model as a correction value, as seen in Figure 2. They were made with combinations in the form of associating the lower dimensions with each other, taking into account the harmony indices of the lower dimensions of the variables.

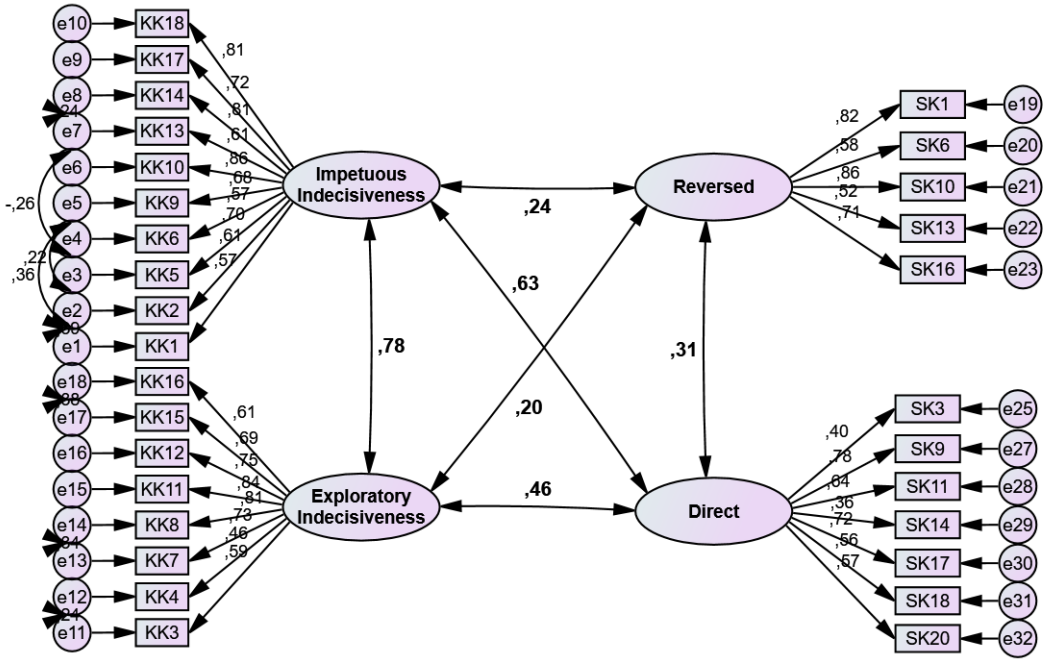
In the model obtained (χ^2=633.991, df=391) there were a total of four (exploratory indecisiveness, impetuous indecisiveness, direct trait anxiety, inverted trait anxiety) subscales of trait anxiety and personal indecisiveness. Chi-square / degree of freedom (χ^2/df), Root Mean Square Error of Approximation (RMSEA), Goodness of Fit Index (GFI), Standardized Root Mean Square Error (Standardized Root Mean Square Residual, SRMR), Comparative Fit Index (CFI), Incremental Fit Index (IFI), fit indices showed that the model was fit at an acceptable level; the results are given in Table 6.

Tablo 6: Yapısal eşitlik modeline ait istatistiksel değerler

Table 6: *Statistical values of the structural equation model*

Measure	Good-fit	Acceptable-fit	Model fit index values
(χ^2/df)	≤ 3	≤ 4-5	1.621**
RMSEA	≤ 0.05	0.06-0.08	0.051**
SRMR	≤ 0.05	0.06-0.08	0.065*
IFI	≥ 0.95	0.94-0.90	0.931*
CFI	≥ 0.97	≥ 0.95	0.930*
GFI	≥ 0.90	0.89-0.85	0.851*
TLI	≥ 0.95	0.94-0.90	0.922*

*Acceptable compliance * good fit ***



Şekil 2: Sürekli kaygı ve kişisel kararsızlığa ait dört alt ölçek arasında etkileşime yönelik YEM modeli

Figure 2: *SEM model for interaction between four subscales of trait anxiety and personal indecisiveness*

The relationships that emerged as a result of the analysis after the improvements were obtained and given in Table 7. Statistically significant and positively directional relationships were found between the sub-dimensions of trait anxiety and the sub-dimensions of personal indecisiveness ($p<0.05$).

Tablo 7: Modifikasyon indekslerine göre yapılan düzeltmeler sonrası oluşan yapısal eşitlik modeli regresyon ağırlıkları

Table 7: *Structural equation model regression weights formed after corrections made according to modification indices*

			Estimates standardized (β)	Estimates (β)	Standard error	Critical value	p
Impetuous indecisiveness	<->	Exploratory indecisiveness	0.782	0.350	0.060	5.812	0.001**
Impetuous indecisiveness	<->	Reversed trait anxiety	0.242	0.135	0.043	3.118	0.002**
Exploratory indecisiveness	<->	Direct trait anxiety	0.459	0.124	0.031	3.950	0.001**
Direct trait anxiety	<->	Reversed trait anxiety	0.311	0.105	0.032	3.326	0.001**
Exploratory indecisiveness	<->	Reversed trait anxiety	0.204	0.107	0.040	2.652	0.008**
Impetuous indecisiveness	<->	Direct trait anxiety	0.626	0.181	0.041	4.392	0.001**

*$p<0.05$ **$p<0.01$

4. Discussion and Conclusion

The KMO test tests whether the distribution is sufficient for factor analysis and the range of it is between 0.80 and 0.90. (29). Therefore, it can be said that the KMO value in this study was at an acceptable level. The Barlett test result was 894.590 ($p<0.05$) for the trait anxiety scale after modification and 2533.440 ($p<0.05$) for the personal indecisiveness scale. In this study, there was no limit on the number of factors, and factors with an eigenvalue greater than 1.50 were included in the scale. Factors with an eigenvalue of 1 or greater than 1 were considered as important factors in factor analysis (9). Considering that variance rates varying between 40% and 60% are considered ideal in factor analysis (27), it is said that the amount of variance obtained in this study was sufficient. According to these results, it was seen that the data set is suitable for factor analysis.

One of the important indicators of whether a factor analysis can be performed on a data set was that the significance of the correlation between variables is sufficient. Kaiser Meyer Olkin (KMO) measure was taken into consideration in the evaluation of this competence (23). This value should be above 0.60 in order to be suitable for factor analysis (22). In our study, this value was found 0.827 for the trait anxiety scale and 0.929 for the personal indecisiveness scale. Tekindal et al. (30) in a study he conducted in the veterinary faculty, the KMO value was 0.70 and the Bartlett test is found to be 1012.414.

The reliability coefficient was found to be 0.789 for the trait anxiety scale used in the study and 0.929 for the personal indecisiveness scale. For personal indecisiveness, the reliability coefficient was found to be 0.920 in the study conducted by Bacanlı (3). Dönmezoğlu (13) found this value as 0.899 in his study, and the reliability coefficient for the trait anxiety scale was found to be 0.872 in the study conducted by Büyüköztürk (8). It was found 0.895 (21) in a study conducted for primary school students, 0.920 (15) in a study for high school students, and 0.810 in a study conducted for primary school students (19). These results support our results.

When these values were examined, in studies to be conducted for trait anxiety and personal indecisiveness scales, first explanatory then confirmatory factor analysis should be performed. These scales may differ for each sample group.

University education is can be seen as the last stage of education for an individual. Uçar and Uysal (32) found in their study that there was a negative relationship between students' trait anxiety and their perception of competence

and lifelong learning tendency. As students' anxiety levels increase, their willingness to learn, their openness to development, and their academic and social competence decrease. For this reason, it is thought that the contribution of similar scientific studies such as the causes of anxiety, strategies for coping with anxiety, and the elimination of the trait anxiety factor can contribute to students' learning and thus increasing the lifelong learning tendency. It is observed that variables such as academic competence, social competence and trait anxiety are effective in planning the future.

In his master's thesis, Öz (26) found a negative relationship between the anxiety level of the person and the state of enjoying their work and, concluded that the high anxiety levels of the students affect their work negatively.

University life brings serious problems that need to be overcome. The decisions made to overcome these problems are very important. Tuncel et al. (31) found negative moderate significance between the impetuous indecision sub-dimension of personal indecisiveness and the value and value/usefulness sub-dimensions of critical thinking motivation in their study for prospective teachers; they also found negative and low-level significant relationships between other sub-dimensions. Furthermore, there is a negative low-level significant relationship between the exploratory indecisiveness sub-dimension of personal indecisiveness and all sub-dimensions of critical thinking motivation. It is noteworthy that problem-solving and decision-making processes are commonly mentioned in definitions related to thinking skills.

In this study, firstly, the personal indecision and permanent anxiety levels of university students were revealed. Then, by using these results, valid and reliable scales were developed to determine the levels of anxiety and uncertainty experienced by students. The results of the analysis to test the validity and reliability of the scales show that the prepared measurement tool was suitable for measuring. In line with these results, it is thought that it can be used by teachers and researchers to obtain information and collect data in determining the effectiveness of the trait anxiety scale on decision-making. Our research findings showed that students ' ongoing concerns affect their decision-making.

This study aims to contribute to the literature on the determination of the relationship between trait anxiety and personal indecisiveness.

Based on the findings from the research, it is possible to make the following recommendations.

This study was conducted with Konya Selçuk University undergraduate students, and it can be applied in different universities and with larger sample sizes in order to obtain more reliable results. In addition to undergraduate students, new research can be conducted by selecting from the high school, graduate or doctorate students of the sample group. In order to support the personality development of university students, it may be very beneficial for students to receive more regular support from Psychological Counseling and Guidance Services. A significant relationship was found between the sub-dimensions of university students' level of personal indecisiveness. This result may bring to mind the question of what factors cause students to experience indecision. Therefore, the individual implementation of the guidance services to be made may help in solving the problems. The fact that researchers focus more deeply on the issue of indecision in their study and conduct multidimensional research may allow the quality of the obtained scientific data to increase. Studies can be conducted by using various variables to influence the decisions students make due to the trait anxiety they have during their university life.

Conflict of Interest

The author declared no conflict of interest.

Funding

No funding.

Authors' Contributions

Idea / concept: Mert DEMİRSÖZ, Mustafa Agah TEKİNDAL, Harun YONAR
Experiment design: Mert DEMİRSÖZ, Harun YONAR
Supervision / Consultancy: Mustafa Agah TEKİNDAL, Mehmet Emin TEKİN
Data collecting: Mert DEMİRSÖZ, Zeynep ÖZEL
Data analysis and interpretation: Mert DEMİRSÖZ, Mustafa Agah TEKİNDAL
Literature search: Mert DEMİRSÖZ, Harun YONAR
Writing the article: Mert DEMİRSÖZ, Zeynep ÖZEL
Critical review: Mehmet Emin TEKİN, Mert DEMİRSÖZ

Ethical Approval

The study is approved by the Local Ethics of Selcuk University, Faculty of Veterinary Medicine (Approval Number: 2020/4-2020/38).

References

1. **Arslan Ç** (2007): *Üniversite öğrencilerinin sürekli kaygı ve kişisel kararsızlık düzeylerinin incelenmesi.* (Doctoral dissertation, Selçuk Üniversitesi Sosyal Bilimler Enstitüsü).
2. **Aydın A, Tiryaki S** (2017): *Üniversite öğrencilerinin kaygı düzeylerini etkileyen faktörleri belirlemeye yönelik bir çalışma (KTÜ örneği)*. Orman Fakültesi Dergisi, **17(4)**, 715-722.
3. **Bacanlı F** (2000): *Kararsızlık Ölçeğinin geliştirilmesi*. Türk Psikolojik Danışma ve Rehberlik Dergisi, **2(14)**, 7-16.
4. **Bacanlı F** (2005a): *Rehberlik ve Psikolojik Danışmada Kullanılan Ölçme Araçları ve Programlar Dizisi PDR' de Kullanılan Ölçekler.* Ankara, Nobel Yayın Dağıtım, **1(2)**, 109-140.
5. **Bollen KA** (1989): *A new incremental fit index for general structural equation models*. Sociological methods and research, **17(3)**, 303-316.
6. **Bozkurt N** (2004): *Bir Grup Üniversite Öğrencisinin Depresyon ve Kaygı Düzeyleri ile Çeşitli*. Eğitim ve Bilim., **29(133)**, 52-59.
7. **Browne MW, Cudeck R** (1993): *Alternative ways of assessing model fit.* 136-162. In: KA Bollen and JS Long (Ed.), Testing structural equation models, Sage Publications, Newbury Park.
8. **Büyüköztürk Ş** (1997): *Araştırmaya yönelik kaygı ölçeğinin geliştirilmesi.* Kuram ve Uygulamada Egitim Yönetimi Dergisi, **3(4)**, 453-464.
9. **Büyüköztürk Ş** (2002): *Faktör analizi: Temel kavramlar ve ölçek geliştirmede kullanımı.* Kuram ve Uygulamada Egitim Yönetimi Dergisi **8(4)**, 470-483.
10. **Büyüköztürk, Ş.** (2007): *Sosyal bilimler için veri analizi el kitabı.* 179-195. In: Geçerlik ve Güvenirlik Analizlerinde Kullanılan Bazı İstatistikler. Pegem A Yayıncılık, Ankara.
11. **Çakmak Ö, Hevedanlı M** (2004): *Biyoloji öğretmen adaylarınınkaygılarını etkileyen etmenler*. XIII. Ulusal Eğitim Bilimleri Kurultayı, (6-9 Temmuz), İnönü Üniversitesi Eğitim Fakültesi, Malatya.
12. **Çolak TS, Eren G, Doğan U** (2017): *İlkokul Öğrencilerinin Sürekli Kaygı, Yetkinlik ve Üzüntü Yönetimine İlişkin Araştırma.* Elementary Education Online **16(1)**, 151-160.
13. **Dönmezoğulları C.** (2020): *Kariyer yapılandırma kuramına dayalı Psikoeğitim programının üniversite öğrencilerinin kariyer kararı ve güçlü yönler öz yeterlilik düzeylerine etkisi.* Ankara Üniversitesi Eğitim Bilimleri Enstitüsü
14. **Ergene T** (2011): *Lise öğrencilerinin sınav kaygısı, çalışma alışkanlıkları, başarı güdüsü ve akademik performans düzeyleri arasındaki ilişkilerin incelenmesi*. Eğitim ve Bilim, **36(160)**, 320-330.
15. **Erktin E, Dönmez G, Özel S** (2006): *Matematik kaygısı ölçeğinin psikometrik özellikleri.* Eğitim ve Bilim, **31(140),** 26-33.
16. **Ford JK, MacCallum RC, Marianne T** (1986): *The application of exploratory factor analysis in applied psychology: A critical review and analysis*. Personnel psychology, **39(2)**, 291-314.
17. **Güneş T, Arıkan R** (1985): *Tarım Ekonomisi İstatistiği.* Ankara Üniversitesi Ziraat Fakültesi Yayınları: 924, Ofset Basım Ders Kitabı, 8, 328.
18. **Hammond, JS, Keeney RL, Howard R** (1998): *The hidden traps in decision making*. Harvard business review, **76(5)**, 47-58.
19. **Hiçyılmaz Y, Karip F** (2020): *Görsel Sanatlar Dersi Kaygı Ölçeği: Geçerlik ve Güvenirlik Çalışması*. Turkish Studies, **15(2)**, 897-909.
20. **Hu LT, Bentler PM** (1999): *Cutoff criteria for fit indexes in covariance structure analysis: Conventional criteria versus new alternatives.* Tructural equation modeling: a multidisciplinary journal, **6(1)**, 1-55.

21. **Kağıtçı B, Kurbanoğlu Nİ** (2013): *Fen ve teknoloji dersine yönelik kaygı ölçeğinin geliştirilmesi: güvenirlik ve geçerlik çalışması.* Journal of Turkish Science Education, **10(3)**, 95-107.
22. **Kaiser HF** (1974): *An index of factorial simplicity*. Psychometrika, **39(1)**, 31-36.
23. **Kırılmaz H** (2013): *Hasta memnuniyetini etkileyen faktörlerin sağlık hizmetlerinde performans yönetimi çerçevesinde incelenmesi: poliklinik hastaları üzerine bir alan araştırması.* ACU Sağlık Bil Derg, **4**, 13-20.
24. **Ocaktan ME, Keklik A, Çöl M** (2002): *Abidinpaşa sağlık grup başkanlığı'na bağlı sağlık ocaklarında çalışan sağlık personelinde Spielberger durumluluk ve sürekli kaygı düzeyi*. Ankara Üniversitesi Tıp Fakültesi Dergisi, **55(1)**, 21-28.
25. **Öner N, LeCompte WA** (1985): *Durumluk-sürekli kaygı envanteri el kitabı*. Boğaziçi Üniversitesi Yayınları.
26. **Öz M** (2019): *Konservatuvar müzik bölümünde okuyan üniversite öğrencilerinde sürekli kaygı ve anksiyete duyarlılığının akış deneyimleri ile ilişkisinin incelenmesi* (Master's thesis, İstanbul Gelişim Üniversitesi Sosyal Bilimler Enstitüsü).
27. **Scherer RF, Luther DC, Wiebe FA, Adams JS** (1988): *Dimensionality of coping: Factor stability using the ways of coping questionnaire.* Psychological Reports, **62(3)**, 763-770.
28. **Stapleton CD** (1997): *Basic Concepts and Procedures of Confirmatory Factor Analysis.* Paper presented at the Annual Meeting of the Southwest educational Research Association, Austin, TX, January 23–25.
29. **Tanaka JS, Huba GJ** (1985): *A fit index for covariance structure models under arbitrary GLS estimation*. British journal of mathematical and statistical psychology, **38(2)**, 197-201.
30. **Tekindal MA, Tekin ME, Çevrimli MB, Aslım G, Mat B, Günlü A, Yaşar A** (2019): *The validity and reliability study for evaluation of factors affecting workplace satisfaction of academic staff: faculty of veterinary medicine.* Veteriner Hekimler Derneği Dergisi, **91(1)**, 15-24.
31. **Tuncel M, Yıldız S, Yıldız K, Yavuz R** (2018): *Öğretmen Adaylarının Kişisel Kararsızlık ve Eleştirel Düşünme Motivasyonları*. Abant İzzet Baysal Üniversitesi Eğitim Fakültesi Dergisi, **18(2)**, 1127-1155.
32. **Uçar MY, Uysal S** (2019): *Üniversite Öğrencilerinin Yaşam Boyu Öğrenme Eğilimi, Yeterlilik Algısı ve Sürekli Kaygı Durumları Arasında İlişki.* Adnan Menderes Üniversitesi Eğitim Fakültesi Eğitim Bilimleri Dergisi, **10(2)**, 72-82.
33. **Urgancı S, Gürgan U** (2019): *Gençlerde Karar Verme Becerileri ile Akademik Öz-Yeterlilik Arasındaki İlişkinin Bazı Değişkenlere Göre İncelenmesi*. Avrasya Sosyal ve Ekonomi Araştırmaları Dergisi, **6(6)**, 243-262.
34. **Varol Ş** (1990): *Lise son sınıf öğrencilerinin kaygılarını etkileyen etmenler.* Yayınlanmamış yüksek lisans tezi, Ondokuz Mayıs Üniversitesi, Samsun.

App (A)

Personal indecisiveness scale

Explanation: There are 18 items on this scale that describe the behavior a person adopts when making decisions. Read these statements and mark the option that suits you by placing (+).

	Items	It's not appropriate	Not exactly	A bit convenient	Appropr iate	Very Appropriate
1	I have a lot of difficulty when I have to make impetuous indecisiveness.	( )	( )	( )	( )	( )
2	I think for hours when I'm making decisions about simple things.	( )	( )	( )	( )	( )
3	As I cannot be patient to exploratory and gather information about the matter, I make an impetuous indecisiveness, and then I give up my decision.	( )	( )	( )	( )	( )
4	I consider myself a hasty person.	( )	( )	( )	( )	( )
5	Most of the time I can't make my decisions for fear of making mistakes.	( )	( )	( )	( )	( )
6	I gather information and research all options when making decisions, but I still can't decide which option suits me best.	( )	( )	( )	( )	( )
7	I make impetuous indecisiveness for fear of missing opportunities, then I give up.	( )	( )	( )	( )	( )
8	I make a quick decision instead of thinking it through, and then I usually give up my decision.	( )	( )	( )	( )	( )
9	I get nervous when I have to make impetuous indecisiveness.	( )	( )	( )	( )	( )
10	I can't decide which is the best option for me.	( )	( )	( )	( )	( )
11	I make a quick decision because I want to make my decision and get out of it, and then I usually give up my decision.	( )	( )	( )	( )	( )
12	I make my decisions quickly and I give up quickly.	( )	( )	( )	( )	( )
13	Among the things I have to do, I have a hard time deciding which one to do first.	( )	( )	( )	( )	( )
14	I can't make a decision when I have to make a decision within a certain time frame.	( )	( )	( )	( )	( )
15	I choose the one I like the most at that moment, and then I give up.	( )	( )	( )	( )	( )
16	When I make a decision, I choose the option where I can get results quickly, and when I don't get what I hoped for, I give up my decision immediately.	( )	( )	( )	( )	( )
17	I think for hours, even when I'm making a decision similar to the one I've made before.	( )	( )	( )	( )	( )
18	I consider myself an ambivalent person.	( )	( )	( )	( )	( )

Size and materials in the created scale: Impetuous indecisiveness; 1-2-5-6-9-10-13-14-17-18, exploratory indecisiveness; 3-4-7-8-11-12-15-16, are numbered items.

App (B)

Trait anxiety scale

Below are some expressions that people use to describe their own feelings. Read each statement, then state how you feel at that moment by marking the appropriate one from the spaces on the right side of the statements. There are no right or wrong answers. Mark the answer that shows how you feel instantly without spending too much time on any statement.

	Items	In almost no time	Some times	A lot of time	Almost always
1	I am generally in a good mood.	( )	( )	( )	( )
2	I usually get tired quickly.	( )	( )	( )	( )
3	I usually cry easily.	( )	( )	( )	( )
4	I want to be as happy as others.	( )	( )	( )	( )
5	I miss opportunities because I cannot make a quick decision.	( )	( )	( )	( )
6	I feel rested.	( )	( )	( )	( )
7	I am generally calm, restrained and cool.	( )	( )	( )	( )
8	I feel that the difficulties have accumulated so much that I cannot overcome	( )	( )	( )	( )
9	I worry about trivial things.	( )	( )	( )	( )
10	I am generally happy.	( )	( )	( )	( )
11	I take everything seriously and worry.	( )	( )	( )	( )
12	I generally lack self-confidence.	( )	( )	( )	( )
13	Generally, I feel safe.	( )	( )	( )	( )
14	I avoid facing difficult and difficult situations.	( )	( )	( )	( )
15	Usually I feel sad.	( )	( )	( )	( )
16	I am generally satisfied with my life.	( )	( )	( )	( )
17	No way thoughts bother me.	( )	( )	( )	( )
18	I take my disappointments so seriously that I will never forget.	( )	( )	( )	( )
19	I am a sane and determined person. I am a sane and determined person.	( )	( )	( )	( )
20	20. The issues that have been on my mind recently make me nervous	( )	( )	( )	( )

Size and materials in the created scale: Direct Trait Anxiety; 2-3-4-5-8-9-11-12-14-15-17-18-20, Reversed Trait Anxiety; 1-6-7-10-13-16-19, are numbered items.

App (C)

Trait anxiety and personal indecisiveness Scales for students of faculty of veterinary medicine

	Items: Personal indecisiveness scale	It's not appropriate	Not exactly	A bit convenient	Appropriate	Very appropriate
1	I have a lot of difficulty when I have to make impetuous indecisiveness.	( )	( )	( )	( )	( )
2	I think for hours when I'm making decisions about simple things.	( )	( )	( )	( )	( )
3	As I cannot be patient to exploratory and gather information about the matter, I make a impetuous indecisiveness, and then I give up my decision.	( )	( )	( )	( )	( )
4	I consider myself a hasty person.	( )	( )	( )	( )	( )
5	Most of the time I can't make my decisions for fear of making mistakes.	( )	( )	( )	( )	( )
6	I gather information and research all options when making decisions, but I still can't decide which option suits me best.	( )	( )	( )	( )	( )
7	I make impetuous indecisiveness for fear of missing opportunities, then I give up.	( )	( )	( )	( )	( )
8	I make a quick decision instead of thinking it through, and then I usually give up my decision.	( )	( )	( )	( )	( )
9	I get nervous when I have to make impetuous indecisiveness.	( )	( )	( )	( )	( )
10	I can't decide which is the best option for me.	( )	( )	( )	( )	( )
11	I make a quick decision because I want to make my decision and get out of it, and then I usually give up my decision.	( )	( )	( )	( )	( )
12	I make my decisions quickly and I give up quickly.	( )	( )	( )	( )	( )
13	Among the things I have to do, I have a hard time deciding which one to do first.	( )	( )	( )	( )	( )
14	I can't make a decision when I have to make a decision within a certain time frame.	( )	( )	( )	( )	( )
15	I choose the one I like the most at that moment, and then I give up.	( )	( )	( )	( )	( )
16	When I make a decision, I choose the option where I can get results quickly, and when I don't get what I hoped for, I give up my decision immediately.	( )	( )	( )	( )	( )
17	I think for hours, even when I'm making a decision similar to the one I've made before.	( )	( )	( )	( )	( )
18	I consider myself an ambivalent person.	( )	( )	( )	( )	( )

	Items: Trait anxiety scale	In almost no time	Some times	A lot of time	Almost always
19	I am generally in a good mood.	( )	( )	( )	( )
20	I usually cry easily.	( )	( )	( )	( )
21	I feel rested.	( )	( )	( )	( )
22	I worry about trivial things.	( )	( )	( )	( )
23	I am generally happy.	( )	( )	( )	( )
24	I take everything seriously and worry.	( )	( )	( )	( )
25	Generally, I feel safe.	( )	( )	( )	( )
26	I avoid facing difficult and difficult situations.	( )	( )	( )	( )
27	I am generally satisfied with my life.	( )	( )	( )	( )
28	No way thoughts bother me.	( )	( )	( )	( )
29	I take my disappointments so seriously that I will never forget.	( )	( )	( )	( )
30	The issues that have been on my mind recently make me nervous	( )	( )	( )	( )

Size and materials in the created scale: Impetuous indecisiveness; 1-2-6-9-10-13-14-17-18, exploratory indecisiveness; 3-4-7-8-11-12-15-16, are numbered items. Direct trait anxiety; 20-22-24-26-28-29-30, reversed trait anxiety; 19-21-23-25-27, are numbered items.

Veteriner fakültesi öğrencilerin için kişisel kararsızlık ve sürekli kaygı ölçekleri Türkçe versiyonu

(Turkish version of Trait anxiety and personal indecisiveness Scales for students of faculty of veterinary medicine)

	Soular: Kişisel kararsızlık ölçeği	Hiç uygun değil	Pek uygun değil	Biraz uygun	Uygun	Tamamıyla uygun
1	Acele karar vermem gerektiğinde çok güçlük çekerim.	( )	( )	( )	( )	( )
2	Basit şeyler hakkında bile karar verirken saatlerce düşünürüm.	( )	( )	( )	( )	( )
3	Karar verirken konuyu araştırmaya ve hakkında bilgi toplamaya sabredemediğim için acele karar veririm, sonra kararımdan vazgeçerim.	( )	( )	( )	( )	( )
4	Kendimi aceleci bir kişi olarak görürüm.	( )	( )	( )	( )	( )
5	Hata yaparım korkusuyla çoğu zaman kararlarımı kesinleştiremem.	( )	( )	( )	( )	( )
6	Karar verirken tüm seçenekler hakkında bilgi toplarım ve araştırma yaparım, fakat yine de bana en uygun seçeneğin hangisi olduğuna karar veremem.	( )	( )	( )	( )	( )
7	Fırsatları kaçırırım korkusuyla acele karar veririm, sonra kararımdan vazgeçerim.	( )	( )	( )	( )	( )
8	Karar verirken iyice düşünmek yerine acele karar veririm, sonra genellikle kararımdan vazgeçerim.	( )	( )	( )	( )	( )
9	Acele karar vermem gerektiğinde telaşlanırım.	( )	( )	( )	( )	( )
10	Karar verirken bana göre en uygun seçeneğin hangisi olduğunu bir türlü belirleyemem.	( )	( )	( )	( )	( )
11	Bir an önce kararımı verip kurtulmak istediğim için acele karar veririm, sonra genellikle kararımdan vazgeçerim.	( )	( )	( )	( )	( )
12	Kararlarımı çabuk verip çabuk ta vazgeçerim.	( )	( )	( )	( )	( )
13	Yapmam gereken işler arasında hangisini önce yapacağıma karar vermekte güçlük çekerim.	( )	( )	( )	( )	( )
14	Belli bir zaman dilimi içinde karar vermem gerektiğinde kararımı kesinleştiremem.	( )	( )	( )	( )	( )
15	Karar verirken tüm seçenekler hakkında araştırma yapmak bana zahmetli geldiğinden o anda en çok hoşuma gideni seçerim, sonra kararımdan vazgeçerim.	( )	( )	( )	( )	( )
16	Karar verirken çabuk sonuç alabileceğim seçeneği seçerim, umduğumu bulamadığımda da kararımdan hemen vazgeçerim.	( )	( )	( )	( )	( )
17	Daha önce verdiğim kararlara benzer bir karar verirken bile saatlerce düşünürüm.	( )	( )	( )	( )	( )
18	Kendimi kararsız bir kişi olarak görüyorum.	( )	( )	( )	( )	( )

	Sorular: Sürekli kaygı ölçeği	Hemen hemen hiçbir zaman	Bazen	Çok zaman	Hemen her zaman
19	Genellikle keyfim yerindedir.	( )	( )	( )	( )
20	Genellikle kolay ağlarım.	( )	( )	( )	( )
21	Kendimi dinlenmiş hissediyorum.	( )	( )	( )	( )
22	Önemsiz şeyler hakkında endişelenirim.	( )	( )	( )	( )
23	Genellikle mutluyum.	( )	( )	( )	( )
24	Her şeyi ciddiye alır ve endişelenirim.	( )	( )	( )	( )
25	Genellikle kendimi emniyette hissederim.	( )	( )	( )	( )
26	Sıkıntılı ve güçlü durumlarla karşılaşmaktan kaçınırım.	( )	( )	( )	( )
27	Genellikle hayatımdan memnunum.	( )	( )	( )	( )
28	Olur, olmaz düşünceler beni rahatsız eder.	( )	( )	( )	( )
29	Hayal kırıklıklarını öylesine ciddiye alırım ki hiç unutamam.	( )	( )	( )	( )
30	Son zamanlarda kafama takılan konular beni tedirgin ediyor.	( )	( )	( )	( )

Oluşturulan ölçek için faktörler ve sorular Aceleci Kararsızlık; 1-2-6-9-10-13-14-17-18, Araştırmacı kararsızlık; 3-4-7-8-11-12-15-16. Direk sürekli kaygı; 20-22-24-26-28-29-30, Tersine dönmüs sürekli kaygı; 19-21-23-25-27.

VETERİNER HEKİMLER DERNEĞİ DERGİSİ
JOURNAL OF THE TURKISH VETERINARY MEDICAL SOCIETY

ISSN: 0377-6395
e-ISSN: 2651-4214
Dergi ana sayfası:
Journal homepage:
http://dergipark.org.tr/vetheder

DOI: 10.33188/vetheder.705992

Derleme/ Review

Non-human animal theories: from mechanism to abolitionism

Sandra Duarte Cardoso [1,a*], Yasemin Salgirli Demirbas [2,b], Ceres Berger Faraco [3,c], Liliana de Sousa [1,d], Gonçalo da Graça Pereira [4,5,e]

[1] Abel Salazar Biomedical Sciences Institute, University of Porto, Rua de Jorge Viterbo Ferreira n.º 228, 4050-313 Porto, Portugal
[2] Department of Physiology, Faculty of Veterinary Medicine, Ankara University, 06110, Ankara, TURKEY
[3] Institute of Animal Health and Psychology (INSPA), Av João Wallig 687/B201, 91340-000, Porto Alegre, RS, Brasil
[4] Center for Animal Knowledge, Avenida dos Bombeiros Voluntários de Algés, 40A, 1495-020 Algés, Portugal
[5] School of Agrarian Studies of Elvas, Polytechnics Institute of Portalegre, Elvas, Portugal
ORCID: 0000-0003-2079-1199 [a]; 0000-0001-6344-5603 [b]; 0000-0001-9811-402X [c]; 0000-0001-6656-4595 [d]; 0000-0003-0540-7435[e]

MAKALE BİLGİSİ / ARTICLE INFORMATION:

Geliş / Received:
18 Mart 20
18 March 20

Kabul / Accepted:
03 Eylül 20
03 September 20

Keywords:
Animal Welfare
Animal Rights
Ethics
Human and Non-human Relationships

Anahtar Sözcükler:
Etik
Hayvan Hakları
Hayvan Refahı
İnsan ve Hayvan İlişkileri

ABSTRACT:

Domestic animals, which started to live with human-animals thousands of years ago, were regarded as “things” which were created for human service and deprived of emotions until the end of the 18th century. This idea of “animals are machines” began to change in 1789, when the British writer, philosopher and social reformer Jeremy Bentham wrote that “The question is not, «Can they reason?» nor «Can they talk?» but, «Can they suffer?» Why should the law refuse its protection to any sensitive being?”. Bentham added that “The time will come when humanity will extend its mantle over everything which breathes.". Today, despite a general tendency for adopting legislation aiming to protect non-human animals and to criminalize animal mistreatment and abuse, the reality is that there are still improvements needed to be done in order to consider animals as sentient beings. This reviewing paper identifies and discusses the different philosophies and theories that are in the basis of the animal rights, which include mechanistic, utilitarianism, welfarism and abolitionism.

İnsan haricindeki hayvanlarla ilgili teoriler: mekanizmden abolisyonist düşünceye

ÖZET:

Binlerce yıl önce insanlarla yaşamaya başlamış olan evcil hayvanlar, 18. yüzyılın sonuna kadar insan hizmeti için yaratılmış ve duygulardan yoksun olan “nesneler” olarak kabul edildi. Bu, “hayvanlar makinedir ” görüşü, İngiliz yazar, filozof ve sosyal reformcu olan Jeremy Bentham 'ın 1789' da yazdığı şu cümlelerle değişmeye başladı: “Asıl soru, Onlar fikir yürütebilirler mi veya konuşabilirler mi değil, acı çekebilirler mi? Yasalar neden herhangi bir hassas varlığı korumayı reddetmeli?”. Bentham bu yazısına şöyle devam etmiştir: “İnsanlığın derisinin nefes alan her şeyi kaplayacağı zaman gelecektir”. Günümüzde, insan olmayan hayvanları da korumayI amaçlayan yasal mevzuatın oluşturulması ve insan dışı hayvanlara kötü muamele ile suistimali cezalandırmak için genel bir eğilim olmasına ragmen; hala daha hayvanlarıncanlı ve duyguları olan varlıklar olarak algılanmaları için iyileştirmelerin yapılması gerekliliği de bir gerçektir. Bu derleme makalesi, mekanistik (mekanizm), faydacılık (utilitarizm), refahçı yönelim ve abolisyonist (köleliğin kaldırılması) akım içeren ve hayvan haklarının savunulmasına dayanan farklı felsefeleri ve teorileri tanımlamakta ve tartışmaktadır.

How to cite this article: **Cardoso SD, Demirbas YS, Faraco CB, Sousa LD, Pereira GDG**: Non-human animal theories: from mechanism to abolitionism. *Veteriner Hekimler Dernegi Dergisi*, 92(1): 76-82, 2021. DOI: 10.33188/vetheder.705992

* *Sorumlu Yazar e-posta adresi / Corresponding Author e-mail address:* sandracardosomedvet@gmail.com

1. Introduction

Philosophically, animal rights are characterised by a concept where some or all animals have a right to live in an environment meeting their physiological, behavioural and emotional needs during their lifetime. Although the concept of animal rights is claimed by most of the authorities, there is still a debate on this topic (13). In several countries, the law already covers basic animal rights, and further, the constitutional consecration of these rights is already present in Germany, Austria and some other countries (3). Those who defend animal rights reject the concept that animals are defined as mere capital goods or property for human use or benefit. However, the opponents of animal rights have sought to identify morally relevant differences between humans and non-human animals in order to justify the allocation of rights and interests of human animals at the expense of non-human ones (16).

Since Pythagoras, Seneca, and Porphyry, it has been said that the condition of every living being designated as an animal has an inseparable bond with the tree of life. In common, equalizing them is of a fragile nature, vulnerable to disease and death, well-being and the good itself, constitutes what can be called "animal life" (10). The philosophy of animal rights does not necessarily support the premise that human and non-human animals are equal. For example, animal rights advocates do not support the right to vote for non-human animals. Some of the authorities differentiate sentient and self-conscious animals and other forms of life (4). Others may extend this right to all animals including those that do not have a high-developed nervous system or self-consciousness (25). There are also activists who support the idea that any human being or institution that uses animals for food, entertainment, cosmetics, clothing, vivisection or any other reason, infringes the rights of animals that deserve possessing their own lives and pursuing their own ends (11).

After all the question is what animals do think and feel. Safina (25) tried to answer these questions by describing his experience and the experience of other academics, by mentioning that the ability to feel, to love, to cry and to pay homage to their dead, is not derived exclusively from human or animal sentience. However, this debate has not always been like this. Animal sentience has long been discussed until recently. On July 7, 2012, some scientists have proclaimed the Cambridge Declaration on Consciousness, where it is stated that "The absence of a neocortex does not appear to preclude an organism from experiencing affective states. Convergent evidence indicates that non-human animals have the neuroanatomical, neurochemical, and neurophysiological substrates of conscious states along with the capacity to exhibit intentional behaviours. Consequently, the weight of evidence indicates that humans are not unique in possessing the neurological substrates that generate consciousness. Nonhuman animals, including all mammals and birds, and many other creatures, including octopuses, also possess these neurological substrates" (18).

The defence of animal rights has its basis on quite different philosophical strands, developed below in this paper, with absolutely antagonistic positions that can be summarised as:

a) The abolitionist position defends that the animal rights should be based on the principle of non-violence and education of veganism as a way to put into practice the changes in the daily life (12);

b) Welfarism that defends a rights-based theory (20, 21);

c) Utilitarianism which focuses on advocating improved treatment of animals, but at the same time accepts that they can be legitimately used for the benefit of humans or other non-human animals (28); and

d) Mechanistic, advocated by Descartes, that reduces animals to automatons and denies the possibility of assigning feelings to animals.

2. Mechanistic

In the seventeenth century, René Descartes argued that "irrational" animals were mere machines and that God was their builder. However, in his view, non-human animals were much more admirable than any other machine that has already been built or may be constructed by human ingenuity (7). Descartes shows, in the penultimate chapter of his work, Discourse on Method (7), the practical application of the method to some scientific questions. This thesis, which is also designated of mechanistic theory of animal nature, was intended to demonstrate the plausibility that any behaviour of the non-human animal can be explained without recourse to a supposed thought or conscience (22). It was

described that non-human animals were complex organic machines. Descartes suggested that even supposing that animals have impulses of anger, fear, and hunger, among others, these are sensations only insofar as they depend on a corporeal organ and, for this reason, can be explained only by means of material movements. Thus, according to this hypothesis, not only can non-human animals be machines insofar as they do not think discursively, but their impulses of anger, fear and thirst, for not involving an idea, can be explained without appeal to the thought. For this very reason, these are not properly sensations. This philosopher admitted the possibility that if there was a man-made machine that imitated a particular animal perfectly, there would be no way of distinguishing the false animal from the true one (7).

Descartes' thesis only recognises to non-human animals the first degree of sensation, which involves only stimuli and bodily movements, and a possible expression of this movement, but denies the possibility of attributing sensations or feelings or affective states to animals. It was denied the second and third degrees of sensation, that is, the consciousness of sensation and the judgment that involves sensation, which implies that he does not even contemplates the possibility of animals being able to feel suffering, hence his well-known experiments with animals to prove his theory (29).

Later, in the work Treatise of Man, Descartes (8) presented his last step of the argument, mentioning that recourse to the sensory and vegetative parts of the soul is unnecessary and added the metaphysical principle according to which "nature always acts by the simplest and easiest means". The conclusion is that animals have neither reason nor, therefore, sensorial experiences (5).

However, the statements of Descartes about animals were not supported by several other philosophers like Jean-Jacques Rousseau. According to Rousseau, in the preface to his work Discourse on Inequality (23), human beings are animals, not meaning that non-human animals have all the same rights that humans do, but only that to harm another sentient creature is universally wrong. Furthermore, since all animals are sentient beings, they should also participate in natural law and man is responsible for the performance of some duties, specifically one has the right not to be unnecessarily mistreated by the other. Voltaire was another philosopher that also refuted Descartes. In his Philosophical Dictionary (32), it can be found the following: "(…), what a sorry thing to have said that animals are machines bereft of understanding and feeling, which perform their operations always in the same way, (…) Is it because I speak to you, that you judge that I have feelings, memory, ideas? Well, I do not speak to you; you see me going home looking disconsolate, seeking a paper anxiously, opening the desk where I remember having shut it, finding it, reading it joyfully. You judge that I have experienced the feeling of distress and that of pleasure, that I have memory and understanding. (…) You discover in it all (for Voltaire "all" were the animals vivisected by Descartes) the same organs of feeling that are in yourself. Answer me, machinist, has nature arranged all the means of feeling in this animal, so that it may not feel? Has it nerves in order to be impassible? Do not suppose this impertinent contradiction in nature."

In this respect, we should also emphasise David Hume's words "Next to the ridicule of denying an evident truth, is that of taking much pains to defend it; and no truth appears to me more evident, than that beasts are endowed with thought and reason as well as men. The arguments are in this case so obvious, that they never escape the most stupid and ignorant." (17).

3. Utilitarianism

In the fourth century BC, Aristotle argued that animals were far from humans in the "great chain of being" or "natural scale". Claiming irrationality, he concluded that animals would have no self-interest, and only exist for the benefit of humans (33).

Later in the eighteenth century, one of the founders of modern utilitarianism, the British philosopher Jeremy Bentham, argues that the ability to suffer, rather than the capacity for reasoning, should be the measure for how we treat other beings. "The question is not, «Can they reason?» nor «Can they talk?» but, «Can they suffer?»" (1). If the ability of reason was a criterion, many human beings, including infants and the severely cognitively disabled, would also have to be treated as such. However, only in the early 1970s, a group of philosophers at Oxford University began to question why the moral status of non-human animals is necessarily lower than that of humans. This group included the psychologist Richard Ryder, who coined the term "speciesism", used on a privately distributed leaflet to describe

discriminatory behaviour of humans towards other animals (24). Despite this, the utilitarian philosophy or position has, as its main representative, the Australian philosopher Peter Singer. Singer is mistakenly considered the founder of the animal rights movement; yet his position on the moral status of animals is not based on the concept of rights but on the utilitarian concept of equal consideration of interests. In his book Animal Liberation, argued that humans should base moral consideration not in intelligence, or on the ability to make moral judgments, or on any other attribute that is inherently human, but in the ability to experience pain (27). Recognising that animals also experience pain, Singer argues that excluding animals from this consideration is a kind of discrimination called "speciesism". Furthermore, he stated that the most common forms where humans uses of animals are not justifiable because the benefits to humans are ignorable compared with the amount of pain that is inflicted to the animal in order to obtain those same benefits. These same benefits could be obtained in ways that do not involve the same degree of animal suffering. However, his position is close to classical welfarism, even defending organic meat and some animal experimentation. In conclusion, Animal Liberation (27), often cited as the "bible" of the animal rights movement, in reality, does not grant moral or legal rights to non-human animals, because it is based on utilitarianism.

4. Welfarism

The term "welfare" refers to the state of an individual in relation to its environment, which can be measured. The failure to cope with the environment is an indicator of poor welfare (2). The term "animal welfare" can have several distinct and even contradictory meanings. For instance, if used by an "animal industry", it may refer to the assurance that non-human animals are treated well and their basic biological needs are satisfied. Animals have a wide range of needs that are a consequence of the many functional systems that make life possible, being minimised by obtaining a particular resource or responding to a particular environmental or bodily stimulus (14). For some opponents of non-humans use, the term "animal welfare" in such context rather denotes ill fare for no matter how "humanely" non-humans are treated, the industry has to compromise on their complex social and biological needs and at the end, they are still slaughtered (30). Apart from this, these voices argue that animals do not have the capacity to enter into a social contract (or exercise contractualism), which is defined by a class of theories that try to explain the paths that lead people to form states and/or maintaining social order (21). However, philosopher Roger Scruton accuses animal rights advocates of "pre-scientific" anthropomorphism (26).

In summary, those who hold this view argue that there is nothing inherently wrong to use animals for food, among entertainment or scientific research, although humans, nevertheless, have a duty to ensure that animals do not suffer unnecessarily (15). These statements have been classified by welfarism and have been advocated and spread by some of the oldest animal protection organisations. However, advocates of animal rights, whom identify in this argument one speciesism position, refute this concept (31).

5. Abolitionism

Abolitionism was the political movement aimed at the abolition of slavery and human trafficking, mostly of African origin. It developed during the illuminism of the eighteenth century and became one of the most representative forms of political activism of the nineteenth century (6).

The animal abolitionism is a social movement that fights against any use of non-human animals, which does not accept that the non-human animals are, in any way, the property of human beings, or used for human benefit or purposes. It is a social movement that advocates not only the regulation of the use of animal by humans, but seeks to include them in the moral community, to ensure that their basic interests are respected and have equal consideration in relation to human interests. Gary Francione was the first academic lecturing on the subject of the animal abolitionism in the USA. He is also known for advocating the inclusion of all sentient animals in the moral community (12). By suggesting the abolition of the condition of animals being property, the term abolitionism is used to designate this idea. The abolitionist position believes that the animal rights movement should be based on the principle of non-violence and education to veganism as a way to put into practice the changes in the daily life (12). The objective is that animals

are not considered, neither legally nor morally, property or "natural resources" and its use is unwarranted. It argues that non-human animals should be respected in the likeness of human rights (9). Furthermore, Francione maintains that giving the status of family members to dogs and cats and at the same time killing chickens, cows and pigs for food, illustrates a society that suffers from "moral schizophrenia" (12).

6. Discussion and Conclusion

If modern moral and political philosophies proclaim the principle of equality as the foundation of human legislation, one must wonder from what criteria equality is being measured. It is no longer the species, in the biological sense, that makes its members equal, but some characteristics selected within the scope of the species, such as soul, intellect, language, autonomy and reason, among others (19). It is not just a matter of defending animals – as one who tires of humanity – but of defending humans with the same vigour: it is in the same moral fabric that the rights of both are sewn together (21).

The philosopher's job is to push reason to the limit, and in return to celebrate the good arguments that compel everyone to new ways of living: changing habits requires changing mindsets, says Regan, betting that his philosophical contribution to the question of the animal and human rights is marked not by emotion and sentimentality, but by reason and the weight of rational argument (19). Tom Regan, therefore, maintains that animals have rights on the grounds that humans have rights. His main objection to theories such as contractualism and utilitarianism is that they produce unacceptable moral results not only for animals but for humans as well. Regan asserts that an adequate moral theory for human beings must include moral rights (the moral rights in question are the right to life, to bodily integrity and to freedom) (20).

Not seriously considering these rights can easily lead to the view that individuals have only instrumental value, that is, that they have value only for the benefits they can provide for others. When moral rights are not taken seriously, the "inherent value" of an individual is ignored. As an ethical theory, therefore, Regan regards utilitarianism as insufficient for the defence of animals, since it is qualified as incompatible with human rights (19).

There are a number of issues at present that consider non-human animals to have rights. Some issues are more concerned with animal liberation and others institutionalise exploitation, but somehow both aim to alleviate the ill-treatment of animals and to protect them. Although the concept of rights creates confusion and sets the distance between the protagonists of animal protection, the discourse of rights has a great practical function: it strengthens the claims of the movement that demands the broadening of the human moral horizon.

Once the argument of non-paradigmatic cases is accepted, the attribution of moral rights to animals imposes itself. The main challenge is to determine what these rights are and how to implement them.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' contributions

Sandra Duarte Cardoso was responsible for the conception and design of the work and for drafting the manuscript. Yasemin Salgirli Demirbas was responsible for the critical revision of the manuscript. Ceres Berger Faraco, Liliana de Sousa and Gonçalo da Graça Pereira were responsible for the critical revision, general coordination, administrative support and supervision of the manuscript.

References

1. Bentham J (1789): *The Principles of Morals and Legislation*. Prometheus Books (February 1, 1988), 40.
2. Broom D (1991): *Animal welfare: concepts and measurement*. J Anim Sci, **69**, 4167-4175.

3. Cardoso SD, Faraco CB, De Sousa L, Da Graça Pereira G (2017*): History and evolution of the European legislation on welfare and protection of companion animals*. J Vet Behav, **19**, 64-68.
4. Cochrane A (2012): *Animal Rights without Liberation*: Applied Ethics and Human Obligations. Columbia University Press, 4-5.
5. Cottingham JA (1978): *Brute to the Brutes?: Descartes' Treatment of Animals.* Philosophy, **53(206)**, 551-559.
6. Curtin P (1969): *The Atlantic Slave Trade*. [S.l.]: The University of Wisconsin Press, 1-58.
7. Descartes R (1637): *Discourse on Method*, Hacket Publishing Company(Ed), Indianapolis, Indiana (third edition, 1988).
8. Descartes R (1662): *Traite De L'Homme*, Ed. GF Flammarion, Paris, 125, 161, 184, 195, 216, 220, 226, 228, 312, 325.
9. Felipe S (1996): *O anarquismo ético de Robert Nozick (Uma critica ao utilitarismo especiezista que predomina na relação dos homens com os animais).* Revista de Ciências Humanas*.* Florianópolis, **14(20)**, 51-62.
10. Felipe S (2009): *Antropocentrismo, sencientismo e biocentrismo: perspectivas éticas abolicionistas, bem-estaristas e conservadoras e o estatuto de animais não-humanos Sônia*. Revista Páginas de Filosofia, **1(1)**, 2.
11. Francione G (2000): *Introduction to Animal Rights*: Your Child or the Dog? Temple University Press, xxix.
12. Francione G (2008): *Animals as Persons: Essays on the Abolition of Animal Exploitation*. Columbia University Press, 16-17, 25-26, 172-184.
13. Francione G, Garner R (2010): *The Animal Rights Debate*. Columbia University Press.
14. Fraser A, Broom D (1990): *Farm Animal Behaviour and Welfare*. Saunders, New York, 1, 263, 400.
15. Frey R (1980): *Interests and Rights: The Case Against Animals*. Oxford University Press, 5, 76, 142.
16. Gruen L (2017): *The Moral Status of Animals*. The Stanford Encyclopedia of Philosophy (Fall 2017 Edition), Edward N. Zalta (ed.).
17. Hume D (1739): *A Treatise of Human Nature*. Reprinted from the Original Edition in three volumes and edited, with an analytical index, by L.A. Selby-Bigge, M.A. (Oxford: Clarendon Press, 1896).
18. Low P (2012): *The Cambridge Declaration on Consciousness*, Ed. Jaak Panksepp, Diana Reiss, David Edelman, Bruno Van Swinderen and Christof Koch, in Francis Crick Memorial Conference on Consciousness in Human and non-Human Animals, Churchill College, University of Cambridge. Available online: http://fcmconference.org/img/CambridgeDeclarationOnConsciousness.pdf. Accessed date: 25.09.2019.
19. Oliveira G (2004): *A teoria dos direitos animais humanos e não-humanos, de Tom Regan.* Ethic@: an International Journal for Moral Philosophy. Florianópolis, **3(3)**, 283-299.
20. Regan T (2004): *Empty Cages: Facing the Challenge of Animal Rights*. New York: Rowman & Littlefield, 2, 27-28.
21. Regan T (2004). *The case for Animal Rights*. 2nd ed. University of California Press, Berkeley and Los Angeles, California.
22. Rocha EM (2004). *Animais, homens e sensações segundo Descartes*. Kriterion: Journal of Philosophy; **45(110)**, 350-364.
23. Rousseau JJ (1754): *Discourse on Inequality & The Social Contract*. Ed. Musaicum Books, 2; 2016.
24. Ryder R (2004): *Speciesism revisited*. Think, **2(6)**, 83-92.
25. Safina C (2015): *Beyond Words: What Animals Think and Feel*. 1st ed., a John Macrae Book. Henry Holt and Company. New York, 3-5.
26. Scruton R (2000): *Animal Rights and Wrongs*. Claridge Press, 3rd Revised edition, 22-31.
27. Singer P (1975): *Animal Liberation: A New Ethics for Our Treatment of Animals*. 2nd. Ed. 2009, New York Review of the Books, 237-239.
28. Singer P (1980): *Utilitarianism and Vegetarianism*. Philosophy & Public Affairs, **9(4),** 325-337.
29. Stoothoff R, Murdoch D (1985): *The Philosophical Writings of Descartes*. Cambridge: Cambridge University press.
30. Sztybel D (2010): *Animal welfare and animal rights, a comparison. In Encyclopedia of animal rights and animal welfare*; 1, 2nd edition, ed. M. Bekoff, Santa Barbara, CA: Greenwood, 49-51.

31. Tuohey J (1992): *Fifteen years after "Animal Liberation": Has the animal rights movement achieved philosophical legitimacy?* Journal of Medical Humanities; **13(2)**, 79-89.
32. Voltaire (1764): *Voltaire's Philosophical Dictionary*. CreateSpace Independent Publishing Platform, 22-24; 2018.
33. Wise S (2016): *Animal rights*. Encyclopedia Britannica. Available online: https://www.britannica.com/topic/animal-rights. Accessed date: 25.09.2019.

VETERİNER HEKİMLER DERNEĞİ DERGİSİ
JOURNAL OF THE TURKISH VETERINARY MEDICAL SOCIETY

ISSN: 0377-6395
e-ISSN: 2651-4214
Dergi ana sayfası:
Journal homepage:
http://dergipark.gov.tr/vetheder

DOI: 10.33188/vetheder.793378

Derleme / Review

Gıda ortamında hayata tutunma: Bakteriyel çoğunluk algılama

Gonca Tülüce [1,a], Seran Temelli [2,b], Ayşegül Eyigör [2,c*]

[1] Erciyes Üniversitesi Veteriner Fakültesi Gıda Hijyeni ve Teknolojisi Anabilim Dalı, Kayseri, Türkiye
[2] Bursa Uludağ Üniversitesi Veteriner Fakültesi Besin Hijyeni ve Teknolojisi Anabilim Dalı, Bursa, Türkiye
ORCID: 0000-0003-2280-1725 [a], 0000-0002-8869-4929[b], 0000-0002-2707-3117[c]*

MAKALE BİLGİSİ:

ARTICLE INFORMATION:

Geliş / Received:
10 Eylül 20
10 September 20

Kabul / Accepted:
02 Kasım 20
02 November 20

Anahtar Sözcükler:
Biyofilm
Çoğunluk algılama
Gıda bozulması

Keywords:
Biofilm
Food spoilage
Quorum sensing

ÖZET:

Bakterilerin hücre içi ve hücreler arası iletişim amacı ile birtakım sinyal molekülleri kullanımına 'Quorum Sensing (QS) / Çoğunluk Algılama' adı verilmektedir. Gıda endüstrisi alanında, çeşitli Gram negatif ve Gram pozitif bakterilerin ürettiği sinyal moleküllerinin varlığının ve QS sistemi ile biyofilm oluşturma mekanizmalarının anlaşılmasına yönelik olarak geliştirilen biyosensör çalışmaları bulunmaktadır. Gıda bozulması ve biyofilm oluşumu, gıda endüstrisinin yüzyüze kaldığı ve geleneksel yaklaşımlarla alt edilmesi neredeyse mümkün olmayan önemli sorunlardan biri olarak karşımıza çıkmaktadır. QS sinyallerinin bakteriyel patojenite ve gıda bozulmasında önemli görevleri olmasından dolayı gıda kaynaklı bakterilerde QS sinyallerinin bloke edilmesi yolu ile gıda bozulmasından sorumlu olan QS ilişkili fenotiplere karşı korunma sağlanabilmesi yolunda adımlar atılmaktadır. Bu derlemede, QS ve mekanizması, gıda endüstrisi yönünden önem taşıyan bazı bakteriler tarafından üretilen sinyal moleküllerinin gıda bozulmasında üstlendikleri roller, QS ve biyofilm oluşumu, biyosensörler ile QS sinyallerinin tespiti, QS inhibitörleri olarak geliştirilen yeni gıda koruyucuları konusunda yapılan çalışmalar hakkında bilgi verilmiştir.

Survival in food environment: Bacterial quorum sensing

ABSTRACT:

The usage of some intracellular and intercellular signal molecules by bacteria for communication is defined as Quorum Sensing (QS). There are biosensor tests that are developed towards understanding the presence of signal molecules, which are produced by various Gram negative and Gram positive bacteria, and to determine the mechanism of biofilm formation via QS mechanism in the field of food industry. The food spoilage and biofilm formation are significant problems in the food industry, and both are almost impossible to overcome with traditional approaches. Since QS signals were proven to have significant roles on bacterial pathogenicity and on food spoilage, blockage of these signals in foodborne bacteria may provide steps for protection against QS related phenotypes responsible for food spoilage.
In this review, information is provided on QS and its mechanism, role of signal molecules produced by food spoilage bacteria that are important for the food industry, QS and biofilm formation, detection of QS signals via biosensors, and the new research on food preservatives developed as QS inhibitors.

How to cite this article: **Tülüce G, Temelli S, Eyigör A:** Gıda ortamında hayata tutunma: Bakteriyel çoğunluk algılama. *Veteriner Hekimler Dernegi Dergisi*, 92(1): 83-94, 2021. DOI: 10.33188/vetheder.793378

* *Sorumlu Yazar e-posta adresi / Corresponding Author e-mail address:* aeyigor@uludag.edu.tr

1. Giriş

Gıda bozulması, karmaşık bir proses olup gıdada doğal olarak meydana gelen biyokimyasal değişiklikler ile mikrobiyal aktivite sonucunda oluşmaktadır. Gıda bozulmaları arasında en yaygın gözlenen mikrobiyal bozulma, gıdanın hem görünüşünde hem de dokusunda bazı değişimler ile ortaya çıkarak endüstride önemli ekonomik kayıplar ve ciddi halk sağlığı sorunlarına neden olmaktadır (26). Mikroorganizmaların, gıda bozulması ile ilişkili olan sakkarolitik, proteolitik, pektinolitik ve lipolitik enzim aktiviteleri sonucu oluşan birtakım metabolitler, arzu edilmeyen bazı tat ve koku oluşumuna bağlı olarak bu gıdaların tüketime sunulmasına engel olmaktadır (40, 54). Son yıllarda, gıda bozulmaları ile ilgili çalışmalar, bozulma basamaklarında Quorum Sensing (QS) / Çoğunluk Algılama sinyallerinin tespiti ile yeni bir boyut kazanmıştır. Yüksek canlılarda doku, organ ve vücudun hücresel bir yanıt üretebilmesi için hücreler arası sinyalleşmeler kullanıldığı bilinmektedir (38). İnsan vücudundaki hücrelerde de gözlendiği gibi mikroorganizmalar ve diğer organizmalar, aktivitelerini kontrol etmek için küçük ve invaziv özellikteki sinyal moleküllerini kullanmaktadır. Hücrelerarası QS mekanizması ile mikroorganizmalar, ürettikleri sinyal moleküllerinin yoğunluğunu ölçebilmekte ve çevrelerindeki diğer mikroorganizmaların miktarını hissedebilmektedir. Bu sayede, koloni olarak göstermeleri gereken davranış şekillerini bir hücreden diğerine sinyal molekülleriyle iletilebilmektedir (4, 30). Kullanılan bu biyosinyallerin yapısına bağlı olarak hücrelere giriş için farklı iz-yollarının kullanıldığı bilinmektedir (38). Hücreler arası iletişimi sağlayan bu mekanizma, kendiliğinden sinyal üretebilen ve 'Auto-Inducer (AI) / Otoindükleyici' adı verilen ve üretildikleri hücrenin metabolizması üzerinde düzenleyici etki gösteren moleküller görev almaktadır (7). Üzerinde en çok çalışma yapılmış olan düşük molekül ağırlığa sahip otoindükleyiciler; Gram negatif bakterilerdeki N-acylhomoserin, Gram pozitif bakterilerdeki oligopeptitler ile otoindükleyicilerin bir sınıfı olan ve birçok vakada 'bilinmeyen yapılar' olarak karşımıza çıkan otoindükleyici-2 (AI-2)'dir (5).

QS ile bakteriyel patojenite ilişkisi konusunda da birçok araştırma olup günümüzde QS'in bakteriyel gıda bozulmasına neden olabildiği ortaya konulmuştur (61). Besinlerin parçalanması sırasındaki proteolitik, lipolitik, kitinolitik ve pektinolitik aktivitelerin QS tarafından düzenlendiği, ayrıca sinyal moleküllerinin birkaç tipinin çeşitli bozulmuş gıda ürününde tespit edildiği saptanmıştır (29). Bu nedenle, QS zincirinin kırılmasının insanlardaki enfeksiyonlar ve gıda bozulmasıyla ilişkili mikrobiyal gen ekspresyonu üzerinde büyük rol oynayabileceği düşünülmektedir. Öncelikle, mikrobiyal bozulmanın önlenebilmesi için gıda bozulmasına neden olan QS sinyal moleküllerinin hücreden hücreye iletişimindeki rolünün anlaşılması gerekmektedir. Bununla birlikte, geliştirilen QS inhibitörleri, hücrenin sinyal moleküllerini inhibe etmeye ya da gıda bozulmasına neden olan sinyal sistemleri ve gıdayla ilişkili bakteriler tarafından oluşturulan biyofilmleri bloke etmeyi amaçlamaktadır. Bu derlemede, gıda kaynaklı bakterilerdeki QS sistemi mekanizmalarının anlaşılması, gıda bozulmaları ve gıda ile ilişkili bakterilerin patojenitesindeki sinyal moleküllerin rolünün belirlenmesi, QS'in biyofilm oluşumundaki rolü, biyosensörler ile gıdadaki QS sinyallerinin tespiti ile gıda bozulmalarını önleyici veya geciktirici gıda koruyucuları olarak QS inhibitörlerinin kullanımı hakkında bilgi verilmektedir.

2. Çoğunluk Algılama (QS)

Mikrobiyoloji alanında son 30 yıldaki en önemli buluşlardan biri, bakteriler arası iletişimdir. Bakterilerde hücreden hücreye iletişim, çeşitli aktiviteleri kontrol eden yaygın bir durumdur. QS ile bakteride gen ekspresyonunun modülasyonu ve bakterinin gelişimi boyunca çevre şartlarına uyum gösterebilmesini sağlayan, fenotipik değişiklikler meydana gelmektedir (69). Hücreden hücreye iletişim, küçük ve invaziv sinyal molekülleri olan AI'ların üretimine, sekresyonuna ve yanıtına bağlı olarak gelişmektedir. Sinyal molekülleri, bakterinin üreme sürecinde bazal seviyede üretilip salgılanmaktadır. Bu moleküllerin çevresel şartlardaki veya bakteri matriksindeki konsantrasyonları, bakteri popülasyonuna bağlı olarak artıp bir eşik değere geldiğinde, hedef genin ekspresyonuna bağlı olan QS tarafından düzenlenen fenotipik özellikleri uyarmaktadır (17). Bu durum, hiçbir dış müdahaleye bağlı olmadan gerçekleşmesi nedeni ile 'otoindüksiyon' olarak tanımlanmaktadır. 1994 yılında QS olarak adlandırılan hücreden hücreye iletişimdeki çeşitli basamakların, genellikle stres koşulları altındaki bakterilere zarar gelmemesi ve hayatta kalmalarının

sağlanmasına yönelik olduğu belirlenmiştir. Ayrıca QS'in, çoğunlukla virulansın düzenlenmesinde, genetik yeteneğin geliştirilmesinde, konjugatif plazmidlerin transferinde, sporulasyonda, biyofilm oluşumunda, antimikrobiyal peptit sentezinde ve simbiyoziste de rol oynadığı bildirilmektedir (61). Bakterilerde çoğunluk algılamada görevli iki grup sinyal molekülü vardır. Bunlardan birincisi, tipik olarak Gram pozitif bakteriler tarafından kullanılan peptit türevleri iken; diğeri Gram negatif bakteriler tarafından kullanılan yağ asidi türevleridir. QS, gıda mikrobiyolojisi yönünden önemli olan birçok bakteri türünde bulunmaktadır. *Agrobacterium, Brucella, Burkholderia, Erwinia, Enterobacter, Pseudomonas, Ralstonia, Serratia, Vibrio* ve *Yersinia* cinslerine ait birçok insan ve bitki patojeni, virulans faktörlerinin sentezinin düzenlenmesi için QS mekanizmasını kullanmaktadır (74). *Bacillus, Enterococcus, Staphylococcus, Streptococcus* ve *Streptomyces* cinsindeki bakteriler ise bu mekanizma ile genetik yeteneklerini geliştirerek, antimikrobiyal peptit ve ekzotoksin üretmekte ve biyofilm oluşturabilmektedir (52).

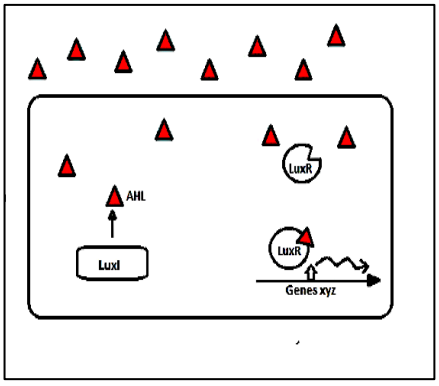
3. Çoğunluk Algılama Mekanizması

Bakteriler tarafından kullanılan QS sinyal molekülleri; Gram negatif bakteriler tarafından kullanılan açil homoserin laktonlar (AI-1, AHL), Gram pozitifler bakteriler tarafından kullanılan linear (düzlemsel) ve siklik (halkasal) otoindüktör peptitler (AIP), hem Gram negatif hem de Gram pozitifler tarafından kullanılan ve bu sayede türler arası iletişimi sağlayan otoindüktör-2 (AI-2, furanosil borat diester)'dir (21,50). Son yıllarda küf (*Aspergillus flavus, Penicillium sclerotiorum*) ve mayalarda (*Sacchharomyces serevisiae, Cryptococcus neoformans*) da QS mekanizmasının henüz net olarak tanımlanmasa bile varlığından bahsedilen çalışmalar bulunmaktadır (8,49).

3.1. AHL mekanizması

Birçok Gram negatif bakteri, AHL sinyal molekülü sentezleyen LuxI enzimlerine sahip olup bu enzim, Şekil 1'de üçgen olarak gösterilen AHL sinyal moleküllerini üretmektedir. Üretilen AHL sinyal molekülleri serbest bir şekilde hücre içerisinden hücre dışına difüze olup, hücre dışındaki AHL sinyal moleküllerinin konsantrasyonu belirli bir seviye ulaştığında AHL molekülleri düzenleyici olarak görev yapan LuxR proteinine bağlanmaktadır. Böylelikle LuxR üzerindeki DNA bağlanma bölgesi açığa çıkmakta, LuxR ve sinyal molekülü kompleksi bakterinin eksprese etmek istediği genlerin düzenleyici bölgelerine yerleşmektedir. Bakteriler ancak topluluk oluşturduğunda gen bölgeleri aktive edilmekte, tek başına olduklarında sadece LuxI enzimi tarafından AHL sinyal molekülü üretimi söz konusu olmaktadır. Üretilen AHL molekülü, her bakteri türünde farklı olup molekülünün yapısındaki karbon zincirinden kaynaklanmaktadır. Böylelikle, sadece aynı AHL molekülünü üreten bakteriler sinyale cevap verebilmektedir. Örneğin *Pseudomonas* sinyali *Vibrio fischeri*'de, *Vibrio fischeri* sinyali de *Pseudomonas*'da hiç bir cevap oluşturmamaktadır. Tür içi iletişim için kullanılan bu moleküller, anahtar kilit gibi kendi LuxR proteinlerine yerleşmekte, çapraz bir iletişim bu sistemde söz konusu olmamaktadır (9).

Gram negatif patojen bakterilerde adhezyon, biyofilm oluşturma, ekzoenzim sekresyonu, pigment üretimi gibi özelliklerin AHL'ye bağlı QS ile regüle edildiği saptanmıştır (58). Gram negatif bakterilerde tipik bir QS sisteminin 2 bileşeni bulunduğu ve bunların (1) AHL sentezinden sorumlu olan AI synthase ve transkripsiyonal aktivasyondan sorumlu AI reseptörü olduğu bilinmektedir. AHL aracılı bakteriyel iletişimde Lux IR sisteminin homoloğu olan genlere sahip 100'den fazla bakteri türü mevcut olduğu, *Serratia marcessens*'deki SmaIR, *Cromobacterium* violaceum'daki CviIR, *Halomonas* anticariensis'deki HanIR ve *Agrobacterium tumefaciens*'deki TraIR, Lux IR prensibi ile çalışmakla birlikte çok küçük değişiklikleri olan AHL'lere örnek oluşturmaktadır (70). Bunun dışında, *Pseudomonas* aeruginosa (*P. aeruginosa*)'da LasIR ve RhIIR'nin birbiri ardına seriler halinde dizildiği QS devreleri LasIR-RhIIR olarak bulunmaktadır (4). Bir diğer QS sistemi ise *Erwinia* carotovora'da gözlemlenen ExpIR'dir (3).

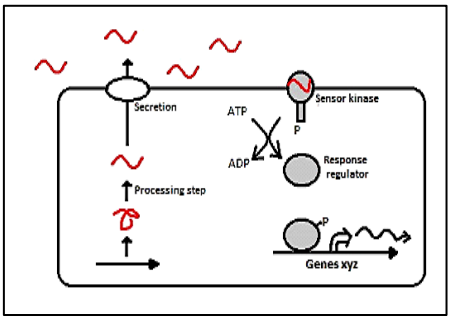


Şekil 1: Gram negatif bakterilerdeki AHL mekanizması (22)
Figure 1: *AHL mechanism in Gram negative bacteria (22)*

3.2. AIP mekanizması

Birçok Gram pozitif bakteri de Gram negatif bakteriler gibi QS sistemine sahip olup, Gram pozitif bakterilerin iletişim için kullandıkları mekanizma çeşitli farklılıklar içermektedir. Gram pozitif bakteriler, indükleyici olarak peptitleri kullanmaktadır. Peptitler genlerde kodlanmakta ve prekürsör olarak sentezlenmektedir. Bu prekürsör proteinler, başlangıçta büyük olup sonrasında bir işleme sistemi tarafından küçük peptitlere parçalanmaktadır. Ardından hücre içerisindeki peptitler, özel bir salgılama mekanizması tarafından hücre dışına gönderilmekte ve ortamda birikmeye başlamaktadır. Ortamdaki peptit konsantrasyonu kritik bir seviyeye ulaştığında ise, hücre içi ile dışı arasında köprü görevi yapan sensör niteliğindeki transmembran protein tarafından bağlanmaktadır. Peptitler bağlandığında, fosforilasyon basamaklarını başlatmaktadır. Transkripsiyon faktörü, fosforile olup ardından aktive olarak, bakterinin istediği gen bölgelerini açığa çıkarmaktadır. Bakteriler sadece topluluk halindeyken bu genleri ekprese edebilmektedir (9) (Şekil 2).

Bacillus subtilis'deki sporulasyon, *Staphylococcus aureus* (*S. aureus*)'un, *Listeria monocytogenes*'in, *Clostridium perfringens* ve *Enterococcus faecalis* (*E. faecalis*)'in virülans genlerininin ekspresyonu, AIP mekanizması ile regüle edilmektedir (70). Birçok Gram pozitif bakteride iletişim sinyal molekülü ve AIP'yi tanıyan membrana bağlı ikili komponent sistemi sayesinde gerçekleşmektedir (48). Buna en iyi örnek *S. aureus*'daki iki komponentli QS sistemidir. Bunun yanında, *Bacillus subtilis*'in virulans faktörlerinden sporulasyon ve enzim üretimi ile *E. faecalis*'deki plazmid transferi bir diğer AIP QS devresi olan ekstraselüler proteazlar tarafından gerçekleştirilmektedir (53). Bunların dışında başka bir AIP QS sistemi ise yarışmacı QS sistemi olarak adlandırılmakta olup daha önce adı geçen 2 sistemin birleşmesi ile ortaya çıkmış ve *Bacillus subtilis*'in böyle bir sisteme sahip olduğu gösterilmiştir (70).



Şekil 2: Gram pozitif bakterilerdeki AIP mekanizması (22)

Figure 1: *AIP mechanism in Gram positive bacteria (22)*

4. Gıda Bozulmasında Çoğunluk Algılama Sinyalleri

Son yıllarda, gıda bozulmasında rol oynayan QS sinyal moleküllerinin etkisi üzerinde artan bir ilginin olduğu ve AI-1 ve AI-2 gibi çeşitli sinyal moleküllerinin süt, et ve sebze gibi farklı gıdaların işlendiği tesislerde tespit edilen bazı psikrotrof bakterilerde bulunduğu bildirilmektedir (51).

Süt ve süt ürünleri, pseudomonaslar gibi psikrotrofik bakteriler tarafından bozulmaya oldukça yatkın olup, bu Gram negatif bakteriler; gıda üzerindeki etkilerini ektraselüler proteinaz, lipaz, lesitinaz ve glikozidaz enzimleri üreterek göstermektedir. Gram pozitif psikrotrofik aerobik *Bacillus* spp. ise bazı süt ürünlerinde bozulmadan sorumlu olan fosfolipazları, *Serratia proteamaculans* suş B5a da ekstraselüler lipolitik ve proteolitik enzimleri üretmektedir (64). Bu enzimlerin üretimi, *Serratia* spp. tarafından sütün bozulmasındaki QS'i gösteren AHL temelli QS sisteminin regülonunun etkisi altında olmaktadır. 2003 yılında Christensen ve ark. (15) tarafından yapılan bir çalışmada, pastörize sütün vahşi tip *Serratia proteamaculans* ile inokulasyonunu takiben oda sıcaklığında 18 saat bekletme sonrasında bozulmaya neden olurken; inaktif *spr*I genine sahip olan bir mutantla yapılan inokulasyonun bozulma oluşturmadığı belirlenmiştir. Benzer şekilde psikrotrofik bakterilerden *Pseudomonas* spp., S*erratia* spp.*, Enterobacter* spp. ve *Hafnia alvei* tarafından hem çiğ hem de pastörize sütteki AHL'lerin üretimi, süt ve süt ürünlerindeki bozulmada QS sisteminin rol oynayabileceği gösterilmiştir (51). Ayrıca, düşük sayıda bakteri içeren (10^2 kob/ml) günlük sütlerde, furanosil BAI-2 sinyallerinin tespiti ile süt bozulmasındaki olası türler arası iletişim fikri öne sürülmüştür (42). *Pseudomonas* spp. aynı zamanda aerobik ve soğuk (3-8 °C) şartlarda depolanmış et ve et ürünlerindeki bozulmadan da sorumlu olan bir bakteridir. 2003 yılında Jay ve ark. (35) tarafından aerobik soğuk koşullar altında depolanan taze et ürünlerinin bozulmasında et yüzeyinde sümüksü tabaka oluşumunda QS'in rol oynadığı bildirilmiştir. *Pseudomonadaceae* (10^8-10^9 kob/g) ve *Enterobacteriaceae* (10^3-10^4 kob/g) tarafından sentezlenen C4-HSL, 3-oxo-C6-HSL, C6-HSL, C8-HSL ve C12-HSL gibi AHL sinyalleri, aerobik ve soğuk şartlarda depolanmış kırmızı et kıyması ve tavukta saptanmıştır (56). Vakumlanarak paketlenmiş ette baskın türler olarak izole edilen *Hafnia alvei* ve *Serratia* spp., AHL üreten *Enterobacteriaceae* olarak tanımlanırlarken; pseudomonasların tespit edilebilir miktarda AHL üretmedikleri saptanmıştır (40). 2004 yılında Lu ve ark. (42)'nın gerçekleştirdiği bir araştırmada, başlangıç bakteriyel yük yüksek olmasına rağmen (6,4-8 kob/ml), dana biftek, dana köfte, tavuk göğsü ve hindi köftesinde çok düşük miktarda BAI-2 aktivitesinin olduğu (negatif kontrol ile karşılaştırıldığında bir kat lüminesans indüksiyonundan daha az) bildirilmiştir. Bunun nedeninin, yağ asitlerinin AI-2 aktivitesini tam veya kısmi olarak inhibe etmesi olduğu düşünülmektedir (62). Soğuk şartlarda aerobik olarak depolanan bozulmuş domuz kıymasından elde edilen hücreden ari ekstraktın, AHL ve BAI-2 sinyalleri içerdiği, ve bu sinyallerin miktarının 5°C'de yapılan ölçümde 20°C'den daha yüksek olduğu tespit edilmiştir (2). 2007 yılında taze etten elde edilen *Pseudomonas fluorescens* (*P. fluorescens)* ve *Serratia marcescens*'in 18 saatlik kültürüne bakteriden ari et özütü ilavesinin, *P. fluorescens*'in lag fazını ve her iki bakteri cinsinin metabolik aktivitesini artırdığı, böyle bir metabolik aktivite artışının, bakteriden ari et özütündeki QS molekülleri gibi bileşenlerin varlığı ile bağlantılı olduğu ortaya konulmuştur (47). AHL'ler 5, 10, 15 ve 20°C'deki modifiye atmosferde depolanmış taze domuz kıymalarında da tespit edilmiştir. AHL üretimi 10 ve 15°C'de 2 ve 7 günlük depolamada maksimumum seviyeye ulaşmış ve bu durum *Enterobacteriaceae* ve *Pseudomonadaceae* üyelerinin ortamda büyümesi ile ilişkilendirilmiştir (10). *Shewenella putrefaciens* ve *Pseudomonas* spp. sırasıyla dondurulmuş deniz balığı ve dondurulmuş tatlı su balıklarında spesifik bozulma yapan mikroorganizmalardır (26). AHL'ler soğuk dumanlanmış somon, balık fileto ve kıyılmış balık gibi ticari olarak farklı çeşitlilikteki balık ürünlerinde tespit edilmiştir. Vakum paketlenmiş ve soğuk dumanlanmış somondaki bozulma, *Enterobacteriaceae* ve miktarları 10^7-10^9 kob/g gibi yüksek konsantrasyonlara çıkan laktik asit bakterilerinden *Carnobacterium* sp. ve/veya *Lactobacillus* sp. sebebiyle meydana gelmektedir (37). Gıda bozulmasına neden olan bakterilerin gıda substratında düşük konsantrasyonda dahi AHL üretebildikleri gösterilmiştir. AHL'ler (başlıca 3-oxo-C6-HSL), 5°C'deki stimule edilmiş gıda ortamında, azaltılmış O_2 ve % 4 NaCl varlığında *Enterobacteriaceae* ailesinin inokule edilmiş ve gıdada doğal olarak bulunan üyeleri tarafından sırasıyla 10^6 ve 10^5-10^6 kob/g gibi düşük konsantrasyonlarda üretilmiştir (25). Paketlenmiş morina filetolarından izole edilen, biyolüminesans vermeyen *Photobacterium phosphoreum* ve *Aeromonas* spp.'deki kitinaz aktivitesini düzenlediği bilinen 3-hidroksi-C8-HSL'nin tespiti, kabukluların bozulmasında AHL temelli bir sistemin olası rolüne işaret etmektedir (23). 3-oxo-C6-HSL, C6-HSL, C8-HSL ve C12-HSL, gökkuşağı alabalık filetolarının bozulmasınaki

proteolitik aktiviteden sorumlu *Hafnia alvei*, *Serratia liquefaciens, P. fluorescens* ve *Pseudomonas putida*'da tespit edilmiştir. Benzer şekilde, AHL kontrolündeki proteolitik aktivite, gıda bozulmasındaki QS AHL sistemlerinin rolünü destekleyen ve soğuk dumanlanmış somondan izole edilen *Serratia proteamaculans* B5a suşunda rapor edilmiştir (15). Meyve ve sebzelerde pektin liyaz, pektat liyaz, poligalakturonaz ve pektin metil esteraz ile pektinolitik aktivite göstererek 10^8-10^9 kob g/L'a kadar üreyebilen *Pseudomonadaceae* veya *Enterobacteriaceae* (çoğunlukla *Erwinia* spp.), bu tip gıdalarda doku bozulması ile beraber enzimatik esmerleşme, kötü tat, kötü kokuya yol açmaktadır. Çoğunlukla 3-oxo-C6-HSL ve C6-HSL varlığı saptanan bu gıdalarda AHL temelli QS sistemlerinin meyve sebze bozulmasındaki ilişkisini desteklemektedir (55).

Gram negatif bakterilerde AHL temelli QS sistemlerinin gıda bozulması ile ilişkisinin incelendiği çalışmalara Tablo 1'de örnekler verilmiştir. Bununla birlikte, AI-2 ile gıda bozulmasında Gram pozitif bakteriler tarafından üretilen AIP'ler hakkında bilgi bulunmamaktadır. Donmuş, vakum paketli ve modifiye atmosfer paketli pastörize süt, et ve balık ürünlerindeki QS sinyal bileşenlerinin varlığı, mevcut olan koruma tedbirlerinin yetersiz olduğunu ortaya koymaktadır.

Tablo 1: Quorum Sensing ile üremeleri regüle edilen fenotipler ve oluşan gıda bozulmaları

Table 1: *Phenotypes with growth regulated by quorum sensing and related food spoilage*

Organizma	Ürün	Sinyale bağımlı fenotipler	Sinyal molekülü	Referans
Pseudomonas fluorescens 395	Süt	Proteolitik	C4-HSL ve 3OCB-HSL	1
Serratia proteonaculans suş B5a	Süt	Lipolitik Proteolitik	3-oxo-C6-HSL	15
Pseudomonas phosohoreum ve *Aeromonas* spp.	Morina filetoları	Kitinolitik	3-hidroksi-C8-HSL	23
Pectobacterium sp. A2JM	Fasülye filizleri	Pektinolitik Proteolitik	3-oxo-C6-HSL	56
Serratia plymuthica RVH1	Sebzeler	Kitinaz ve Proteaz aktv.	3-oxo-C6-HSL ve C6-HSL	72
Pseudomonas spp.	Et	Biyofilm oluşumu Proteolitik	AHLler	35
Photobacterium phosphoreum ve *Aeromonas* spp.	Morina filetoları	Kitinolitik	3-hidroksi-C8-HSL	23

5. Çoğunluk Algılama ve Biyofilm Oluşumu

Biyofilm; mikroorganizmaların birbirleriyle, bulundukları yüzeylere veya bulundukları yüzeylerden daha alt tabakalara yani ara yüzeylere geri dönüşümsüz olarak tutunmalarını sağlayan, aynı zamanda büyüme oranı ve gen transkripsiyonuna bağlı olarak farklı fenotipik özellikler kazanarak salgıladıkları Extracellular Polymeric Substance (EPS) / hücre dışı polimerik madde matriksi olarak adlandırılmaktadır (60). Oluşumunda QS'in de rol aldığı düşünülen biyofilmde EPS içinde gömülü olarak calılıklarını sürdürebilen mikroorganizmalar, immun sistem elemanlarından, antibiyotiklerden, patojenlerin üretmiş olduğu antimikrobiyal ürünlerden, fiziksel, kimyasal ve biyolojik streslerden korunmaktadır (45). Biyofilm içindeki bakterilerin antibiyotiklere karşı 1000 kat daha dirençli olduğu, farklı mikroorganizmaların ise yaşadıkları çevrede planktonik formlarına göre daha fazla hayatta kalma ve büyüme şansına sahip olduğu bildirilmektedir (32).

Biyofilm oluşumunun tüm aşamalarında rol alan QS ile, olgunlaşmış bir biyofilmde, besin ihtiyacı ve kaynakların yeterliliğine göre uyum sağlamak için popülasyon yoğunluğu ve metabolik aktivite düzenlenmektedir. Biyofilmdeki bakteriler, serbest yaşayan aynı soydaki planktonik bakterilerden farklı tranksripsiyonel programlara sahiptir (6). Bakteriler, çevrelerinde yer alan diğer bakteri hücrelerini biyofilm oluşturma yönünde uyarabilmek için,

ortama küçük, diffuze olabilen moleküller yaymaktadır. Böylece QS, bakteriyel populasyonun davranışlarını kontrol etmektedir (18). Bu aşamada gerçekleşen yüzeye tutunma, kimyasal bağlanmadan ziyade elektrostatik bir etkileşimle olduğu için geri dönüşümlüyken, hücrelerin bazılarının daha sıkı bağ kurmak amacıyla bazı yapılar oluşturması sonucu biyofilmin diğer bir basamağı olan geri dönüşümsüz bağlanmaya geçilebilmektedir (16). EPS üretimi ile bakterilerin yüzeye geri dönüşümsüz bağlanması, membrana bağlı uyarıcı proteinlerin uyarılması sonucu şekillenmektedir. Böylelikle hücreler arası köprüler kurularak bakteri kümeleri oluşmaktadır (31). Bakteriler biyofilm bileşiminde bulunan Biyofilm Associated Protein (BAP) / Birleşmiş Protein yapısı ile yüzeye kolonize olabilmekte ve burada sürekli kalabilmektedir (67). Tutunma sonrasında, biyofilm oluşturma yönünde farklılaşmanın başlaması, QS sisteminden gelen sinyallere bağlı olarak şekillenmektedir. QS ile bakteriler çevrelerindeki bakteri popülasyon yoğunluğunu belirlemekte ve yüzeye tutunan her bakteri ortama sinyal veren bir molekül salgılamaktadır. Yüzeye tutunan bakterilerin sayısı arttıkça, bu sinyalin lokal konsantrasyonları da artmaktadır. Bu sinyal molekülünün (AI) konsantrasyonundaki artış ile birlikte biyofilm oluşumuna yönelik bir dizi işlem başlatılmış olmaktadır. Hücre yoğunluğuna bağlı olarak QS, küçük işaret moleküllerinin birikimine yanıt verip, ortamı tarayıp salınımda bulunmakta, bu tür etkileşimler sonucunda da bir grup hedef gen regülasyonu ile belirli bir sinyal yoğunluğuna erişildiğinde bazı genlerin ekspresyonu sağlanmaktadır (19). Sonuçta oluşan biyofilm ile sağlanan izole ortam, bakterinin yer aldığı ektraselüler matriksten ayrılmasına engel olmaktadır. Biyofilm içi popülasyon yoğunluğu arttığında ise bakteriler biyofilm tabakasından ayrılarak ortama salınabilmektedir. Stafilokokların polisakkarit intraselüler adezin üretimini azaltmak için AI-2 sinyallerini kullandığı ve böylelikle bakterilerin biyofilmden ayrılmasına izin verdiği düşünülmektedir. Ayrıca hücre yoğunlukları yüksek olduğunda, çekirdek algılama kontrolü altında olan deterjan özelliklerine sahip kısa peptitler, biyofilmdeki bakterilerin ortama verilmesi için kullanılmaktadır. Biyofilmdeki bakteriler, bakteri popülasyonu genişlerken planktonik bakteriler için sürekli bir kaynak sağlamaktadır (46). Süt, et ve balık ürünlerinden sıklıkla izole edilen *Hafnia alvei* (73)'nin biyofilm oluşumunda önemli bir potansiyele sahip olduğu Viana ve ark. (72) tarafından rapor edilmiştir. *Vibrio cholerae* ve *Serratia liquefaciens* sinyal moleküllerinin EPS ve biyofilm oluşumu için gerekli olan hücre birikimini kontrol ettiği görüşünün aksine Van Houdt ve ark. (71), gıda işleme ortamından izole edilen Gram negatif bakterilerde QS üretimi ile biyofilm oluşumu arasında bir korelasyon olmadığını bildirmiştir. Biyofilmlerdeki sinyal moleküllerinin tespit edilmesine rağmen QS'in henüz biyofilm oluşumundaki rolü kesin olarak açıklanamamaktadır. Biyofilmler, gıdanın işlendiği ortamlarda bulunan inatçı bir problem olup QS'in inhibe edilmesi ile biyofilm oluşumu önlenebilmektedir. Böylece gıda bozulmaları geciktirilerek gıda üretimi ve güvenliği için faydalı olunabilecektir (4).

6. Biyosensörler ile Gıdada Çoğunluk Algılama Sinyallerinin Tespiti

QS sinyal molekülleri; hücreden ari süpernatant (cell-free supernatant), gıda örneği özütleri ile gıdadan izole edilmiş bakterilerin süpernatantında (spent culture supernatant) tespit edilebilmektedir (2). Yüksek performanslı sıvı kromatografi-MS, gaz kromatografi-MS ve nükleer manyetik rezonans spektroskopisi gibi kütle spektrometrileri (MS) kullanılarak daha önce tanımlanmış AHL'lerin yapıları belirlenmiştir (12). Ancak farklı AHL tiplerine spesifik geliştirlen biyosensörler sayesinde ölçümü daha kolay, ekonomik ve hızlı bir hale gelmiştir. Biyosensörler; sadece eksojen AHL'lerin varlığında fenotipik bir yanıtı kodlayan haberci bir genin ekspresyonunu düzenleyen soydaş bir hedef promotörüyle (genellikle soydaş LuxI sentazın promotörü) klonlanmış fonksiyonel bir LuxR familya proteininden oluşmaktadır. Sinyal moleküllerini üretmeyip onların eşlenik reseptörlerine sahiptir (63).

7. Çoğunluk Algılama İnhibitörlerinin Gıda Endüstrisinde Kullanımı

Günümüzde QS sistemine müdahale etme amacı ile bakterilerin, AI aktivite blokajında QS inhibitörleri (QSI)'nin ve sinyal moleküllerini parçalamada ise Quorum Quenching (QQ) enzimlerinin kullanıldığına dair bulgular mevcuttur (57). Yakın geçmişe ait bu stratejilerin uygulanabilirliğinin test edildiği çalışmalar bakteriyel patojenitenin azaltılması, biyofilm oluşumunun minimize edilmesi, antimikrobiyal ajanlara ve makrofajlara karşı duyarlılığın artırılması yönünde umut verici sonuçlar vermektedir. Bazı fırsatçı insan ve bitki patojenlerindeki virulans faktörlerinin

regülasyonuna AHL'lerin katılması, spesifik olarak AHL iletişimini bloke eden bileşenlerin yoğun olarak araştırılmasının önünü açmıştır. Hücreler arası QS'in engellenmesinde önemli bir yaklaşım olan QQ, özellikle klinik mikrobiyoloji ve gıda mikrobiyolojisinde istenmeyen mikrobiyal gelişim ve biyofilm oluşumunun bertaraf edilmesi açısından oldukça önemlidir. QS mekanizmasının işleyişinin engellenmesi amacı ile (a) QS reseptör inaktivasyonu, (b) sinyal sentezi inhibisyonu, (c) sinyal degredasyonu ile (d) sinyal blokajı'nın hedeflendiği stratejiler bulunmaktadır (36).

QS reseptör inaktivasyonunda; flavonoidlerin QS reseptörlerine bağlanması sonrası *P. aeruginosa*'nın virulans gen ekspresyonunda belirgin azalma görüldüğü, böylelikle etkenin neden olduğu enfeksiyonlarda kullanılan antibiyotiklerin terapötik dozlarının azaltılabildiği belirlenmiştir (11). Bu tür reseptör inhibitörlerinde karşılaşılan en önemli sorunun, bu maddelerin alkali ortamda yapılarının instabil hale gelmesi ve degrede olması olduğu bildirilmiştir (36). Sinyal sentezi inhibisyonuna ilişkin olarak; AHL aracılı virülans faktörlerinin inhibe edilmesi ile ökaryotik hücrelerdeki patolojik hasarın azaltılması hedef alınmıştır (65). Sinyal degredasyonu; QS sinyallerinin parçalanmasında (enzimatik QQ) sinyal moleküllerini parçalayan enzimler (1) lactonase enzimleri (lactonase aracılı QQ), (2) acylase enzimleri (acylase aracılı QQ) ile (3) oxydoreductase enzimleri (oxydoreductase aracılı QQ) olmak üzere 3 kategoride incelenmektedir (70). Bunlar içerisinde özellikle, AHL lactonase' ların antibiyotiklere karşı bakteriyel duyarlılığı artırıp, *P. aeruginosa* ve *Acinetobacter baumanii*'nin üremesini etkilemediği belirlenmiştir (14, 28). Bu enzimin ayrıca *P. aeruginosa*'nın (59), ve karideslerde *Vibrio parahaemolyticus*'un biyofilm oluşturmasının önlenmesi (66) ile balıklarda *Aeromonas hydrophila* enfeksiyonunun engellenmesinde kullanıldığı bildirilmiştir (41). İnsan hekimliğinde problem yaratan *P. aeruginosa* ile *S. aures*'un inhibisyonuna yönelik olarak hedef antikor kulanımı ile QS sinyal blokajı (39); ayrıca anti QS ajanları ile antibiyotiklerin kombine kullanıldığı ve bu iki etken maddenin sinerjestik etkisinin olduğu tespit edilmiştir (24).

QS inhibitörlerinden biri olan Avustralya kırmızı algi (*Delisea pulchra*) tarafından sentezlenen halojenlenmiş furanonlar AHL reseptör proteinlerine müdahale ederek sinyal sentezinin blokajına neden olmaktadır (43). Bu bileşenlerin uygulanması ile, AHL tarafından regüle edilen *P. aeruginosa* ve *Serratia liquefaciens* virulans faktörlerinin ekspresyonu engellenerek biyofilm oluşumu azaltılmıştır (33). Halojenlenmiş furonanların sitotoksik özellikleri ve kimyasal olarak kararsız yapıda olmaları, diğer doğal kaynaklarda toksik olmayan başka QS inhibitörlerinin aranmasına neden olmuştur. Biyolojik olarak aktif bileşenlerin araştırılmasında en umut verici olanlardan biri, geleneksel tedavide kullanılan bitkilerdir (34). 2015 yılında Truchado ve ark (68) tarafından yapılan bir çalışmada, bitkilerde sağlığa yararlı ve antimikrobiyal aktivitesi olduğu bilinen bazı fitokimyasalların subletal konsantrasyonlarda QS inhibitör aktivitesi gösterdikleri bildirilmiştir. Benzer şekilde Duarte ve ark (20) tarafından kişniş esansiyel yağı ile bu yağ içerisinde en yüksek düzeyde bulunan linaloolun *Campylobacter jejuni* ve *Campylobacter coli* üzerinde antinmikrobiyal aktivitesinin yanısıra biyofilm oluşumunu engellenmesi ve oluşan biyofilmin dağılmasının sağlaması üzerinde etkili olduğu ortaya konulmuştur. Baharat ve çeşni olarak yaygın kullanım alanı bulan vanilyanın da bakteriyel QS'i inhibe edebildiği tespit edilmiştir (13). 2008 yılında Girennavar ve ark (27) tarafından greyfurt suyundan elde edilen doğal furokumarinlerin, AI-1 ve AI-2 aktivitesi ve *Salmonella* Typhimurium, *Escherichia coli* ile *P. aeruginosa* tarafından oluşturulan biyofilmlerin potansiyel inhibitörü olarak davrandıkları rapor edilmiştir. Bu durum greyfurt suyunun, halojenlenmiş furonanlara alternatif olarak mikrobiyal QS'de hedef strateji geliştirilmesinde kullanılabileceğini göstermektedir. Ayrıca insanlarda bulunan bazı hormonların da mikroorganizmalar üzerine inhibisyon etkisi ve QS'i baskılayıcı durumlarının olduğu belirtilmektedir. Yapılan bir çalışmada, Enterohemorajik *Escherichia coli* (EHEC)'nin QS mekanizmasının insan vücudunda sentezlenen hormonlar ile çapraz etkileşimi olduğu gösterilmiştir (1). QS inhibitör bileşenlerinin bitkilerden bu çeşitlilikte elde edilebiliyor olması gıda koruma yönünden oldukça umut vericidir. QS inhibitörleri, gıda kaynaklı bakterilerin gıda yüzeylerinde kolonizasyonu, toksin oluşumu ve çoğalmasını engellemektedir. Bununla birlikte, QS inhibitörlerinin doğal oluşu, toksikolojik etkilerinin ayrıca değerlendirilmesi gerektirmektedir.

8. Sonuç

Biyokimyasal değişiklikler ve mikrobiyal aktivite sonucu oluşan gıda bozulması karmaşık bir süreçtir. Son yıllarda yapılan çalışmalar, önceleri sadece mikrobiyal virulans ve patojenite ile ilgili olduğu düşünülen QS'in, gıda

bozulmasında da önemli bir role sahip olabileceğini ortaya koymaktadır. Gram negatif bakteriler ile ilişkili AHL ve AI-2 temelli QS sistemleri farklı gıda ekosistemlerinde tespit edilmiştir. Bununla birlikte, Gram pozitif bakterilerin oynadığı rolün tam olarak anlaşılabilmesi için daha fazla araştırmaya ihtiyaç duyulmaktadır. QS inhibitörlerinin gıda güvenliğinin sağlanması ve raf ömrünün uzatılması için gıda koruyucusu olarak kullanılabilme potansiyeli bulunmaktadır. Mikroorganizmalar arası QS'de olumlu ve olumsuz tüm etkenler göz önüne alındığında, tıp alanında ve gıda endüstrisinde patojen ve gıda bozulmasına yol açan mikroorganizmaların inhibisyonunun sağlanması ve yeni antimikrobiyel maddelerin geliştirilmesi için mikroorganizmalara ait QS ve inhibisyon sistemlerinin iyi bilinmesi gerekmektedir.

Çıkar Çatışması Beyanı

Makalenin yazarları arasında bu derleme çalışması kapsamında herhangi bir kişisel ve finansal çıkar çatışması bulunmamaktadır.

Yazar Katkısı Beyanı

Fikir/kavram: Ayşegül Eyigör
Deney tasarımı: Seran Temelli
Denetleme/Danışmanlık: Ayşegül Eyigör
Veri toplama: Gonca Tülüce
Veri analizi ve yorum: Seran Temelli
Kaynak taraması: Gonca Tülüce
Makalenin yazımı: Gonca Tülüce, Seran Temelli, Ayşegül Eyigör
Eleştirel inceleme: Ayşegül Eyigör

Kaynaklar

1. Açıkgöz E (2012): *Quorum quenching.* Orlab Online Mikrobiyol Derg TR, **10**, 27-44.
2. Ammor MS, Michaelidis C, Nychas EGJ (2008): *Insights into the role of quorum sensing in food spoilage*. J Food Prot, **71**, 1510-1525.
3. Andersson RA, Eriksson ARB, Heikinheimo R, Mäe A, Pirhonen M, Kõiv V, Hyytiäinen H, Tuikkala A, Palva ET (2014): *Quorum sensing in the plant pathogen Erwinia carotovora subsp. carotovora: the role of expR Ecc.* Mol Plant Microbe Interact*,* **13**, 384-393.
4. Annous BA, Fratamico PM, Smith JL (2009): *Quorum sensing in biofilms: why bacteria behave the way they do*. J Food Sci, **74**, 24-37.
5. Antunes LC, Ferreira RB (2009): *Intercellular communication in bacteria*. Crit Rev Microbiol, **35**, 69-80.
6. Asad S, Opal SM (2008): *Bench-to-bedside review: quorum sensing and the role of cell-to-cell communication during invasive bacterial infection*. Crit Care, **12**, 236-246.
7. Bai AJ, Rai VR (2011): *Bacterial quorum sensing and food industry.* Compr Rev Food Sci Food Saf, **10**, 184-194.
8. Barriuso J, Hogan DA, Keshavarz T, Martinez MJ (2018): *Role of quorum sensing and chemical communication in fungal biotechnology and pathogenesis*. FEMS Microbiol Rev, **42**, 627-638.
9. Bassler B (2010): *Bonnie Bassler (Princeton) Part 1: Bacterial Communication via Quorum Sensing.* https://www.youtube.com/watch?time_continue=17&v=saWSxLU0ME8&feature=emb_logo (date accessed: 01 09 2020)
10. Blana V, Stamatiou A, Michaelidis C, Stergiou V, Nychas EGJ (2007): *Qualitative evaluation of QS compounds produced in pork and beef samples at different storage conditions; possible effect on kinetic characteristics on spoilage bacteria.* Athens, Greece: Second Panhellenic Congress: Biotechnology and Food Technology.
11. Capilato JN, Philippi SV, T. Reardon, **McConnell , Oliver DC, Warren A, Adams JS, Wu C, Perez LJ** (2017): *Development of a novel series of non-natural triaryl agonists and antagonists of the Pseudomonas aeruginosa LasR*

quorum sensing receptor. Bioorg Med Chem, **25**, 153-165.
12. Cataldi TR, Bianco G, Palazzo L, Quaranta V (2007): *Occurrence of N-acyl-L-homoserine lactones in extracts of some gram-negative bacteria evaluated by gas chromatography–mass spectrometry*. Anal Biochem, **361**, 226-235.
13. Choo JH, Rukayadi Y, Hwang JK (2006): *Inhibition of bacterial quorum sensing by vanilla extract*. Lett Appl Microbiol, **42**, 637-641.
14. Chow JY, Yang Y, Tay SB, Chua KL, Yew WS (2014): *Disruption of biofilm formation by the human pathogen acinetobacter baumannii using engineered quorum-quenching lactonases*. Antimicrob Agents Chemother, **58**, 1802-1805.
15. Christensen AB, Riedel K, Eberl L, Flodgaard LR, Molin S, Gram L, Givskov M (2003): *Quorum sensing-directed protein expression in Serratia proteamaculans B5a.* Microbiology, **149**, 471-483.
16. Costernon JW, Montanaro L, Arciola CR (2004): *Biofilm in implant infections: its production and regulation.* Int Journal of Articial Organs, **28**, 1062-1068.
17. Czajkowski R, Jafra S (2009): Quenching of acyl-homoserine lactone-dependent quorum sensing by enzymatic disruption of signal molecules. Acta Biochim Pol, **56**, 1-16.
18. Davies D (2003): *Understanding biofilm resistance to antibacterial agents*. Nat Rev Drug Discov, **2**, 114-122.
19. Dong YH, Zhang LH (2005): *Quorum sensing and quorum-quenching enzymes*. J Microbiol, **43**, 101-109.

20. Duarte A, Luis A, Oleastro M, Domingues FC (2016): *Antioxidant Properties of coriander essential oil and linalool and the irpotential to control Campylobacter spp*. Food Control, **61**, 115-122.
21. Eickhoff MJ, Bassler BL (2018): *SnapShot: Bacterial Quorum Sensing*. Cell, **174**, 1328-1328.
22. Federle MJ, Bassler BL (2003): *Interspecies communication in bacteria.* J Clin Invest, **112**, 1291-1299.
23. Flodgaard LR, Dalgaard P, Andersen JB, Nielsen KF, Givskov M, Gram L (2005): *Nonbioluminescent strains of Photobacterium phosphoreum produce the cell-to-cell communication signal N-(3-hydroxyoctanoyl)homoserine lactone.* Appl Environ Microbiol, **71**, 2113-2120.
24. Fong J, Yuan M, Jakobsen TH, Mortensen KT , May Santos MSD, Chua SL, Yang L, Tan CH, Nielsen TE, Givskov M (2017): *Disulfide bond-containing ajoene analogues as novel quorum sensing inhibitors of Pseudomonas aeruginosa.* J Med Chem, **60**, 215-227.
25. Gram L, Christensen AB, Ravn L, Molin S, Givskov M (1999): *Production of acylated homoserine lactones by psychrotrophic members of the Enterobacteriaceae isolated from foods*. Appl Environ Microbiol, **65**, 3458-3463.
26. Gram L, Ravn L, Rasch M, Bruhn JB, Christensen AB, Givskov M (2002): *Food spoilage interactions between food spoilage bacteria.* Int J Food Microbiol, **78**, 79-97.
27. Girennavar B, Cepeda ML, Soni KA, Vikram A, Jesudhasan P, Jayaprakash GK, Pillai SD, Patil BS (2008): *Grapefruit juice and its furocoumarin inhibits autoinducer signaling and biofilm formation in bacteria*. Intl J Food Microbiol, **125**, 204-208.
28. Guendouze A, Plener L, Bzdrenga J, Jacquet P, Remy B, Elias M, Lavigne JP, Daude D, Chabriere E (2017): *Effect of quorum quenching lactonase in clinical isolates of pseudomonas aeruginosa and comparison with quorum sensing inhibitors*. Front Microbiol, **8**, 227.
29. Gülgör G, Korukluoğlu M (2014): *Mikroorganizmalar Arasında Çoğunluk Algılanması (Quorum Sensing)*. J Agr Fac Uludag Univ, **28**, 83-92.
30. Gün İ, Ekinci FY (2009): *Biyofilmler: yüzeylerdeki mikrobiyal yaşam*. GIDA, **34**, 165-173.
31. Hall-Stoodley L, Costernon JW, Stoodley P (2004): *Bacterial biofilms: from the natural environment to infectious diseases.* Nat Rev Microbiol, **2**, 95-108.
32. Hassan A, Usman J, Kaleem F, Omair M, Khalid A, Iqbal M (2011): *Evaluation of different detection methods of biofilm formation in the clinical isolates.* Braz J Infect Dis, **15**, 305-311.
33. Hentzer M, Riedel K, Rasmussen TB, Heydorn A, Andersen JB, Parsek MR, Rice SA, Eberl L, Molin S, Hoiby n, Kjellberg S, Givskov M (2002): *Inhibition of Pseudomonas aeruginosa quorum sensing biofilm bacteria by a halogenated furanone compound.* Microbiology, **148**, 87-102.
34. Jamuna Bai A, Rai VR, Pradeepa VS (2011): *Evaluation of the antimicrobial activity of three medicinal plants*

of South India. Malays J Microbiol, **7**, 14-18.
35. Jay JM, Vilai JP, Hughes ME (2003): *Profile and activity of the bacterial biota of ground beef held from freshness to spoilage at 5-7 °C*. Int J Food Microbiol, **81**, 105-111.
36. Jiang Q, Chen J, Yang C, Yin Y, Yao K (2019): *Quorum Sensing: A Prospective Therapeutic Target for Bacterial Diseases*. Hindawi BioMed Res Int, **2019**, 1-15.
37. Jorgensen LV, Huss HH, Dalgaard P (2000): *The effect of biogenic amine production by single bacterial cultures and metabiosis on cold-smoked salmon*. J Appl Microbiol, **89**, 920-934.
38. Karaboz İ, Sukatar A (2004): *Bakterilerde sosyal davranışlar (bakterilerde iletişim mekanizmaları)*. Orlab On-Line Mikrobiyol Derg, **2**, 23-32.
39. Koul S, Prakash J, Mishra A, Kalia VC (2016): *Potential emergence of multi-quorum sensing inhibitor resistant (MQSIR) bacteria*. Indian J Microbiol, **56**, 1-18.
40. Liu M, Gray JM, Griffiths MW (2006): *Occurrence of proteolytic activity and N-acyl-homoserine lactone signals in the spoilage of aerobically chill-stored proteinaceous raw foods.* J Food Protect **69**, 2729-2737.
41. Liu W, Ran C, Liu Z, Gao Q, Xu S, Ringø E, Myklebust R, Gu Z, Zhou Z (2016): *Effects of dietary Lactobacillus plantarum and AHL lactonase on the control of Aeromonas hydrophila infection in tilapia* Microbiologyopen, **5,** 687-699.
42. Lu L, Hume ME, Pillai SD (2004): *Autoinducer-2-like activity associated with foods and its interaction with food additives.* J Food Protect, **67**, 1457-1462.
43. Manefield M, Rasmussen TB, Hentzer M, Andersen JB, Steinberg P, Kjellberg S, Givskov M (2002): *Halogenated furanones inhibit quorum sensing through accelerated LuxR turnover*. Microbiology, **148**, 1119-1127.
44. Mukherjee S, Moustafa D, Smith CD, Goldberg JB, Bassler L (2017): *The RhlR quorum-sensing receptor controls Pseudomonas aeruginosa pathogenesis and biofilm development independently of its canonical homoserine lactone autoinducer.* PLOS Pathog, **2017**, 13:e1006504.
45. Nouraldin AAM, Baddaour MM, Harfous AH, Essa AAM (2016): *Bacteriophage-antibiotic synergism to control planktonic and biofilm producing clinical isolates of Pseudomonas aeruginosa*. Alexandria J Med, **52**, 99-105.
46. Novick RP (2003): *Autoinduction and signal transduction in the regulation of staphylococcal virulence*. Mol Microbiol, **48**, 1429-1449.
47. Nychas GJE, Douglas LM, Sofos JN (2007): *Meat, poultry, and seafood*, 105-140. In: Doyle MP, Beuchat LR (Ed). Food microbiology: fundamentals and frontiers. Washington DC: ASM Press.
48. Olson ME, Todd DA, Schaeffer CR, Paharik AE, Van Dyke MJ, Büttner H, Dunman PM, Rohde H, Cech NB, Fey PD, Horswill AH (2014): *Staphylococcus epidermidis agr quorum-sensing system: signal identification, cross talk, and importance in colonization*. J Bacteriol, **196**, 3482-3493.
49. Padder SA, Prasad R, Shah AH (2018): *Quorum sensing: a less known mode of communication among fungi.* Microbiol Res, **210**, 51-58.
50. Papenfort, K, Bassler BL (2016): *Quorum sensing signal-response systems in Gram-negative bacteria*. Nat Rev Microbiol, **14**, 576-588.
51. Pinto UM, de Souza VE, Martins ML, Vanetti MCD (2007): *Detection of acylated homoserine lactones in gram-negative proteolytic psychrotrophic bacteria isolated from cooled raw milk*. Food Control, **18**, 1322-1327.
52. Podbielski A, Kreikemeyer B (2004): *Cell density-dependent regulation: basic principles and effects on the virulence of Gram-positive cocci*, Int J Infect Dis, **8**, 81-95.
53. Pottathil M, Lazazzera B (2003): *The extracellular Phr peptide-Rap phosphatase signaling circuit of Bacillus subtilis*. Front Biosci, **8**, 32-45.
54. Ragaert P, Devlieghere F, Debevere J (2007): *Role of microbiological and physiological spoilage mechanisms during storage of minimally processed vegetables*. Postharvest Biol Technol, **44**, 185-194.
55. Rasch M, Rasmussen TB, Larsen TO, Givskov M, Gram L (2005): *Effect of quorum sensing inhibitors on food spoilage*, 42-43 [abstract]. In: Institute of Food Technologists Annual Meeting. Chicago: Institute of Food Technologists.
56. Ravn L, Christensen AB, Molin S, Givskov M, Gram L (2003): *Influence of food preservation parameters and*

associated microbiota on production rate, profile and stability of acylated homoserine lactones from food-derived Enterobacteriaceae. Int J Food Microbiol, **84**, 145-156.57

57. Rémy B, Mion S, Plener L, Elias M, Chabrière E, Daudé D (2018): *Interference in Bacterial Quorum Sensing: A Biopharmaceutical Perspective*. Front Pharmacol, **9**, 203.

58. Schuster M, Sexton D, Diggle S, Greenberg E (2013): *Acyl-homoserine lactone quorum sensing: from evolution to application*. Annu Rev Microbiol, **67**,43-63.

59. Sakr MM, Aboshanab KM, Elkhatib WF, Yassien MA, Hassouna NA (2018): *Overexpressed recombinant quorum quenching lactonase reduces the virulence, motility and biofilm formation of multidrug-resistant Pseudomonas aeruginosa clinical isolates*. Appl Microbiol Biotechnol, **102**, 10613-10622.

60. Shunmugaperumal, T (2010): *Biofilm Eradication and Prevention: A Pharmaceutical Approach to Medical Device Infections*, John Wiley and Sons, Inc.

61. Smith JL, Fratamico PM, Novak JS (2004): *Quorum sensing: a primer for food microbiologists*. J Food Protect, **67**, 1053-1070.

62. Soni KA, Jesudhasan P, Cepeda M, Widmer K, Jayaprakasha GK, Patil BS, Hume ME, Pillai SD (2008): *Identification of ground beef-derived fatty acid inhibitors of autoinducer-2 based cell signaling*. J Food Protect, **71**, 134-138.

63. Steindler L, Venturi V (2007): *Detection of quorum-sensing N-acyl homoserine lactone signal molecules by bacterial biosensors*. FEMS Lett Microbiol, **266**, 1-9.

64. Stepaniak L (2004): *Dairy enzymology*. Int J Dairy Technol, **57**, 153-171.

65. Tomioka H, Sano C, Tatano Y (2017): *Host-directed therapeutics against mycobacterial infections*, Curr Pharm Des, **23**, 2644-2656.

66. Torres M, Reina JC, Fuentes-Monteverde JC, Fernández G, Rodríguez J, Jiménez C, Llamas I (2018): *AHL-lactonase expression in three marine emerging pathogenic Vibrio spp. reduces virulence and mortality in brine shrimp (Artemia salina) and Manila clam (Venerupis philippinarum)*. PLoS One, **13**, ID e0195176.

67. Tormo MA, Marti M, Valle J, Manna AC, Cheung AL, Lasa I, Penadés JR (2005): *SarA is an essential positive regulator of Staphylococcus epidermidis biofilm development.* J Bacteriol, **187**, 2348-2356.

68. Truchado P, Larrosa M, Castro-Ibanez I, Allende A (2015): *Plant food extracts and phytochemicals: their role as quorum sensing inhibitors.* Trends Food Sci Technol, **43**, 189-204.

69. Turovskiy Y, Kashtanov D, Paskhover B, Chikindas ML (2007): *Quorum sensing: fact, fiction, and everything in between.* Adv Appl Microbiol, **62**, 191-234.

70. Vadakkan K, Choudhury AA, Gunasekaran R, Hemapriya J, Vijayanand S (2018): *Quorum sensing intervened bacterial signaling: Pursuit of its cognizance and repression.* J Genet Eng Biotechnol, **16**, 239-252.

71. Van Houdt R, Aertsen A, Jansen A, Quintana AL, Michiels CW (2004): *Biofilm formation and cell-to-cell signaling in gram-negative bacteriaisolated from a food processing environment.* J Appl Microbiol, **96**, 177-184.

72. Viana ES, Campos MEM, Ponce AR, Mantovani HC, Vanetti MCD (2009): *Biofilm formation and acyl homoserine lactone production in Hafnia alveiisolated from raw milk*. Biol Res, **42**, 427-436.

73. Vivas J, Padilla D, Real F, Bravo J, Grasso V, Aacosta F (2008): *Influence of environmental conditions on biofilm formation by Hafnia alvei strains.* Vet Microbiol, **129**, 150-155.

74. Williams P (2007): *Quorum sensing, communication and cross-kingdom signaling in the bacterial world.* Microbiology, **153**, 3923-3938.

VETERİNER HEKİMLER DERNEĞİ DERGİSİ
JOURNAL OF THE TURKISH VETERINARY MEDICAL SOCIETY
ISSN: 0377-6395
e-ISSN: 2651-4214
Dergi ana sayfası:
Journal homepage:
http://dergipark.org.tr/vetheder

Corrected and Republished Abstract

The vascularity of preovulatory follicle: The Colour–Doppler assessment and its predictive value in the early pregnancy outcome in Arabian Mares

Zeynep Günay Uçmak [1,a], İbrahim Kurban [2,b], Melih Uçmak [1,c*]

[1] University of Istanbul-Cerrahpasa, Faculty of Veterinary Medicine, Department of Obstetrics and Gyneacology, Istanbul, Turkey
[2] University of Istanbul- Cerrahpasa,Vocational School of Veterinary Medicine, Istanbul, Turkey
ORCID: 0000-0003-2530-1291 [a]; 0000-0002-8391-905X [b]; 0000-0002-8688-127X [c]

MAKALE BİLGİSİ / ARTICLE INFORMATION:

Geliş / Received:
09 Ocak 20
09 January 20

Kabul / Accepted:
25 Nisan 20
25 April 20

Yayın /Published:
15 Haziran 20
15 June 20

Düzeltme/Corrected:
11 Eylül 20
11 September 20

Anahtar Sözcükler:
Gebelik
Kısrak
Preovulatör folikül
Vaskülarizasyon

Keywords:
Mare
Pregnancy
Preovulatory follicle
Vascularization

ABSTRACT:

The aim of this study is to determine the relationship between the amount of vascularization in the preovulatory follicle wall and pregnancy establisment with colour Doppler ultrasonography. Colour Doppler ultrasonography images from 26 Arabian mares in breeding season were evaluated in the study. Mares no abnormalities in the reproductive system and mild-manner mares were handled. Preovulatory follicle (>35mm) was monitored twice in a day by transrectal B-mode ultrasonography and colour Doppler ultrasonography until the ovulation day. Follicular vascularization images which were incorporated into the study, were monitored 18 hours before the ovulatio Also amount of pixels in colour Doppler images were evaluated with computer-based image analysis program. The mares were mated naturally with a stallion. Pregnancy diagnosis was performed by ultrasonography on day 14 to day 30 after mating. As a result of ultrasonography examination, mares were divided into two groups as pregnant (n=13) and non-pregnant (n=13). The statistical difference between the amount of vascularization in the preovulatory follicle wall of pregnant mares and the amount of vascularization in the preovulatory follicle wall of non-pregnant mares was compared by t-test. As a result of the study, there were no significant differences between pregnant and non-pregnant mares in terms of area, volume and intensity units of coloured pixels in the preovulatory follicle wall (P> 0.05). In conclusion, it was observed that the quantative evaluation of the colour Doppler images of the preovulatory follicle wall in mares in the breeding season cannot be used to estimate the early pregnancy outcome.

Preovulatör folikülün vaskülarizasyonu: Arap kısraklarda erken gebelik sonuçlarında renkli Doppler değerlendirmesi ve öngörülen değeri

ÖZET:

Sunulan makalede renkli Doppler ultrasonografi ile preovulatör folikül çeperindeki damarlaşma düzeyinin gebelik oluşumuyla ilişkisinin belirlenmesi amaçlandı. Üreme sezonundaki sağlıklı 26 adet Arap kısraktan alınan renkli Doppler ultrasonografi görüntüleri değerlendirildi. Sakin mizaçlı ve reprodüktif açıdan problemi olmayan kısraklar seçildi. Preovulatör folikül (>35mm), transrektal B-mod ultrasonografi ve renkli Doppler ultrasonografi ile ovulasyon gününe dek günde iki kere izlendi. Çalışmaya dahil edilen foliküler vaskülarizasyon görüntüleri yumurtlamadan 18 saat önce izlendi. Ayrıca, renkli Doppler üzerinden akım görülen alanlardaki piksel sayısı bilgisayar destekli görüntü analiz programı ile değerlendirildi. Kısraklar, bir aygırla doğal aşım yoluyla çiftleştirildi. Aşımı izleyen 14-30 günlerde ultrasonografi ile gebelik muayeneleri yapıldı. Bunun neticesinde kısraklar gebe (n=13) ve gebe olmayan (n=13) olmak üzere iki gruba ayrıldı. Gebe kısraklara ait preovulatör folikül çeperindeki damarlaşma miktarı ile gebe olmayan kısraklara ait preovulatör folikül çeperindeki damarlaşma miktarı arasındaki istatistiksel fark t-testi ile karşılaştırıldı. Çalışmanın sonucunda, preovulatör folikül çeperindeki renkli piksellere ait alan, hacim ve yoğunluk düzeylerinin gebe ve gebe olmayan kısraklarda farklı olmadığı görüldü (P>0,05). Sonuç olarak, üreme sezonundaki kısraklarda preovulatör folikül duvarına ait renkli Doppler görüntülerinin kantitatif değerlendirilmesinin erken gebelik sonucunu tahmin etmek amacıyla kullanılamayacağı görüldü.

Access to the original article: "Uçmak ZG, Kurban İ, Uçmak M: The vascularity of preovulatory follicle: The colour–Doppler assessment and its predictive value in the early pregnancy outcome in Arabian Mares. *Veteriner Hekimler Dernegi Dergisi*, 91(2): 104-109, 2020, DOI: 10.33188/vetheder.672255"

* *Sorumlu Yazar e-posta adresi / Corresponding Author e-mail address:* melih.ucmak@istanbul.edu.tr

VETERİNER HEKİMLER DERNEĞİ DERGİSİ
YAYIM KOŞULLARI

1. Dergi, Veteriner Hekimler Derneğinin yayın organı olup, yılda iki kez (Ocak ve Haziran) yayımlanır. Derginin kısaltılmış resmi adı "**Vet Hekim Der Derg**" olup derginin yayım dili Türkçe ve İngilizcedir.

2. Dergide, tamamı daha önce başka bir yerde yayımlanmamış güncel konulara ilişkin özgün bilimsel araştırmalar, derlemeler, olgu sunumları ve kısa bilimsel çalışmalar yayımlanır. **Derleme niteliğindeki çalışmalar, ilgili bilim insanlarından davet usulü ile talep edilir.**

3. Dergide yayımlanmak üzere gönderilen makaleler Editörler Kurulunca değerlendirilerek konu ile ilgili hakemlere gönderilir. Hakemlerin görüşü alındıktan sonra önerilen değişiklik ve düzeltmelerin yapılması için makale yazara/yazarlarına geri gönderilir; düzeltmeler yapıldıktan sonra yayımlanır. Hakemlerin önerileri dışında makalelerde sonradan ekleme ve çıkartma yapılamaz. Yayımlanması uygun bulunmayan makalelerle ilgili herhangi bir iade yapılmaz.

4. Dergide yayımlanması istenen yazılar uygun formata göre hazırlanmış ve dergi web sitesinde erişime sunulan "şablon"a göre düzenlenmelidir. Yazar; Dergide yayımlanması istenen yazıyı ilgili şablonu kullanarak uygun formata getirdikten sonra Dergipark sistemini kullanarak 3 dosya yükleyecektir. Bu dosyalar:

(1) Mevcut şablon uygun şekilde doldurularak elde edilen Word dosyası (tablo, şekil, kaynaklar dahil).

(2) Mevcut şablondan "yazar isimleri, kurum adları, sorumlu yazar iletişim bilgileri" vs. silinerek elde edilen Word dosyası (tablo, şekil, kaynaklar dahil)

(3) "Yazar isimleri, kurum adları, sorumlu yazar iletişim bilgileri" olmayan versiyonun pdf dosyasına çevrilmiş hali.

- ÖNEMLİ BİLGİ: Makaleyi sisteme yükleme adımları sırasında ulusal dizin ve atıf takibi için makalede yer alan kaynakçanın "ayrıca" bir kez daha girilmesi istenmektedir. Dolayısıyla hem ana metin hem de ileriki adımlarda belirtilen kaynaklar kısmına giriş yapılmalıdır. Sistemde bu kısım için kaynakça sıra numarası "olmaksızın" her bir kaynakçayı "enter" tuşuna basarak ayırmalı (her bir kaynakça arasında bir satır olacak şekilde) ve belirtilen alana kopyalamanız gerekmektedir). Sisteme yüklenecek makale, sistemde "Makale Dosyaları" kısmından yüklenecek olup, "Dosya Tipi"ni tam metin olarak seçtikten sonra hemen altındaki seçenekten dosya başlığı kısmına "makalenizin adını" yazmanız gerekmektedir. Bu aşamada "Dosya başlığını metinsel olarak girmek istiyorum"u tıklatmayı unutmayınız. (Bu şekilde sisteme "**makale kısa adı- yazarlı.docx**"; "**makale kısa adı-yazarsız.docx**"; "**makale kısa adı-yazarsız.pdf**" şeklinde üç dosya yüklemeniz beklenmektedir. Lütfen sisteme yüklediğiniz dosyaların adını verirken kendi adınızı veya kurumunuzu belli edecek isim kullanmayınız.)

5. Yazıların tamamı, şekil ve tablolar dâhil olmak üzere orijinal bilimsel araştırmalarda ve derlemelerde **15**, kısa bilimsel çalışmalarda **10**, olgu sunumlarında **8** sayfayı geçmemelidir.

6. Makaleler; **başlık, yazar/yazarların isimleri, Türkçe öz ve anahtar sözcükler, yabancı dilde başlık, yabancı dilde öz ve anahtar sözcükler, giriş, gereç ve yöntem, bulgular, tartışma ve sonuç, teşekkür ve kaynaklar** sırası ile hazırlanmalıdır. Anadili Türkçe olmayan iletişim yazarının çalışmasında Türkçe özet şartı aranmaz. Sosyal bilimler alanındaki çalışmalar ile sağlık ve fen bilimleri alanındaki kısa bilimsel çalışmalarda, giriş, gereç ve yöntem, bulgular, tartışma ve sonuç bölümlemesi yapılmayabilir.

7. Makalenin başlığı kısa ve açık olmalı; ilk sözcüğün başlangıcı büyük, diğerleri küçük harflerle olacak şekilde, yazılmalıdır ("Köpek ve kedilerde uterus patolojileri" gibi). Varsa çalışmaya ilişkin açıklama dipnot işareti ile gösterilmelidir.

8. Yazar/yazarların, ad ve soyadları makale başlığının altına yazılmalıdır; adresleri ve unvanları ilk sayfada dipnot şeklinde belirtilmelidir.

9. Öz, makalenin önemli noktalarını içerecek tarzda kısa ve açık olmalıdır. Türkçe Öz, en az **150**, en fazla **250** sözcük olmalıdır. Anahtar sözcükler **MeSH** (Medical Subject Headings) terimlerine uygunluk açısından Türkiye Bilim Terimleri'nden seçilmeli ve en az **3**, en fazla **5** adet olacak şekilde alfabetik olarak sıralanmalıdır. Yabancı dilde Öz (Abstract, Zusammenfassung, Resume), en az **150**, en fazla **300** sözcük olmalıdır. Yabancı dilde anahtar sözcükler MeSH terimlerine uygun olmalı ve en az **3**, en fazla **5** adet olacak şekilde alfabetik olarak sıralanmalıdır.

10. Giriş bölümünde, çalışma ile doğrudan ilgili kısa literatür bilgisi ve çalışmanın orjinalliği ile ilgili bilgi verildikten sonra, son paragrafta çalışmanın amacı vurgulanmalıdır. Bu bölüm 2 sayfayı geçmemelidir.

11. Gereç ve Yöntem, gereksiz ayrıntıya girilmeden, öz ve anlaşılır biçimde yazılmalıdır. Etik kurul izni gerekli ise mutlak suretle belirtilmelidir. (Kurum, Tarih, sayı numarası ile birlikte)

12. İstatistik analiz sonuçlarının gösteriminde P değerleri tam olarak raporlanmalıdır. P değeri için virgülden sonra 3 hane, tanımlayıcı istatistiklerin raporlanmasında ise virgülden sonra 2 hane yeterlidir. Anadili Türkçe olan makaleler için ondalık ayracı olarka vürgül (,), İngilizce olanlar için ise nokta (.) kullanılmalıdır.

13. Bulgular bölümünde, veriler kısa bir şekilde açıklanmalıdır. Tablolarda verilen bulguların metinde tekrarlanmasından kaçınılmalıdır.

14. Bölüm başlıkları sola yaslı biçimde, kalın yazı karakteri ile sözcüklerin ilk harfleri büyük olacak şekilde yazılmalıdır. İkinci derecedeki alt başlıklar sola dayalı olarak kalın yazı karakteri ile sadece ilk harf büyük olacak şekilde küçük harflerle yazılmalıdır. Üçüncü derecedeki başlıklar ise paragraf başında yer almalı ve italik olarak sadece ilk harf büyük olacak şekilde küçük harflerle yazılmalıdır (Bkz. Şablon).

15. Tablo ve şekil başlıkları, Türkçe ve yabancı dilde dergi formatı dikkate alınarak yazılmalıdır. Başlıkların tabloyu yeterli düzeyde açıklayıcı olmasına özen gösterilmelidir. Tablolarda dikey çizgi kullanımından kaçınılmalıdır. Yatay çizgiler ise gerektiğinde yalnızca tablonun ilk satırı ve son satırından sonra kullanılabilir.

16. Yazarlar her bir bilimsel kısaltmanın açılımını metinde ilk geçtiği yerde açıklamalıdırlar. Latince cins ve tür isimleri italik yazı tipi ile yazılmalıdır. Tüm ölçüler SI (Systeme Internationale)'ye göre verilmelidir.

17. Tartışma ve Sonuç bölümünde, veriler literatür bilgilerinin ışığında tartışılmalı ve yorumlanmalıdır.
18. Kaynaklar bölümünde, bibliyografik bilgi, alfabetik sıra ile verilmeli, çok yazarlı çalışmalarda yazar adlarının arasına sadece virgül konulmalıdır. Kaynaklar alfabetik ve kronolojik dizin dikkate alınarak sıralanmalı ve numaralandırılmalıdır. Kaynak yazımında yazar adları kalın, konu başlığı italik yazı tipi ile yazılmalıdır. Dergi adlarının kısaltması kullanılmalı ve dergi adı kısaltılmasında "Periodical Title Abbreviations: By Abbreviation"ın son baskısı esas alınmalıdır. Dergi kısaltması içerisinde nokta (.) kullanılmamalıdır. Metin içerisinde kaynak, parantez içerisine alınmış sıra numarası ile belirtilmelidir. Metin içerisinde kaynak kullanımında, aynı konuyu bildiren 1'den çok kaynak varsa bunlar sıraları itibariyle küçükten büyüğe doğru sıralanmalı ve sayıları 5'i geçmemelidir. Kaynakta belirtilen yazar isimlerinin tamamı verilmeli, kaynakçada et. al. veya ve ark. şeklinde kısaltma kullanılmamalıdır. et al veya ve ark yalnızca metin içerisindeki kaynak gösteriminde ikiden fazla yazar olması durumunda kullanılabilir.
Metin içerisinde örnek kaynak gösterimi: "Lizin amino asiti yumurta kütlesini oluşturan protein sentezi ile doğrudan ilişkilidir (1). Lizin amino asitinin rasyonda doğru olarak dengelenmesi kanatlılarda yemden yararlanma oranını artırır. Aynı zamanda yumurta kalitesi ile de yakından ilişkilidir (2). Smiricky-Tjardes ve ark (3), lizin sülfat içerisinde bulunan kurumuş mikroorganizmaların hayvanların performansını olumsuz etkileyebileceği fikrini savunmuştur."
Yukarıda verilen örneğe ilişkin uygun kaynak gösterimi:
1. Bailleul PJD, Bernier J, Milgen JV(2000): *The utilization of prediction models to optimize farm animal production systems: the case of a growing pig model.* 379–392 In: Mc Namara JP, France J, Beever DE (Eds.), Modelling Nutrient Utilization in Farm Animals, CAB International, Wallingford.

2. Özpınar AA(1997): *The variations in blood ionized calcium, sodium and potassium concentrations with age and laying cycle and the relationships of these ions with eggshell quality.* Arch Geflügelk, **61(6)**,287-290.
3. Smiricky-Tjardes MR, Mavromichalıs I, Albin DM (2004): *Bioefficacy of L-lysine sulfate compared with feed-grade L-lysine HCl in young pigs.* J Anim Sci, **82**, 2610–2614.

Çeşitli kaynak gösterimlerine örnekler:

Kaynak, bilimsel çalışma ise:

Kasperowicz A, Michalowski T (2002): *Assessment of the fructanolytic activities in the rumen bacterium Treponema saccharophilum strain S*. J Appl Microbiol, **92**, 140–146.
Christy RC, Thirunavukkarasu, M (2006): *Emerging importance of animal health economics: A note*. Turk J Vet Anim Sci, **2(3),** 113–117.

Kaynak, kitap ise:

Falconer DS (1960): *Introduction to Quantitative Genetics*. Oliver and Boyd Ltd, Edinburgh

Kaynak kitaptan bir bölüm ise:

Bahk J, Marth EH (1990): *Listeriosis and Listeria monocytogenes.* 248-256. In: DO Cliver (Ed), Foodborne Diseases. Academic Press, San Diego.

Kaynak internette yer alıyor ise erişim tarihi ile birlikte yazılmalıdır;

Otte MJ, Chilonda P (2007): *Animal Health Economics: An introduction.* Erişim: http://www.fao.org/ag/againfo/resources/en/publications/agapubs/pproc01.pdf. Erişim Tarihi: 11.05.2008

19. Yazışma adresi, çalışmada şablon içerisinde verilen kısımda yer almalıdır. Çok yazarlı çalışmalarda yazarlardan sadece birinin adı, yazışma adresi olarak belirtilmelidir.
20. Veteriner Hekimler Derneği Dergisinde yayımlanacak olan, hayvan deneylerine dayalı bilimsel çalışmalarda "Etik Kurul Onayı Alınmıştır" ifadesi aranır.
21. Araştırmaya konu olan maddelerin ve ürünlerin ticari adları kullanılmamalıdır.
22. Dergide yayınlanan her türlü makalenin sorumluluğu yazarlarına aittir.
23. Gönderilen makaleler geliş tarihine göre hakeme gönderilir ve yayın kurulunun aldığı kararla yayımlanır. Makale yayımlandıktan sonra yayın hakkı dergiye aittir.

Veteriner Hekimler Derneği Dergisi

Journal of the Turkish Veterinary Medical Society

ISSN: 0377-6395
e-ISSN: 2651-4214

http://dergipark.org.tr/vetheder

YAYIN HAKKI DEVRİ FORMU

Bu formu imzalayan yazarlar, basıldığı takdirde, gönderdikleri ve aşağıda başlığı olan yazının içeriği ile ilgili hiçbir konuda Veteriner Hekimler Derneği Dergisinin sorumlu olmadığını kabul ederler.

Makale Başlığı:

.....
.....
.....
.....

Bu formu imzalamakla, yazıları basılan yazarlar;

- yazının, İçerdiği verilerin, resimlerin ve çizimlerin orijinal olduğunu,
- verileri ve yayın üzerinde yazar(lar) dışında başka kişi ve kurumların hak sahibi olmadığını,
- başka bir dergiye gönderilmemiş olduğunu,
- daha önce yayınlanmadığını, veya
- kısmen de olsa, veriler daha önce yayınlandıysa bunların Veteriner Hekimler Derneği Dergisi'nde yayınlanması için gerekli iznin alınıp bu forma eklendiğini kabul ederler.

Yazarların saklı hakları şunlardır:

- Yayın hakkı (copyright) dışında kalan, hasta haklan da dâhil olmak üzere içeriğe ait tüm mülkiyet haklan,
- Yazının içeriğinin tamamı veya kısımlarını, kendilerine ait başka çalışmalarda karşılıksız kullanma hakkı,

Bu yazının yayınlanmasına dair sorumlulukları kabul ediyor ve imzalıyoruz. Böylece, yazının yayın hakkını (copyright) Veteriner Hekimler Derneği Dergisine devrediyoruz.

Yazarlar ve İmza

Ad	Soyad	İmza	Tarih

Bu form eksiksiz olarak doldurulup yayın başvurusu sırasında makale dosyalarına eklenmelidir.

Veteriner Hekimler Derneği Dergisi

Journal of the Turkish Veterinary Medical Society

ISSN: 0377-6395
e-ISSN: 2651-4214

http://dergipark.org.tr/vetheder

COPYRIGHT RELEASE FORM

Manuscript title:

.....
.....
.....
.....

On the behalf of all authors, we warrant that;

i) the manuscript submitted is our own original work,
ii) all authors participated in the work in a substantive way and are prepared to take public responsibility for the work
ii)all authors have seen and approved the manuscript as submitted,
iii) the manuscript has not been published and is not being submitted or considered for publication elsewhere,
iv) the text, illustrations and any other materials included in the manuscript do not infringe upon any existing copyright or other rights of anyone
v) we transfer all financial rights, especially processing, reproduction, representation, printing, distribution and online transmittal to Veteriner Hekimler Derneği with no limitation whatsover.

Name and Surname	Date	Signiture

Please submit this document in addition to your manuscript via Dergipark submission system

Veteriner Hekimler Derneği Dergisi
Journal of The Turkish Veterinary Medical Society

ETİK BEYAN FORMU / ETHICAL STATEMENT FORM

Ethic Decleration (EN)

In this thesis / research article / case case presentation / invited review article, which was prepared for Veteriner Hekimler Derneği Dergisi *(Journal of Turkish Veterinary Medical Sciences)* ;

- I/We have obtained the data, information and documents in the framework of academic and ethical rules,
- I/We provide all the information, documents, evaluations and results in accordance with scientific ethics and moral codes,
- I/We referred to all of the articles I used in this study with appropriate references,
- I/We have not made any changes to the data used and the results,
- The information and findings specified in this study are original.

I/We declare above mentioned issues and accept all rights losses that may arise against me.

Name of The Author(s) (Title)	Date	Signiture

Etik Kurul Raporu & Beyanı: Araştırmada hayvan kullanılmış ise araştırma etik kurul tarafından onaylanmalı ilgili belge çevrimiçi makale değerlendirme sistemine yüklenmelidir. Hayvan kullanılmayan veri toplanarak gerçekleştirilmiş çalışmalar için verileri, bilgileri ve dokümanları akademik ve etik kurallar çerçevesinde elde ettiğine ilişkin ilgili kurum&kuruluşlardan alınmış izin belgesi veya etik beyan formunun doldurulması ve sisteme yüklenmesi gerekmektedir.

Ethics Committee Report & Statement: If animals were used in the study, the research should be approved by the ethics committee and the relevant document should be uploaded to the online manuscript evaluation system. For studies carried out by collecting data without animals, it is necessary to fill in the permission document or ethical declaration form obtained from the relevant institutions and organizations that they have obtained the data, information and documents within the framework of academic and ethical rules.

Veteriner Hekimler Derneği Dergisi
Journal of The Turkish Veterinary Medical Society

ETİK BEYAN FORMU / ETHICAL STATEMENT FORM

ETİK BEYANI (TR)

Veteirner Hekimler Derneği Dergisi'nde yayınlanmak üzere hazırladığım bu tez/araştırma makalesi/olgu vaka sunumu/davetli derleme çalışmasında;

- Sunduğum verileri, bilgileri ve dokümanları akademik ve etik kurallar çerçevesinde elde ettiğimi/ettiğimizi,
- Tüm bilgi, belge, değerlendirme ve sonuçları bilimsel etik ve ahlak kurallarına uygun olarak sunduğumu/sunduğumuzu,
- Çalışmada yararlandığım eserlerin tümüne uygun atıfta bulunarak kaynak gösterdiğimi/gösterdiğimizi,
- Kullanılan verilerde ve ortaya çıkan sonuçlarda herhangi bir değişiklik yapmadığımı/yapmadığımızı,
- Bu çalışmada belirtilen bilgilerin ve bulguların özgün olduğunu,

bildirir, aksi bir durumda aleyhime doğabilecek tüm hak kayıplarını kabullendiğimi beyan ederim/ederiz.

Yazarların Adı Soyadı (Ünvanı)	Tarih	İmza

Etik Kurul Raporu & Beyanı: Araştırmada hayvan kullanılmış ise araştırma etik kurul tarafından onaylanmalı ilgili belge çevrimiçi makale değerlendirme sistemine yüklenmelidir. Hayvan kullanılmayan veri toplanarak gerçekleştirilmiş çalışmalar için verileri, bilgileri ve dokümanları akademik ve etik kurallar çerçevesinde elde ettiğine ilişkin ilgili kurum&kuruluşlardan alınmış izin belgesi veya etik beyan formunun doldurulması ve sisteme yüklenmesi gerekmektedir.

Ethics Committee Report & Statement: If animals were used in the study, the research should be approved by the ethics committee and the relevant document should be uploaded to the online manuscript evaluation system. For studies carried out by collecting data without animals, it is necessary to fill in the permission document or ethical declaration form obtained from the relevant institutions and organizations that they have obtained the data, information and documents within the framework of academic and ethical rules.

Veteriner Hekimler Derneği Dergisi

Journal of the Turkish Veterinary Medical Society

Cilt / Volume : 92 - Sayı / Issue : 1 - Yıl / Year : 2021

İÇİNDEKİLER / *CONTENTS*