online

7TH INTERNATIONAL CONGRESS ON VETERINARY AND ANIMAL SCIENCES

20–22 October 2022

www.icvas.gen.tr

ANT ACADEMY

online

7TH INTERNATIONAL CONGRESS ON VETERINARY AND ANIMAL SCIENCES

20–22 October 2022

CONGRESS BOOK

E - ISBN: 978-605 - 73778-4-5

Editors

Assoc. Prof. Dr. Mustafa YİPEL
Assoc. Prof. Dr. Hüsamettin EKİCİ

www.icvas.gen.tr

ANT ACADEMY

THIS PAGE INTENTIONALLY LEFT BLANK

CONTENT

Dear Colleagues,

We are pleased to announce that the 7th International Congress on Veterinary and Animal Sciences (ICVAS 2022) has been held online on 20–22 October 2022. This event has been brought together researchers to present their research innovations, to share ideas and knowledge, and discuss future perspectives of Veterinary Medicine and Animal Sciences.

In the congress program, clinical sciences such as surgery, internal diseases, obstetrics and gynecology, reproduction; preclinical sciences such as pathology, parasitology, microbiology, fundamental sciences such as biochemistry, physiology, and histology and embryology as well as zootechnics, animal nutrition, food hygiene, technology and other relevant topics presentations have been involved in.

We would like to thank all participants, collaborators, audiences, invited speakers, scientific committee members of the 7th ICVAS 2022 congress where the latest scientific findings to address current and future challenges have been presented and discussed.

With our best regards.

Sincerely,

Assoc. Prof. Dr. Hüsamettin EKİCİ

Kırıkkale University, Türkiye

Assoc. Prof. Dr. Mustafa YİPEL

Hatay Mustafa Kemal University, Türkiye

ORGANIZING COMMITTEE

Heads of Organizing Committee

Assoc. Prof. Dr. Hüsamettin EKİCİ DVM, PhD, Kırıkkale University, Faculty of Veterinary Medicine, TÜRKİYE

Assoc. Prof. Dr. Mustafa YİPEL DVM, PhD, Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, TÜRKİYE

Members of Organizing Committee

Prof. Dr. Narcisa MEDERLE, DVM, PhD, Banat Uni. of Agr. Sci. and Veterinary Med. of Timisoara, ROMANİA

Assoc. Prof. Dr. Ishraga Gaafar IBRAHIM, Central Vet. Research Lab. SUDAN

Assoc. Prof. Dr. Shahram SAGHAEI, DVM, PhD, Orumieh Azad University, IRAN

Assoc. Prof. Dr. Chuleui JUNG, Andong National University, KORE

Sultan Nurhan AYIKOL, DVM, Ph.D student, Ankara University, Faculty of Veterinary Medicine, TÜRKİYE

Leila KEBAL, Ph.D student, University of Mostaganem, Faculty of Nature and Life Sciences, ALGERIA

Marin Ana MARIA, DVM, PhD student, Banat Uni. of Agr. Sci. and Veterinary Med. of Timisoara, ROMANIA

Moraru FLORINA, DVM, PhD student, Banat Uni. of Agr. Sci. and Veterinary Med. of Timisoara, ROMANIA

Beren KABASAKAL, student, Kırıkkale University, Faculty of Veterinary Medicine, TURKİYE

Palagan Senopati SEWOYO, DVM, M.V.Sc. student, Faculty of Veterinary Medicine, Udayana University, INDONESIA

Golara KOR, Ph.D student, University of South Bohemia, Faculty of Fisheries and Protection of Waters, CZECH REPUBLIC

SCIENTIFIC COMMITTEE

Prof. Dr. Aleš GREGORC, University of Maribor, SLOVENIA

Prof. Dr. Aneliya MILANOVA, Trakia University, Stara Zagora, BULGARIA

Prof. Dr. Attila KARSİ, Mississippi State University, USA

Prof. Dr. Ebru YILDIRIM, Kırıkkale University, TÜRKİYE

Prof. Dr. Ender YARSAN, Ankara University, TÜRKİYE

Prof. Dr. Fusun TEMAMOGULLARI, Harran University, TÜRKİYE

Prof. Dr. Herman VIOREL, Faculty of Veterinary Medicine BUASVM Timisoara, ROMANIA

Prof. Dr. Koula DOUKANI, University of Ibn Khaldoun, ALGERIA

Prof. Dr. Kader YILDIZ, Kırıkkale University, TÜRKİYE

Prof. Dr. Ki-Young KIM, Kyung Hee University, KOREA

Prof. Dr. Levent ALTINTAŞ, Ankara University, TÜRKİYE

Prof. Dr. Luigi INTORRE, University of Pisa, ITALY

Prof. Dr. Marcos PÉREZ-LÓPEZ, Extremadura University, SPAIN

Prof. Dr. Meryem EREN, Erciyes, TÜRKİYE

Prof. Dr. Miyase ÇINAR, Kırıkkale University, TÜRKİYE

Prof. Dr. Murat YUKSEL, Hatay Mustafa Kemal University, TÜRKİYE

Prof. Dr. Narcisa MEDERLE, Banat Uni. of Agr. Sci. and Vet. Med. of Timisoara, ROMANIA

Prof. Dr. Pınar DEMİR, Kafkas University, TÜRKİYE

Prof. Dr. Sevil Dincer ISOGLU, Abdullah Gul University, TÜRKİYE

Prof. Dr. Siyami KARAHAN, Kırıkkale University, TÜRKİYE

Prof. Dr. Şule Yurdagül ÖZSOY, Aydın Adnan Menderes University, TÜRKİYE

Prof. Dr. Zeynep PEKCAN, Kırıkkale University, TÜRKİYE

Assoc. Prof. Dr. Adnan AYAN, Van Yuzuncu Yil University, TÜRKİYE

Assoc. Prof.Dr. Ali SOLEIMANZADEH, Urmia University, IRAN

Assoc. Prof. Dr. Andreja PREVENDAR, Crnic University of Zagreb, CROATIA

Assoc. Prof. Dr. Begüm YURDAKÖK-DİKMEN, Ankara University, TÜRKİYE

Assoc. Prof. Dr. Behnam ROSTAMI, University of Zanjan, IRAN

Assoc. Prof. Damla ARSLAN ACARÖZ, Afyon Kocatepe University, TÜRKİYE

Assoc. Prof. Dilek DÜLGER, Karabük University, TÜRKİYE

Assoc. Prof Dr. Fulya ALTINOK YİPEL, Hatay Mustafa Kemal University, TÜRKİYE

Assoc. Prof Dr. Hidayet TUTUN, Burdur Mehmet Akif Ersoy University, TÜRKİYE

Assoc. Prof.Dr. Ibrahim KUÇUKASLAN, Dicle University, TÜRKİYE

Assoc. Prof. İbrahim Ozan TEKELİ, Hatay Mustafa Kemal University, TÜRKİYE

Assoc. Prof. Dr. Kıvılcım SONMEZ, İstanbul University, TÜRKİYE

Assoc. Prof. Dr. Latif Emrah YANMAZ, Atatürk University, TÜRKİYE

Assoc. Prof. Dr. Mehmet CENGİZ, Muğla Sıtkı Koçman University, TÜRKİYE

Assoc. Prof. Mehmet GÜVENÇ, Hatay Mustafa Kemal University, TÜRKİYE

Assoc. Prof. Dr. Mian Muhammad AWAIS, Bahauddin Zakariya University, PAKISTAN

Assoc. Prof. Dr. Mokhtar BENHANIFIA, University Mustapha Stambouli, ALGERIA

Assoc. Prof. Dr. Nilay SEYİDOĞLU, Tekirdağ Namık Kemal University, TÜRKİYE

Assoc. Prof. Dr. Noureddine DJEBLI, University of TÜRKIYE, ALGERIA

Assoc. Prof. Dr. Sami GOKPINAR, Kırıkkale University, TÜRKİYE

Assoc. Prof. Dr. Şeyda CENGİZ, Muğla Sıtkı Koçman University, MUĞLA

Assoc. Prof. Dr. Şerife TÜTÜNCÜ, Ondokuz Mayıs University, TÜRKİYE

Assoc. Prof. Dr. Şinasi AŞKAR, Cankiri Karatekin University, TÜRKİYE

Assoc. Prof. Dr. Ulaş ACARÖZ, Afyon Kocatepe University, TÜRKİYE

Assoc. Prof.Dr. Yuksel AGCA, University of Missouri, USA

Assist. Prof. Dr. Abdur RAHMAN Sial,University of Veterinary &Animal Sciences, PAKISTAN

Assist. Prof Dr. Ahmad ALI, University of Mumbai, INDIA

Assist. Prof Dr Farnaz MALEKIFARD, Urmia University, IRAN

Assist. Prof. Dr. Fatgzim LATIFI, University of Prishtina, Pristina, KOSOVA

Assist. Prof Dr Firas ALALI, University of Kerbala, IRAQ

Assist. Prof. Dr. Mahendra Pratap Singh TOMAR, Sri Venkateswara Veterinary Univ. INDIA

Assist. Prof. Dr. Yaşar ŞAHİN, Kırıkkale University, TÜRKİYE

Dr. Abdul Shakoor CHAUDHRY, Newcastle University, Newcastle upon Tyne, UK

Dr. İbrahim ABBAS, Faculty of Veterinary Medicine, Mansoura Univ., Mansoura, EGYPT

Dr. Seda EKİCİ, Veterinary Control Central Research Institute, TÜRKİYE

Dr. Sedat SEVİN, Ankara University, TÜRKİYE

INVITED SPEAKERS

Prof. Dr. Kerem URAL

Shell Deformation: Intestinal Permability Targetted Natural Therapy in Veterinary Internal Medicine

Adnan Menderes University, Faculty of Veterinary Medicine, Dep. of Internal Medicine, Aydın, Türkiye

Assoc. Prof. Dr. Begüm YURDAKÖK-DİKMEN

Veterinary forensic toxicology

Ankara University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Ankara, Türkiye

Prof. Dr. Malgorzata KOTULA BALAK

Telocytes in the male reproductive system

University Center of Veterinary Medicine, Agriculture University in Krakow, POLAND

Prof. Dr. Abdurrahman AKSOY

Pros & Cons of Experimental Laboratory Animal Studies

Ondokuz Mayıs University, Director of Experimental Animal Application and Research Center, Samsun, Türkiye

Dr. Cristina Botías TALAMANTES

Understanding The Effect of Global Change Stressors on Bee Health

Universidad de Alcalá, Department of Life Sciences, Madrid, Spain

SCIENTIFIC PROGRAM

7th International Congress on Veterinary and Animal Sciences (ICVAS)-Online

20 October, Thursday, 2022

09:45	*OPENNING SPEECH*
10:00	**Chair: Assoc. Prof. Dr. Hasan ERDOĞAN** *Aydın Adnan MenderesUniversity, Faculty of Veterinary Medicine, Department of Internal Medicine, Aydın, Türkiye*
10:00-10:20	**Variation of Intraocular Pressure Measurements Using Tono-Pen Vet® and TonoVet® Tonometry in Calves** **Artina PRASTIWI Mümin Gökhan ŞENOCAK** *Ataturk University, Faculty of Veterinary Medicine, Department of Veterinary Surgery, Erzurum, Turkiye*
10:25-10:45	**Successful treatment of portosystemic shunt in two dogs** **Zeynep BİLGEN Büşra KİBAR KURT** *Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Surgery Department, Aydın, Türkiye*
10:50-11:20	**Prevalence and Molecular Characterization of *Giardia duodenalis* in Lambs in Hakkari Region** **Erdal BİNGÖL[1] Abdulalim AYDIN[1] Yaşar GÖZ[2] Adnan AYAN[3] Özlem ORUNÇ KILINÇ[4] Özgür Yaşar ÇELİK[5] Fatma ERTAŞ OĞUZ[6] Gürkan AKYILDIZ[7] Burçak ASLAN ÇELİK[8] Alp ATAY[1] Özge OKTAY AYAN[9]** *[1]Çölemerik Vocational School, Hakkari University, Hakkari, Türkiye* *[2]Department of Nutrition and Dietetics, Faculty of Health Sciences, Van Yuzuncu Yil University, Van, Türkiye* *[3]Department of Genetics, Faculty of Veterinary Medicine, Van Yuzuncu Yil University, Van, Türkiye* *[4]Özalp Vocational School, Van Yuzuncu Yil University, Van, Türkiye* *[5]Department of Internal Medicine, Faculty of Veterinary Medicine, Siirt*

University, Siirt, Türkiye

[6]Department of Medical Services and Techniques, Tuzluca Vocational School, Iğdır University, Iğdır, Türkiye

[7]Faculty of Health Sciences, Marmara University, İstanbul, Türkiye

[8]Department of Parasitology, Faculty of Veterinary Medicine, Siirt University, Siirt, Türkiye

[9]Institute of Health Sciences, Van Yuzuncu Yil University, Van, Türkiye

---

Gut-Heart Axis

11:25-11:45

Cansu BALIKÇI Gamze GÖKÇAY Songül ERDOĞAN Hasan ERDOĞAN Kerem URAL

Aydın Adnan Menderes University, Faculty of Veterinary, Department of İnternal Medicine, Aydın, Türkiye

---

Effect of Fenoksi-2-Metil-2-Propionic Acid (Hepagen®) Treatment on Reproductive Performance in Ewes During the Early and Late Postpartum Period

11:50-12:10

Halef DOĞAN[1] Metehan KUTLU[2]

[1] Tekirdag Namik Kemal University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology, Tekirdag, Türkiye

[2]Adana Metropolitan Municipality, Department of Agriculture and Animal Services, Adana, Türkiye

---

Total Knee Replacement Applications in Veterinary Surgery

12:15-12:35

Ziya YURTAL[1] Kadri KULUALP[2] İbrahim ALAKUŞ[1] Halil ALAKUŞ[1] Ömer KIRGIZ[1] Muhammed Enes ALTUĞ[1]

[1]Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Surgery, Hatay, Türkiye

[2] Dokuz Eylül University, Faculty of Veterinary Medicine, Department of Surgery, İzmir, Türkiye

---

12:40-13:00	**Prevalence and Molecular Characterization of Cryptosporidium spp. in Lambs in Hakkari Region** **Erdal BİNGÖL[1] Abdulalim AYDIN[1] Yaşar GÖZ[2] Adnan AYAN[3] Özlem ORUNÇ KILINÇ[4] Özgür Yaşar ÇELİK[5] Fatma ERTAŞ OĞUZ[6] Gürkan AKYILDIZ[7] Burçak ASLAN ÇELİK[8] Alp ATAY[1] Özge OKTAY AYAN[9]** *[1]Çölemerik Vocational School, Hakkari University, Hakkari, Türkiye* *[2]Department of Nutrition and Dietetics, Faculty of Health Sciences, Van Yuzuncu Yil University, Van, Türkiye* *[3]Department of Genetics, Faculty of Veterinary Medicine, Van Yuzuncu Yil University, Van, Türkiye* *[4]Özalp Vocational School, Van Yuzuncu Yil University, Van, Türkiye* *[5]Department of Internal Medicine, Faculty of Veterinary Medicine, Siirt University, Siirt, Türkiye* *[6]Department of Medical Services and Techniques, Tuzluca Vocational School, Iğdır University, Iğdır, Türkiye* *[7]Faculty of Health Sciences, Marmara University, İstanbul, Türkiye* *[8]Department of Parasitology, Faculty of Veterinary Medicine, Siirt University, Siirt, Türkiye* *[9]Institute of Health Sciences, Van Yuzuncu Yil University, Van, Türkiye*
13:00-14:00	Break
14:00-14:30	**INVITED SPEAKER** **Prof. Dr. Kerem URAL** **Shell Deformation: Intestinal Permability Targetted Natural Therapy in Veterinary Internal Medicine** *Adnan Menderes University, Faculty of Veterinary Medicine, Dep. of Internal Medicine, Aydın, Türkiye*

14:30	**Chair: Assoc. Prof. Dr. Hidayet TUTUN** *Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology Burdur, Türkiye*
14:30-14:50	**Stereotypic Behaviours of Horses and Their Effects on Horse Welfare** **Erva ESER[1] Serkan ERAT[2]** *[1]Kırıkkale University Graduate School of Health Science Department of Animal Breeding and Husbandry and Animal Nutrition, Kırıkkale, Türkiye* *[2]Kırıkkale University Faculty of Veterinary Medicine Department of Animal Breeding and Husbandry, Kırıkkale, Türkiye*
14:55-15:15	**Comparative Sensitivity of Eosin Nigrosin and Propidium Iodide Staining Procedures to Detect Viability of Frozen Thawed Rabbit Sperm** **Niyazi KÜÇÜK** *Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Aydın, Türkiye*
15:20-15:40	**A Pre-Investigation for The Position of Kangal Turkish Shepherd Dog in Turkish Hair Goat Flocks in Burdur Province** **Aykut Asım AKBAŞ[1] Mustafa SAATCI[2]** *[1]Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Animal Science, Burdur, Türkiye* *[2]Muğla Sıtkı Koçman University, Fethiye Faculty of Agriculture, Department of Animal Science, Muğla, Türkiye*
15:45-16:05	**Implementation of HACCP System: A Case Study of a Chicken Luncheon Meat Manufacturing in Türkiye** **İrem KARAASLAN** *Hatay Mustafa Kemal University, Technology and Research and Development Center, Hatay, Türkiye*

16:10-16:30	**The Effect of Addition of Hempseed Oil (*Cannabis Sativa* L.) to Broiler Rations on Performance Parameters** **Mehmet DEMİRCİ** **Şevket EVCİ** *Kırıkkale University, Vocational School, Laboratory and Veterinary Health Department, Kırıkkale, Türkiye*
16:30-17:00	*End*

7th International Congress on Veterinary and Animal Sciences (ICVAS)-Online

21 October, Friday, 2022

10:00-10:30	**INVITED SPEAKER** **Assoc. Prof. Dr. Begüm YURDAKÖK-DİKMEN** **Veterinary forensic toxicology** *Ankara University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Ankara, Türkiye*
10:30	**Chair: Prof. Dr. Füsun TEMMAOĞULLARI** *Harran University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Harran, Türkiye*
10:30-10:50	***Pharmacological Effect of Rheum ribes*** **Ecenur TUNÇKILIÇ[1]** **Hüsamettin EKİCİ[2]** *[1]Kırıkkale University, Graduate School of Health Sciences, Department of Pharmacology and Toxicology Kırıkkale, Türkiye* *[2] Kırıkkale University, Faculty of Veterinary Medicine Department of Pharmacology and Toxicology Kırıkkale, Türkiye*
10:55-11:15	**Prokinetic Effect of Metoclopramid** **Sara Buşra EMİROĞLU** *Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Hatay, Türkiye*

11:20-11:40	**Alternative Methods to Animal Experiments in Toxicity Testing** **Zeyno NUHOĞLU ÖZTÜRK** **Abdurrahman AKSOY** *Ondokuz Mayıs University, Faculty of Veterinary Medicine, Department of Veterinary Pharmacology and Toxicology, Samsun, Türkiye*
11:45-12:05	**Determination of Cd and Hg Levels in Infant Formulas and Supplementary Foods** **Hicran NURAL[1]** **İbrahim Ozan TEKELİ[2]** *[1]Hatay Mustafa Kemal University, Instit. of Health Science, Department of Pharmacology and Toxicology, Hatay, Türkiye* *[2]Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Hatay, Türkiye*
12:10-12:30	**Evaluation of Bisphenol Analogs: In Terms of Veterinary Toxicology** **Aysun İLHAN[1]** **Mustafa YİPEL[2]** *[1] Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Hatay, Türkiye* *[2] Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Hatay, Türkiye*
12:30-13:00	**INVITED SPEAKER** **Prof. Dr. Malgorzata KOTULA BALAK** **Telocytes in the male reproductive system** University Center of Veterinary Med., Agriculture University in Krakow, Poland
13:00-14:30	Break
14:30-15:00	**INVITED SPEAKER** **Prof. Dr. Abdurrahman AKSOY** **Pros & Cons of Experimental Laboratory Animal Studies** *Ondokuz Mayıs University, Director of Experimental Animal Application and Research Center, Samsun, Türkiye*

15:00	**Chair: Assoc Prof. Dr. Mehmet GÜVENÇ** *Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Physiology, Hatay, Türkiye*
15:00-15:20	**Antiviral Effects of Flavonoids** **Dilek Nur BESTIL** **Hamdi UYSAL** *Ankara. University, Faculty of Veterinary, Department of Biochemistry, Ankara, Türkiye*
15:25-15:45	**Methicillin and Vancomycin Resistance Profiles of Staphylococcus aureus strains Isolated from Cow's Mastitic Milk Samples** **Orkun BABACAN** *Balıkesir University, Kepsut Vocational School, Department of Veterinary, Kepsut/Balıkesir City, Türkiye*
15:50-16:05	**Effects of Horse Chestnut Extract (*Aesculus Hippocastanum* L.) on Bone and Calcium Metabolism in Rats Fed a High Protein Diet** **Erten AKBEL[1]** **Abdullah ERYAVUZ[2]** *[1] Usak University, Faculty of Health Scienses, Department of Physiotherapy and rehabilitation, City, Usak, Türkiye* *[2] Afyon Kocatepe University, Faculty of Veterinary, Department of Physiology, Afyonkarahisar, Türkiye*
16:10-16:30	**Investigation of yeast, mold detection and aflatoxin types by HPLC method in raw naturel nuts in Sakarya province and near location** **Samet KORKMAZ** **Murat YILDIRIM** **Sibel KIZIL** *Kırıkkale University, Faculty of Veterinary Medicine, Department of Microbiology, Kırıkkale, Türkiye*
16:35-16:50	**Pathological Findings of Unilateral Chronic Granulomatous Orchitis and its Management in Mini Pomeranian Dog with Cryptorchid: A Case Report** **Dzikri Nurma'rifah TAKARIYANTI[1]** **Archie Leander MASLIM[2]** **Palagan Senopati SEWOYO[3]** **Marissa Divia DAYANTI[4]** **Wayan BATAN[5]** *[1] Veterinary Practitioner, Healthy Pet Clinic, Bandung, Indonesia* *[2] Veterinary Practitioner, Archie Veterinary Clinic, Bandung, Indonesia* *[3]Master's Student, Postgraduate School, Fac. of Veterinary Med., Udayana*

Univ., Bali, Indonesia

[4] Veterinary Student, Faculty of Veterinary Medicine, Udayana University, Bali, Indonesia

[5] Laboratory of Veterinary Clinical Diagnosis, Clinical Pathology and Radiology, Faculty of Veterinary Medicine, Udayana University, Bali, Indonesia

16:55-17:10	**Immunolocalization of Actin and Vimentin Proteins in Fetal Bovine Liver** **Uğur TOPALOĞLU[1]** **Nurşin AYDIN[2]** *[1] Dicle University, Faculty of Veterinary Medicine, Department of Histology and Embriyology, Diyarbakır, Türkiye* *[2*] Dicle University, Faculty of Veterinary Medicine, Department of Histology and Embriyology, Diyarbakır, Türkiye*
17:10	*End*

7th International Congress on Veterinary and Animal Sciences (ICVAS)-Online

22 October, Saturday, 2022

Chair: Prof. Dr. Kader YILDIZ

Kırıkkale University, Faculty of Veterinary Medicine, Department of Parasitology, Kırıkkale, Türkiye

10:30	
10:30-10:45	**Investigation of The Prevalence of Varroasis and Nosemosis in Honey Bee (*Apis Mellifera*) Colonies in Aksaray Province and Determination of Risk Potentials for Local Beekeeping** **Neslihan SURSAL SIMSEK[1]** **Emrah SIMSEK[2]** *[1] Mugla Sitki Kocman University, Milas Faculty of Veterinary Med., Dep. of Parasitology, Mugla, Türkiye* *[2] Mugla Sitki Kocman University, Milas Faculty of Veterinary Med., Preclinical Sciences, Mugla, Türkiye*

10:50-11:05	***Cryptosporidiosis* in Lambs** **Sinem AKDENIZ[1] Aycan Nuriye GAZYAĞCI[2]** *[1] Kırıkkale University, Graduate Scholl of Health Science, Department of Parasitology, Kırıkkale, Türkiye* *[2] Kırıkkale University, Faculty of Veterinary Medicine, Department of Parasitology, Kırıkkale, Türkiye*
11:10-11:25	**Hatchling of infective stage larvae of *Toxocara canis* in vitro** **Gözde Nur AKKUŞ[1] Kader YILDIZ[2]** *[1] Kırıkkale University, Graduate Scholl of Health Science, Department of Parasitology, Kırıkkale, Türkiye* *[2] Kırıkkale University, Faculty of Veterinary Medicine, Department of Parasitology, Kırıkkale, Türkiye*
11:30-11:45	**Public and Medical Use of *Arum Rupicola* Boiss Var. *Rupicola*** **Tuğrul ATALAY[1,2] Kader YILDIZ[3]** *[1]Bozok University, Şefaatli Vocational Training School Laboratory and Veterinary Health, Yozgat, Türkiye* *[2]Kirikkale University, Graduate School of Health Science, Department of Parasitology, Kırıkkale, Türkiye* *[3]Kırıkkale University, Faculty of Veterinary Medicine, Department of Parasitology, Kırıkkale, Türkiye*
11:50-12:20	**INVITED SPEAKER** **Dr. Cristina Botías TALAMANTES** **Understanding The Effect of Global Change Stressors on Bee Health** *Universidad de Alcalá, Department of Life Sciences, Madrid, Spain*
12:30	*ANNOUNCEMENT of SCIENTIFIC AWARDS* *&* *CLOSING SPEECH*

SCIENTIFIC AWARDS

- **BEST ORAL PRESENTATION**

  1) Halef DOĞAN

  2) Niyazi KÜÇÜK

  3) İrem KARAASLAN

- **YOUNG SCIENTIST**

  1) Zeyno NUHOĞLU ÖZTÜRK

  2) Gözde Nur AKKUŞ

  3) Artina PRASTIWI

online

7TH INTERNATIONAL CONGRESS ON VETERINARY AND ANIMAL SCIENCES

20–22 October 2022

INTERNATIONAL CONGRESS ON VETERINARY AND ANIMAL SCIENCES
www.icvas.gen.tr

Presentations

online

7TH INTERNATIONAL CONGRESS ON VETERINARY AND ANIMAL SCIENCES

20–22 October 2022

INTERNATIONAL CONGRESS ON VETERINARY AND ANIMAL SCIENCES
www.icvas.gen.tr

Full Texts

COMPARATIVE SENSITIVITY OF EOSIN NIGROSIN AND PROPIDIUM IODIDE STAINING PROCEDURES TO DETECT VIABILITY OF FROZEN THAWED RABBIT SPERM

Niyazi KÜÇÜK

Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Aydın, Türkiye

*E-mail: niyazikucuk@adu.edu.tr

Viability assessment is an important step to evaluate the quality of semen. The eosin nigrosin (EN) and propidium iodide (PI) staining procedures are used to determine live sperm rate, widely. Thus, the present study aimed to detect comparative sensitivity of those staining procedures in frozen thawed rabbit sperm samples. Pooled sperm samples (n=6) were cryopreserved in an extender including 250 mmol/L Tris-hydroxymethylaminomethane, 88 mmol/L citric acid, 47 mmol/L glucose, 1% sucrose and 8% DMSO in the present study. After thawing process, the sperm samples were stained both EN and PI staining procedures. The dead sperm rate were found higher in PI group ($P<0.05$). In conclusion, the PI staining procedure was found more sensitive than EN staining procedure under the present experimental conditions.

Keywords: Cryopreservation; Eosin nigrosine; Propidium iodide, Rabbit; Sperm; Viability.

INTRODUCTION

The determination of sperm viability is one of the basic part of semen examination. The eosin nigrosin (EN) and propidium Iodide (PI) staining procedures are the most common techniques that are used to detect sperm viability in different species. The EN staining method is well known, widely used and a simple test (Björndahl et al., 2003). In eosin nigrosin staining technique, when eosin stains membrane

damaged dead sperm cells, the nigrosine stains background and subsequently; the dead red stained and live non-stained sperm cells could be easily distinguished under bright field microscope (Agarwal et al., 2016: Björndahl et al., 2004). Similarly, PI stains only membrane damaged (dead) sperm cells. The red stained (dead) sperm cells are detected under fluorescents microscope. Additionally, the combination of PI and SYBR-14 is also used to detect sperm viability with flow cytometry (Garner and Johnson, 1995). Thus, the present study was designed to investigate the comparative sensitivity of EN and PI staining procedures to detect viability of frozen thawed rabbit sperm.

MATERIAL AND METHOD

Sperm Collection and Cryopreservation

Five mature New Zealand rabbits were used in this study. Sperm samples were collected with artificial vagina. Collected semen samples were pooled after initial semen evaluation. The pooled semen samples (n=6) were diluted at a ratio of 1:3 in a sperm extender including 250 mmol/L Tris-hydroxymethylaminomethane, 88 mmol/L citric acid, 47 mmol/L glucose, 1% sucrose and 8% DMSO (Iaffaldano et al., 2012). The semen were filled in 0.25 mL straws and then cooled from 36 °C to 4 °C in 90 min with a programmable incubator. The straws were incubated further 10 min at 4 °C for equilibration. The cooled straws were frozen in liquid nitrogen vapors 6-7 cm above the surface for 10 min, just before directly plunging into the liquid nitrogen. The frozen sperm samples were stored in liquid nitrogen until the start of sperm analyses. Thawing of frozen sperm samples were performed in a water bath at 37 °C for 30 seconds (Küçük et al., 2021).

Evaluation of viability with EN Staining Procedures: Equal volume (3 µL) sperm sample and EN stain (%0.67 Eosin Y and 10% Nigrosin dissolved in saline solution) were gently mixed on microscope slide where it was smeared by sliding a cover slip (Björndahl et al., 2003). At least 200 sperm were observed at a magnification of 400x under bright field microscope. When stained sperm cells were counted as dead, non-stained sperm cells were counted as live.

Evaluation of viability with PI Staining Procedures: 50 µL sperm sample was stained with 2.5 µL PI (500 µg/mL) for 10 min at room temperature. After staining procedure, a fixative (2.5 µL; 4% Paraformaldehyde dissolved in PBS) was added the sperm just before evaluation. The 3µL sperm sample stained with PI was placed on slide and covered with cover slip. The sperm samples were analyzed with epifluorescence microscope (Olympus BX53) equipped with a differential interference contrast (DIC) and multiple-fluorescence filters (U-FBW, BP460-495, BA510IF, DM505). The sperm images in the same field were captured both DIC and epifluorescent optic (×400 magnification) using a digital camera (DP26- Olympus 5.0 MP). 200 sperm cells were examined. When unstained sperm cells were evaluated as live sperm, the stained sperm cells were counted as dead sperm (Küçük et al., 2021).

Statistical Analyses: The live sperm rates obtained from two different staining procedures were compared with independent samples *t test* (SPSS). The results were presented as mean ± S.E.M.

RESULTS

The live (unstained) sperm rate was found higher in EN group than PI group (Table 1). The PI staining procedure was found more sensitive than the EN staining procedure in evaluation of frozen thawed rabbit sperm (P<0.05).

Table 1. The live sperm rates were obtained from eosin nigrosine (EN) and propidium iodide (PI) staining procedures.

Staining Procedures	Live Sperm Rate (%)
EN Staining	32,5 ± 3,19*
PI staining	19 ± 0,89

*P<0.05.

DISCUSSION AND CONCLUSION

The PI is a DNA specific and red-fluorescent dye that cannot penetrate membrane of the viable cells. However, it can penetrate the membrane of dead cells. Therefore, PI is widely used to evaluate cell viability (Garner and Johnson, 1995). The principle of EN staining procedure is similar. The similarity is that the membrane damaged dead sperm cells intake eosin that stains the sperm head red. The nigrosine stain background of slide to ease to visualization of unstained viable cells (Björndahl et al., 2004). Thus, the present study was designed to investigate the comparative sensitivity of PI and EN staining procedures. The result of present study indicated that the unstained (live) sperm rate was higher in EN group (Table 1). The damage caused to sperm cells by freezing thawing process more effectively was revealed by PI staining procedure than EN staining procedure. Similarly, a previous study reported that the damage caused to pigeon spermatozoa by storage at 5° C was more visible in PI stained group than EN stained group (Klimovicz-Bodys et al., 2012). In conclusion, the PI staining procedure was found more sensitive than EN staining procedure under the present experimental conditions.

FINANCIAL SUPPORT

This research received no grant from any funding agency/sector.

CONFLICT OF INTEREST

The author declared that there is no conflict of interest.

REFERENCES

Björndahl L, Söderlund I, Kvist U. 2003. Evaluation of the one-step eosin-nigrosin staining technique for human sperm vitality assessment. Human Reproduction, 18(4): 813-816.

Björndahl L, Söderlund I, Johansson S, Mohammadieh M, Pourian MR, Kvist U. 2004. Why the WHO recommendations for eosin-nigrosin staining techniques for human sperm vitality assessment must change. Journal of Andrology, 25(5): 671-678.

Agarwal A, Gupta S, Sharma R. 2016. Andrological Evaluation of Male Infertility, A Laboratory Guide, Springer, Switzerland.

Garner DL, Johnson LA. 1995. Viability assessment of mammalian sperm using SYBR-14 and propidium iodide. Biology of Reproduction, 53(2): 276-284.

Laffaldano N, Di Iorio M, Rosato MP. 2012. The cryoprotectant used, its concentration, and the equilibration time are critical for the successful cryopreservation of rabbit sperm: dimethylacetamide versus dimethylsulfoxide, Theriogenology, 78: 1381–1389.

Küçük N, Raza S, Matsumura K, Uçan U, Serin İ, Ceylan A, Aksoy M. 2021. Effect of different carboxylated poly L-lysine and dimethyl sulfoxide combinations on post thaw rabbit sperm functionality and fertility. Cryobiology, 102: 127–132.

Klimowicz-Bodys MD, Batkowski F, Ochrem AS, Savic MA. 2012. Comparison of assessment of pigeon sperm viability by contrastphase microscope (eosin-nigrosin staining) and flow cytometry (SYBR-14/propidium iodide (PI) staining) [evaluation of pigeon sperm viability]. Theriogenology, 77: 628–635.

METHICILLIN AND VANCOMYCIN RESISTANCE PROFILES OF *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED FROM COW'S MASTITIC MILK SAMPLES

Orkun BABACAN

Balıkesir University, Kepsut Vocational School, Department of Veterinary, Kepsut/Balıkesir City, Türkiye

*E-mail: orkun.babacan@balikesir.edu.tr

In this study, it was aimed to determine the prevalence of *Staphylococcus aureus* and the presence of methicillin and vancomycin resistance in *S. aureus* isolates in dairy cows's milk samples in Balıkesir province, which is have high cow milk production in Turkey. In the study, 107 cow milk samples collected from the various different private dairy farms and sent to laboratory by veterinarians for microbiological examinations in terms of mastitis infection in Balıkesir city during the period of September 2021 and May 2022 were examined. For isolation and identification of *S. aureus*, initially, milk samples were gently shaken for homogenization and then were inoculated onto Rabbit Plasma Fibrinogen- Baired-Parker (RPF-BP) Agar (Oxoid-UK) and were incubated at 37°C for up to 48 hours. Methicillin and vancomycin resistance profiles of *S. aureus* isolates was investigated and evaluated phenotypically according to EUCAST standard. Methicillin resistance in *S. aureus* isolates was also investigated by PCR in terms of *mecA* and *mecC* genes. Methicillin resistance was determined phenotypically in 5 of 15 *S. aureus* isolates by disc diffusion test according to EUCAST procedure, while Vancomycin resistance was not detected in all isolated *S. aureus* strains by MIC E-test. By PCR method, *mecC* gene was detected in all MRSA strains (n:5), while *mecA* gene was not detected. it was thought that the isolates determined to be MRSA phenotypically should also be analyzed in terms of the *mecC* gene. In this study, it was thought that the detection of the *mecC* gene in all MRSA isolates emphasized the

importance of this situation. Considering that the absence of VRSA in isolated *S. aureus* strains is similar to the findings in the world, but antibiotic resistance is spreading rapidly nowadays, it was thought that vancomycin resistance should be monitored periodically in *S. aureus* isolates from an epidemiological point of view.

Keywords: Cow; Mastitis; MRSA; PCR; VRSA

INTRODUCTION

Staphylococcus aureus is one of the important strain causes of subclinical, clinical and chronic infectious bovine mastitis all over the world and causes serious economic losses in dairy farms due to its effect on milk production, milk quality and bulk tank somatic cell count (SCC) (Aslantaş et al., 2011; Brahma et al., 2022; Roshan et al., 2022; Selim et al., 2022; Zhang et al., 2022). *S. aureus* may also cause public health problems due to its contamination with animal products such as milk and raw milk products, called Milk-borne Disease (Campos et al., 2022; Khairullah et al., 2022; Neelam et al., 2022; Siriken et al., 2022). MRSA causes nasocomial infections, hospital-acquired illness, endocarditis and haemolytic pneumonia in humans (Nielsen et al., 2022; Roshan et al., 2022). Control of *S. aureus* infections is difficult due to its multi-drug resistance, which facilitates *S. aureus* infiltration into the host's immune system (Selim et al., 2022). Moreover, the World Health Organization (WHO) has listed MRSA as a “priority pathogen” (Nandhini et al., 2022).

Methicillin-Resistant *S. aureus* (MRSA) strains are resistant to beta-lactam antibiotics (Mehrotra et al., 2000; Tavşanlı and Cıbık, 2022; Zaatout and Hezil, 2022). The MRSA strain is associated with the acquisition of a mobile genetic element called Staphylococcal Cassette Chromosomal mec (SCCmec), which encodes low-affinity penicillin-binding protein 2a (PBP2a) and carries the *mecA* and *mecC* genes that confer resistance to beta-lactam antibiotics (Mehrotra et al., 2000; Rusenova et al., 2022; Şeker et al., 2022; Siriken et al., 2022; Tavşanlı and Cıbık, 2022).

Due to the emergence and spread of multi-drug-resistant zoonotic pathogens, scientists are becoming increasingly concerned about the frequent use of antibiotics.

Antibiotic-resistant bacteria do not respond to routine antibiotic treatment and prolong the course of the disease. Therefore, the resistance of *S. aureus* to antibiotic agents may complicate the treatment of infections. (Yimane and Tesfaye, 2022).

MRSA strains are classified according to their SCCmec type into three groups. These are hospital-associated (HA-MRSA), community-associated (CA-MRSA), and livestock-related (LA-MRSA). (Rusenova et al., 2022; Tavşanlı and Cıbık, 2022). The European Food Safety Authority (EFSA) has reported the role of foods of animal origin as possible sources of MRSA (Rusenova et al., 2022).

High rates of MRSA contamination in dairy farms may be the result of overuse of antibiotics in the treatment of dairy cows. The spread of these bacteria can be caused not only by the sanitation and hygiene management during milking, but also by the milk from the udder or by the hands of the farmers during the milking process (Khairullah et al., 2022).

It has been suggested in the 1990s that the susceptibility of *S. aureus* to vancomycin has changed. In May 1996, the first infection in which Vancomycin-Intermediate *S. aureus* (VISA) was detected in a patient in Japan was reported. In 2015 on May, 14 Vancomycin-Resistant *S. aureus* (VRSA) infections have been reported in patients in the United States. They reported *vanA* vancomycin resistance gene, which find in vancomycin-resistant enterococci, was found in all VRSAs. VRSA is thought to originate from specific precursor organisms, such as MRSA containing a plasmid of the pSK41 type and VRE containing *vanA* encoded on a plasmid like Inc18 (Walters et al., 2015).

In this study, it was aimed to determine the prevalence of *S. aureus* and the presence of methicillin and vancomycin resistance in *S. aureus* isolates in dairy cows's milk samples in Balıkesir province, which is have high cow milk production in Turkey.

MATERIALS

In the study, 107 cow milk samples collected from the various different private dairy farms and sent to laboratory by veterinarians for microbiological examinations in terms of mastitis infection in Balıkesir city during the period of September 2021 and

May 2022 were examined. The milk samples were taken into sterile sample containers approximately 5-10 ml after teat dipping and first milk discarded by veterinarians, suitable for microbiological examination. These samples were delivered to the laboratory under cold chain conditions. Samples not to be analyzed immediately were frozen and stored at (-20) °C (Grima et al., 2021).

METHODS

For isolation and identification of *S. aureus*, initially, milk samples were gently shaken for homogenization and then were inoculated onto Rabbit Plasma Fibrinogen-Baired-Parker (RPF-BP) Agar (Oxoid-UK) and were incubated at 37°C for up to 48 hours (Baired-Parker, 1962).

Gram staining, catalase, and growth spesifications on RPF-BP Agar were done and evaluated on the colonies that grew after incubation and isolates were identified as *S. aureus* (Baired-Parker, 1962; Baired-Parker, 1962). The isolates determined as *S. aureus* were put the beads and were stored in cryotubes at (-20) °C. Methicillin and vancomycin resistance profiles of *S. aureus* isolates was investigated and evaluated phenotypically according to EUCAST standard (EUCAST, 2017). Methicillin resistance was investigated by disc diffusion method using cefoxitin (30 µg disk, Oxoid, UK) (EUCAST, 2017). Vancomycin resistance was investigated by E-test using Vancomycin strips (Liofilchem, Italy) (EUCAST, 2017). *S. aureus* strains with < 22 mm zone diameter was recorded as Methicillin-resistant and >MIC 2 value was recorded as Vancomycin-resistant (EUCAST, 2017).

For to investigate Methicillin-resistance by PCR, DNA was purified from the *S. aureus* isolates with DNA extraction kit (GeneJET Genomic DNA Purification kit, Thermo, USA) and using lysis buffer according to manufacturer procedure. Methicillin resistance genes, *mecA* and *mecC*, were investigated by polymerase chain reaction method by used previously described primers and amplification conditions (Table 1) (García-Álvarez et al., 2022; GarcÍa-Garrote et al., 2014; Mehrotra et al., 2000). For *mecA* and *mecC* genes, PCR mix was prepared in a total volume of 50 µl using with Taq polymerase Master Mix (Ampliqon, Denmark) and contained 5µl of DNA extract.

PCR amplicons were electrophoresed on 1.5% agarose (Prona, USA) gel using Novel juice dye (Thermo Scientific, USA) and DNA molecular weight marker (Gene Ruler 100bp DNA Ladder plus, Thermo Scientific, USA) and visualized on the gel imaging system (EBOX CX5 TS EDGE, Vilber).

Methicillin resistant *S. aureus* NCTC 12493 (*mecA*) and Methicillin resistant *S. aureus* NCTC 13552 (*mecC*) were used as a reference strains, which was obtained form Ministry of Health, General Directorate of Public Health, Microbiology Reference Laboratory.

Table 1. Primer sequences, target genes and references for *mecA and mecC* genes.

Primers	Sequences	Target genes	References
GMECAR-1 GMECAR-2	ACTGCTATCCACCCTCAAAC CTGGTGAAGTTGTAATCTGG	*mecA*	Mehrotra M. et al. (16)
Primer-F Primer-R	5′ -GAA AAA AAG GCT TAG AAC GCC TC-3′ 5′ GAA GAT CTT TTC CGT TTT CAG C-3	*mecC*	Garcı´a-Alvarez et al. (12) and Garcı´a-Garrote F. et al. (13)

RESULTS

A total of 15 *S. aureus* isolated and identified from 107 cow's mastitic milk samples (Figure 1). Methicillin resistance was determined phenotypically in 5 of 15 *S. aureus* isolates by disc diffusion test according to EUCAST procedure, while Vancomycin resistance was not detected in all isolated *S. aureus* strains by MIC E-test (Figure 2, Figure 3).

By PCR method, *mecC* gene was detected in all MRSA strains (n:5) (Figure 4), while *mecA* gene was not detected.

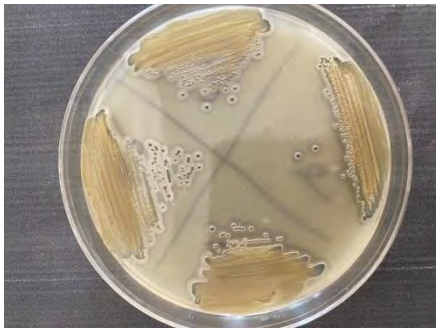


Figure 1. Isolated *S. aureus* strains on RPF-BP agar.

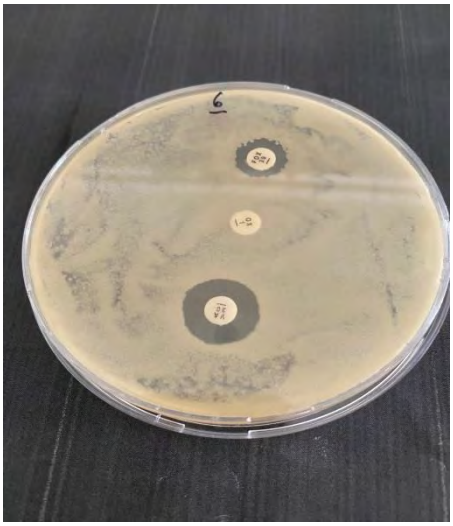


Figure 2. Methicillin resistance in isolated *S. aureus* strain by disc diffusion test.

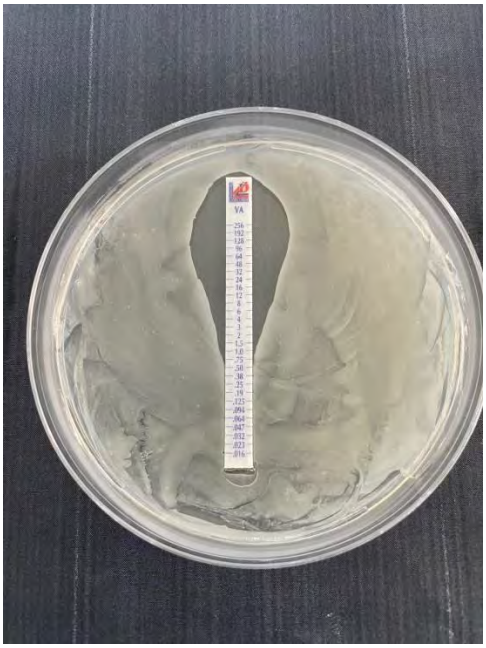


Figure 3. E-Test (MIC) for Vancomycin in isolated *S. aureus* strain.

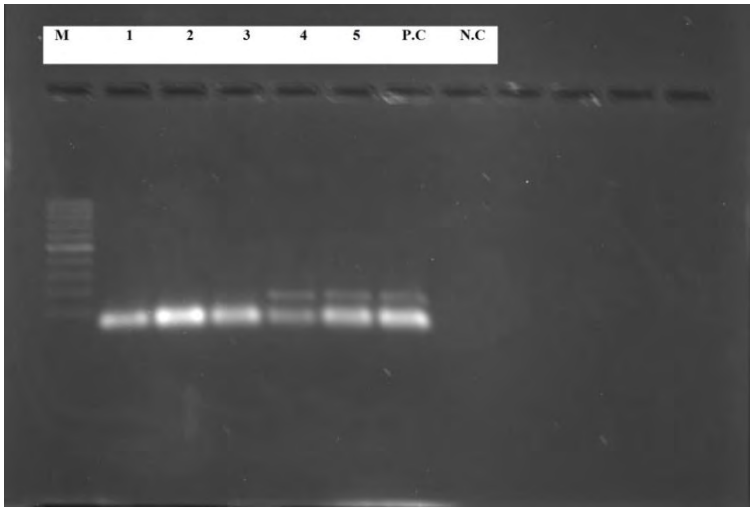


Figure 4. PCR image of *mecC* gene in isolated S. aureus strains.
M: Marker; Line 1,2,3: *S. aureus* isolates negative for *mecC* gene; Line 4,5: *S. aureus* isolates with *mecC* gene positive; P.C: Positive Control; N.C: Negative Control

DISCUSSION AND CONCLUSION

MRSA is increasingly being reported as a new problem in veterinary medicine. The emergence of MRSA causes serious zoonotic diseases (Yimana and Tesfaye, 2022). Additionally, presence of MRSA in bovine mastitis is a possible risk and a source of infection for for people, especially veterinarians and livestock workers (Zaatout and Hezil, 2022) With all this information, MRSA can also be evaluated with the One Health approach (Campos et al., 2022).

Risk factors for carriage of *mecC* MRSA in humans are contact with animals and the presence of an underlying disease (EFSA and ECDC, 2022). According to the EFSA 2019-2020 report, MRSA was found to be 7.7% in 366 cow's milk samples (EFSA and ECDC, 2022).

Especially, in Balıkesir province, authors were previously study and reported MRSA in mil*k samples.* Tavşanlı and Cibik, 2022 reported that they detected MRSA in 6 (6.3%) of 95 *S. aureus* strains isolated from 725 milk samples with subclinical mastitis, which they examined for microbiological examination for mastitis in the province of Balıkesir.

Ektik et al., 2017 reported that they detected MRSA in 3 of 26 coagulase-positive Staphylococ strains isolated from 175 cow bulk tank milk and dairy products' samples, which they examined for microbiological examination for mastitis in the province of Balıkesir. Also they and reported that one of these isolates carried the *mecA* gene.

Büyükcangaz et. al., 2013 reported that they isolated 151 *S. aureus* from 480 milk samples with subclinical mastitis collected from cows in 6 different cities, including Balıkesir province between 2010 and 2012, and 62 of them were found to be resistant to cephoxitin by disc diffusion test. Also, they reported that they detected the *mecA* gene in 24 of 151 *S. aureus* isolates with their PCR test.

Aslantaş et al., 2016 reported that they isolated 112 *S. aureus* from milk samples between 2008 and 2010 and they diagnosed MRSA by both detecting *mecA* gene in PCR and disc diffusion in 5 of these isolates.

Çiftçi et al., 2009 reported that 59 strains were detected as *S. aureus* by both conventional tests and PCR isolated from bovine subclinic mastitic milk samples, and 13 of them were found to be methicillin resistant and 4 (30.7%) were positive for *mecA* gene.

Türüoğlu et. al., 2009 reported that they detected *mecA* gene in 3 of 18 MRSA strains isolated from milk samples with cow mastitis between 2002-2004.

MRSA was detected by both disc diffusion and PCR in 5 of 15 *S. aureus* isolated in this study. Considering the cited studies in Balıkesir (Büyükcangaz et. al., 2013; Ektik et al., 2017; Tavşanşı and Cıbık, 2022), it was determined that the presence of MRSA is still present in mastitis infections of dairy cows in Balıkesir province.

Unlike the other cited studies including some of the ones made in Balıkesir (Aslantaş et al., 2016; Büyükcangaz et. al., 2013; Çiftçi et al., 2009; Ektik et al., 2017; Türüoğlu et. al., 2009) the *mecC* gene was detected in all MRSA isolates in the PCR test in this study. This situation was found to be similar with Degaim et. al., 2019 and Sakmanoğlu et. al., 2016's studies. Degaim et. al., 2019 reported that in their study established the significance of *mecC* gene in MRSA recognition than *mecA* gene and highlighted the increasing manner of its frequency in south of Iraq. Sakmanoğlu et. al.,

2016 reported that in their study they had identified unexpectedly high prevalence of *mecC* MRSA in cows with mastitis.

However, VRSA has not yet been reported in Europe and is is rarely reported all over the world today (EUCAST, 2017). Similarly, in this study, vancomycin resistance was not detected in all *S. aureus* isolates as a result of MIC evaluation.

As a conclusion, the presence of both *S. aureus* and MRSA was detected in this study, as can be seen in the findings of other cited studies (Büyükcangaz et. al., 2013; Ektik et al., 2017; Tavşanşı and Cıbık, 2022) in cow milk with mastitis in Balıkesir province. Moreover, in this study, it was determined that the rate of *mecC*-MRSA was high.

As seen in the results of Ektik et al., 2017, Büyükcangaz et. al., 2017 and Çiftçi et al., 2009's studies, they could not detect genotypic resistance genes in some isolates that were found to be phenotypically resistant in the PCR test performed only for the detection of the *mecA* gene. For this reason, it was thought that the isolates determined to be MRSA phenotypically should also be analyzed in terms of the *mecC* gene, not only *mecA* gene. In this study, it was thought that the detection of the *mecC* gene in all MRSA isolates emphasized the importance of this situation.

Considering that the absence of VRSA in isolated *S. aureus* strains is similar to the findings in the world, but antibiotic resistance is spreading rapidly nowadays, it was thought that vancomycin resistance should be monitored periodically in *S. aureus* isolates from an epidemiological point of view.

ACKNOWLEDGEMENT

The author thanks the Republic of Turkey Ministry of Health General Directorate for Public Health, Microbiology Reference Laboratory for control strains used in this study.

FINANCIAL SUPPORT

This research received no grant from any funding agency/sector.

CONFLICT OF INTEREST

The author declared that there is no conflict of interest.

REFERENCES

Aslantaş, Ö., & Demir, C. (2016). Investigation of the antibiotic resistance and biofilm-forming ability of *Staphylococcus aureus* from subclinical bovine mastitis cases. Journal of Dairy Science, 99(11), 8607–8613. https://doi.org/10.3168/jds.2016-11310

Aslantaş, Ö., Öztürk, F., & Ceylan, A. (2011). Prevalence and molecular mechanism of macrolide and lincosamide resistance in staphylococci isolated from subclinical bovine mastitis in Turkey. Journal of Veterinary Medical Science, 73(12), 1645–1648. https://doi.org/10.1292/jvms.11-0003

Baird-Parker, A. C. (1962). An improved diagnostic and selective medium for isolating coagulase positive Staphylococci. Journal of Applied Bacteriology, 25(1), 12–19. https://doi.org/10.1111/j.1365-2672.1962.tb01113.x

Brahma, U., Suresh, A., Murthy, S., Bhandari, V., & Sharma, P. (2022). Antibiotic resistance and molecular profiling of the clinical isolates of *Staphylococcus aureus* causing bovine mastitis from India. Microorganisms, 10(4). https://doi.org/10.3390/microorganisms10040833

Buyukcangaz, E., Kahya, S., & Sen, A. (2013). *mecA* gene prevalence in *Staphylococcus aureus* isolates from dairy cows in Turkey. J. Biol. Environ. 7(21), 183–190. http://jbes.uludag.edu.tr/PDFDOSYALAR/21/mak08.pdf

Campos, B., Pickering, A. C., Rocha, L. S., Aguilar, A. P., Fabres-Klein, M. H., de Oliveira Mendes, T. A., Fitzgerald, J. R., & de Oliveira Barros Ribon, A. (2022). Diversity and pathogenesis of *Staphylococcus aureus* from bovine mastitis: current understanding and future perspectives. BMC Veterinary Research, 18(1), 1–16. https://doi.org/10.1186/s12917-022-03197-5

Ciftci, A., Findik, A., Onuk, E. E., & Savasan, S. (2009). Detection of methicillin resistance and slime factor production of *Staphylococcus aureus* in bovine mastitis. Brazilian Journal of Microbiology, 40(2), 254–261. https://doi.org/10.1590/s1517-83822009000200009

Degaim, Z. D., Mater, A. D., & Al-Malky, K. (2019). mec A and mec C genes profile of clınıcal ısolates of *Staphylococcus aureus*. Basrah J Vet Res. 675-682. http://www.basjvet.com/wp-content/uploads/2018/12/675-682.pdf

EFSA and ECDC. (2022). The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2019–2020. EFSA Journal, 20(3). https://doi.org/10.2903/j.efsa.2022.7209

Ektik, N., Gökmen, M., & Çibik, R. (2017). The prevalence and antibiotic resistance of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in milk and dairy products in Balikesir, Turkey. Journal of the Hellenic Veterinary Medical Society, 68(4), 613–620. https://doi.org/10.12681/jhvms.16062

European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2017). EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 2.0. Available at https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Resistance_mechanisms/EUCAST_detection_of_resistance_mechanisms_170711.pdf. (Accessed September 29, 2020).

García-Álvarez, L., Holden, M. T. G., Lindsay, H., Webb, C. R., Brown, D. F. J., Curran, M. D., Walpole, E., Brooks, K., Pickard, D. J., Teale, C., Parkhill, J., Bentley, S. D., Edwards, G. F., Girvan, E. K., Kearns, A. M., Pichon, B., Hill, R. L. R., Larsen, A. R., Skov, R. L., Holmes, M. A. (2011). Meticillin-resistant Staphylococcus aureus with a novel *mecA* homologue in human and bovine populations in the UK and Denmark: A descriptive study. The Lancet Infectious Diseases, 11(8), 595–603. https://doi.org/10.1016/S1473-3099(11)70126-8

García-Garrote, F., Cercenado, E., Marín, M., Bal, M., Trincado, P., Corredoira, J., Ballesteros, C., Pita, J., Alonso, P., & Vindel, A. (2014). Methicillin-resistant *Staphylococcus aureus* carrying the *mecC* gene: Emergence in Spain and report of a fatal case of bacteraemia. Journal of Antimicrobial Chemotherapy, 69(1), 45–50. https://doi.org/10.1093/jac/dkt327

Grima, L. Y. W., Leliso, S. A., Bulto, A. O., & Ashenafi, D. (2021). Isolation, identification, and antimicrobial susceptibility profiles of *Staphylococcus aureus* from clinical mastitis in Sebeta Town dairy farms. Veterinary Medicine International, 2021. https://doi.org/10.1155/2021/1772658

Khairullah, A. R., Sudjarwo, S. A., Effendi, M. H., Ramandinianto, S. C., Gelolodo, M. A., Widodo, A., Riwu, K. H. P., Kurniawati, D. A., & Rehman, S. (2022). Profile of Multidrug Resistance and Methicillin-Resistant *Staphylococcus aureus* (MRSA) on dairy cows and risk factors from farmer. Biodiversitas, 23(6), 2853–2858. https://doi.org/10.13057/biodiv/d230610

Mehrotra, M., Wang, G., & Johnson, W. M. (2000). Multiplex PCR for detection of genes for Staphylococcus aureus enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. Journal of Clinical Microbiology, 38(3), 1032–1035. https://doi.org/10.1128/jcm.38.3.1032-1035.2000

Mushtaq, M., Agrawal, R., Bhat, M. A., & Pande, N. (2022). Susceptibility pattern in methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from bovine mastitis. Applied Veterinary Research, 1(1), 1–5. https://doi.org/10.31893/avr.2022003

Nandhini, P., Kumar, P., Mickymaray, S., Alothaim, A. S., Somasundaram, J., & Rajan, M. (2022). Recent developments in Methicillin-Resistant *Staphylococcus aureus* (MRSA) treatment: A review. Antibiotics, 11(5), 1–21. https://doi.org/10.3390/antibiotics11050606

Neelam, Jain, V. K., Singh, M., Joshi, V. G., Chhabra, R., Singh, K., & Rana, Y. S. (2022). Virulence and antimicrobial resistance gene profiles of *Staphylococcus aureus* associated with clinical mastitis in cattle. PLoS ONE, 17(5 May), 1–11. https://doi.org/10.1371/journal.pone.0264762

Nielsen, S. S., Bicout, D. J., Calistri, P., Canali, E., Drewe, J. A., Garin-Bastuji, B., Gonzales Rojas, J. L., Gortázar, C., Herskin, M., Michel, V., Miranda Chueca, M. Á., Padalino, B., Pasquali, P., Roberts, H. C., Spoolder, H., Ståhl, K., Velarde, A., Viltrop, A., Winckler, C., Alvarez, J. (2022). Assessment of listing and categorisation of animal diseases within the framework of the Animal Health Law (Regulation (EU) No 2016/429): antimicrobial-resistant *Staphylococcus aureus* in cattle and horses. EFSA Journal, 20(5). https://doi.org/10.2903/j.efsa.2022.7312

Roshan, M., Parmanand, Arora, D., Behera, M., Vats, A., Gautam, D., Deb, R., Parkunan, T., & De, S. (2022). Virulence and enterotoxin gene profile of methicillin-resistant *Staphylococcus aureus* isolates from bovine mastitis. Comparative Immunology, Microbiology and Infectious Diseases, 80(July 2021), 101724. https://doi.org/10.1016/j.cimid.2021.101724

Rusenova, N., Vasilev, N., Rusenov, A., Milanova, A., & Sirakov, I. (2022). Comparison between some phenotypic and genotypic methods for assessment of antimicrobial resistance trend of bovine mastitis *Staphylococcus aureus* isolates from Bulgaria. Veterinary Sciences, 9(8). https://doi.org/10.3390/vetsci9080401

Sakmanoglu, A., Ucan, U. S., Pinarkaya, Y., Uslu, A., Aras, Z., Erganis, O., & Sayın, Z. (2016). Detection of methicilline resistant *Staphylococcus aureus* carrying *mecC* gene in mastitic milk samples of cattle in Turkey. Eurasian Journal of Veterinary Sciences, 32(3), 182–182. https://doi.org/10.15312/eurasianjvetsci.2016318398

Seker, E., Ozenc, E., Turedi, O. K., & Yilmaz, M. (2022). Prevalence of *mecA* and pvl genes in coagulase negative staphylococci isolated from bovine mastitis in smallholder dairy farms in Turkey. Animal Biotechnology, 0(0), 1–6. https://doi.org/10.1080/10495398.2022.2094802

Selim, A., Kelis, K., AlKahtani, M. D. F., Albohairy, F. M., & Attia, K. A. (2022). Prevalence, antimicrobial susceptibilities and risk factors of Methicillin resistant *Staphylococcus aureus* (MRSA) in dairy bovines. BMC Veterinary Research, 18(1), 1–7. https://doi.org/10.1186/s12917-022-03389-z

Siriken, B., Yildirim, T., Güney, A. K., Erol, I., & Durupinar, B. (2016). Prevalence and molecular characterization of methicillin-resistant *Staphylococcus aureus* in foods of

animal origin, Turkey. Journal of Food Protection, 79(11), 1990–1994. https://doi.org/10.4315/0362-028X.JFP-16-161

Tavsanli, H., & Cibik, R. (2022). The prevalence, genetic diversity and antibiotic resistance of *Staphylococcus aureus* associated with subclinical bovine mastitis in Balikesir, Turkey. Veterinarski Arhiv, 92(1), 17–25. https://doi.org/10.24099/vet.arhiv.1315

Türütoğlu, H., Hasöksüz, M., Öztürk, D., Yıldırım, M., & Sağnak, S. (2009). Methicillin and aminoglycoside resistance in *Staphylococcus aureus* isolates from bovine mastitis and sequence analysis of their *mecA* genes. Veterinary Research Communications, 33(8), 945–956. https://doi.org/10.1007/s11259-009-9313-5

Walters M, Lonsway D, Rasheed K, Albrecht, V, McAllister, S, Limbago B, Kallen A. (2015). Investigation and control of Vancomycin-resistant *Staphylococcus aureus*: A guide for health departments and infection control personnel. Atlanta, GA. Erişim adresi: http://www.cdc.gov/hai/pdfs/VRSA-Investigation-Guide-05_12_2015.pdf Erişim Tarihi: 01/10/2022.

Yimana, M., & Tesfaye, J. (2022). Isolation, identification and antimicrobial profile of methicillin-resistant *Staphylococcus aureus* from bovine mastitis in and around Adama, Central Ethiopia. Veterinary Medicine and Science, 1–9. https://doi.org/10.1002/vms3.902

Zaatout, N., & Hezil, D. (2022). A meta-analysis of the global prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from clinical and subclinical bovine mastitis. Journal of Applied Microbiology, 132(1), 140–154. https://doi.org/10.1111/jam.15192

Zhang, Z., Chen, Y., Li, X., Wang, X., & Li, H. (2022). Detection of antibiotic resistance, virulence gene, and drug resistance gene of *Staphylococcus aureus* isolates from bovine mastitis. Microbiology Spectrum, 10(4). https://doi.org/10.1128/spectrum.00471-22.

EFFECTS OF HORSE CHESTNUT EXTRACT (*AESCULUS HIPPOCASTANUM* L.) ON BONE AND CALCIUM METABOLISM IN RATS FED A HIGH PROTEIN DIET

Erten AKBEL[1]* **Abdullah ERYAVUZ[2]**

[1] Usak University, Faculty of Health Scienses, Department of Physiotherapy and rehabilitation, Usak, Türkiye

[2] Afyon Kocatepe University, Faculty of Veterinary, Department of Physiology, Afyonkarahisar, Türkiye

*E-mail:erten.akbel@usak.edu.tr

The purpose of the study was to investigate the effects of a horse chestnut (Aesculum hippocastanum L.) seed extract given specifically on calcium and bone metabolism in feeded rats with high protein diet. The study included 5 groups: a control group, and 4 trials (high protein, high protein and extract, extract, and ethanol-water). Control and experimental groups' serum and plasma samples were collected. The ethanol group has lower calcium levels than the control and study groups ($p<0.001$). The applications had no effect on parathyroid hormone (PTH), calcitonin, osteocalcin and alkaline phosphatase (ALP) levels when compared to the control group. When high protein and extract were applied together as well as in the ethanol group, vitamin D levels increased compared to the control group, but this increase did not result in a statistically significant difference ($p<0.01$). It was found that high protein application increased plasma levels of inorganic phosphorus (Pi) ($p<0.001$) and urea nitrogen ($p<0.001$) while high protein and extract application decreased these values. While high protein and extract applications didn't show a significant difference in total protein level when compared to the control group, the difference between high protein and ethanol groups was statistically significant. The animals' glucose levels were unaffected by the extract when given a high-protein diet, but they increased in the ethanol group ($p<0.001$).

Keywords: Aescin; *Aesculus hippocastanum* L.; Calcium; Rat; Saponin

INTRODUCTION

Saponins, which occur naturally and are frequently found in many plants and plant products, are steroid or triterpenoid glycosides that are significant for both human and animal nutrition. Saponins are steroid or triterpenoid glycosides that are frequently found in various plants and exist naturally and they are important for both human and animal nutrition. Studies have demonstrated that saponins have properties such as hypocholesterolemic, anticoagulant, neuroprotective, anti-carcinogenic, hypoglycemic, hepatoprotective, immunomodulatory, anti-inflammatory, antioxidant activity and immunostimulant, as well as effective on cell membrane permeability and immune system (Das et al. 2012; Addisu et al. 2016).

In addition to these biological effects, saponins also have anti-inflammatory, antibacterial (Fidan and Dündar, 2007), and blood pressure-lowering properties (Hosstettman and Marston 1995; Küçükkurt and Fidan, 2008). Saponins can prevent other nutrients from being digested and absorbed because of their limited absorption and increased action in the digestive tract. (Cheeke, 1998).

Aesculus hippocastanum L., commonly known as horse chestnut, was frequently used to cure fractures as well as respiratory and nutrition problems in injured horses. Its name comes from the Latin word "esca," which means "meal" (Yadav et al. 2022). The horse chestnut, sometimes known as the European horse chestnut, is a species of Aesculus that originated in Europe but it is now grown in parks, gardens, and along roadsides all over the world (Drăghici et al. 2020). Horse chestnut seeds contain aescin, which is derived from the coumarin glycoside esculin and its aglycone aesculetin, oxycoumarinic glycosides fraxin and scopolin, and their aglycones fraxetine and scopoletin, allantoin and quercetin, is the main compound of Aesculus hippocastanum. Aescin is used in treatment of hemorrhoids, varicose veins, hematoma and venous congestion (Koçkar 1989; Gallelli, 2019; Sağdıçoğlu, 2000) in addition to in the field of cosmetics forthe therapies of cellulitis (Yoshikawa et al. 1994) (Figure 1). Aescin, a combination of triterpene saponins, has anti-edema, anti-inflammatory, and venotonic effects. Thereare two different variations of it: α and β (Sirtori, 2001).

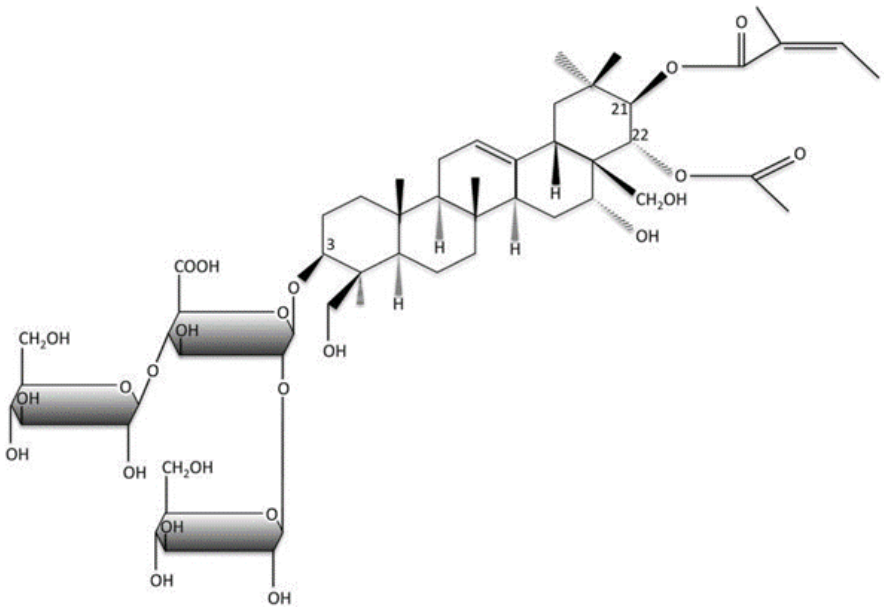


Figure 1. Aescin's chemical composition (Gallelli 2019).

Two of the most major, manageable factors that influence people's health are diet andnutritional status (Turnbaugh et al. 2009). Obesity has continuously increased in recent decades, and it is now one of the leading causes of death globally. (Rakhra et al. 1996). Today, consuming less calcium and fiber raises the risk of developing insulin resistance and obesity (Linn at al. 1996). Studies on people have revealed a connection between high-protein diet use and a person's deterioration in renal function. Proteinuria, glomerular damage, and intraglomerular hypertension have all been connected to diets high in protein. It is said that consuming a lot of protein over the long term can cause chronic renal disease (Ko et al. 2020). According to Chow et al. (1994), eating large amounts of protein elevated the excretion of calcium (Ca) in the urine, which was associated witha n increased risk of fracture and renal cell carcinoma (Chow et al. 1994). It is well recognized that a high protein diet will increase the production of endogenous acid and the excretion of calcium in the urine, which will result in hypercalciuria (Kerstetter et al. 2005). It is claimed that saponins reduce protein digestibility, possibly by the formation of poorly digestible saponin-protein complexes during the digestion of proteins (Desai et al. 2009).

The results of adding "aescin" (escin), which is a mixture of triterpenic saponin found in the seeds of the horse chestnut tree (*Aesculus hippocastanum* L.), which is prevalent both in our nation and around the world, to the diet with increased protein content on bone and calcium metabolism were examined in this study.

MATERIALS AND METHODS

Plant materials and Preparation of plant extract: In this research, in accordance with the "United States Patent-3,609,137" patent, horse chestnut seeds were extracted from the garden of the Uşak Forest Management Directorate and shade-dried. In the extraction process, the seeds were extracted with a 2% acetic acid- water solution to remove oil, and then extracted with a mixed extraction method with 50% ethanol-water solvent system at room temperature. The quantity of active substance contained in the extract, which was concentrated under vacuum and turned into powder, was determined by HPLC method. The results of the analysis are shown in Table 2 and Graphics 1, 2, and 3.

Animals and test samples: Fifty male Sprague-Dawley rats weighing 173-287 g, 2-3 months old, and from the "Isparta Süleyman Demirel University Faculty of Medicine Experimental Animals Laboratory" were used in the study. The animals were kept At "Afyon Kocatepe University Experimental Animals Application and Research Center" in a 12:12 h light:dark cycle at room temperature (25 ± 3 °C). Standard rodent feed and water were constantly available. The study was permitted by "Afyon Kocatepe University Animal Ethics Committee", dated 15.04.2008, number B.30.2. AKÜ.0.8Z.00.00/182 and reference number AKÜHEK-26- 08. They were given a standard pellet diet and a high-protein diet with more protein (casein) than standard feed for 30 days (Tatar, 2003), the content of which was determined by the "Afyon Kocatepe University Faculty of Veterinary Medicine Department of Animal Nutrition" (Table 1).

Table 1. Composition of rat feed (%)

	Standart rat food	High Protein Rat Food
Vegetable oil	3	3
Molasses	1.5	1.5
Yellow corn	55	34
Meat-bone meal, 34% Crude Protein	2	0
Soybean meal, 46%	19.19	26.95
Full-fat soybeans	17.64	10
Dicalcium phosphate	1.3	0.4
DL- Methionin	0,26	0
Limestone	0.75	1.5
L-Lysine hydrochloride	0.26	0
Sodium bicarbonate	0.20	0.25
Salt	0.1	0.1
Vitamin-mineral mix	0.3	0.3
Chemical analysis		
Dry Matter,%	89.3	90
Crude protein,%	20.0	40.2
Metabolic Energy, kcal/kg	3062	3062
Calcium, %	0.93	0.90
Ash %	0.45	0.43

In each group, there were 10 experimental animals. The rats were given horse chestnut extract for a period of one month via gastric gavage. It was dissolved in 20 mL of a 50/50 solvent mixture of ethanol and water at a concentration of 100 mg/kg/day. This dose was calculated using previous research (Sirtori, 2001). Experimental groups and applications are given below:

I. Control Group (C): Standard rat diet+ drinking water
II. High Protein Group (HP): High protein rat diet + drinking water
III. High Protein and Horse Chestnut Extract Group (HP + HC): High protein rat diet

+ 20 ml horse chestnut extract (dissolved in 50% ethanol and 50% water) with gastric gavage + drinking water

IV. Horse Chestnut Extract Group (HC): Standard rat diet + 20 ml horse chestnut extract (dissolved in a solvent system containing 50% ethanol and 50% water) with gastric gavage +drinking water

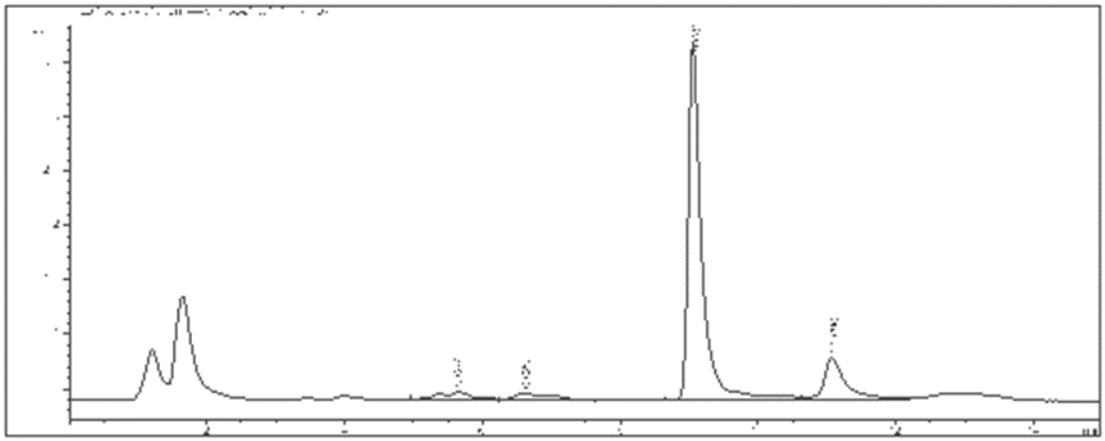
V. ethanol Group (EA): Standard rat diet + 20 ml 50% ethanol and 50% water with gastric gavage + drinking water

Following the one-month trial period, serum and plasma were separated from blood samples taken from the beating hearts of the experimental animals while they were anesthetized with injections of 10 mg/kg xylazine and 50 mg/kg ketamine HCl (Cigerci et al. 2009). In serum; calcium level was determined by the colorimetric method, and phosphorus and alkaline phosphatase levels were determined by the photometric method. Using commercially available kits, analyses of PTH (450 nm) (DRG- PTH Intact, EIA-3645), calcitonin (450 nm) (Biosource, Cat. No: Cap.0421), Vitamin D (450 nm) (Immune Diagnostics, 25-OH Vitamin EIA), osteocalcin (450 nm) (Biosource, Cat. No: Cap.1381), protein (540 nm) (Chema Diagnostica, Italy. Cat. No: TP500CH), urea nitrogen (600 nm) (BioSystems, Spain. Cat. No: 11536) and glucose (510 nm) (Chema Diagnostica, Italy. Cat. No: GLF400CH) were carried out on plasma samples. The Shimadzu UV-1601 brand spectrophotometer was used to determine these parameters using the specified commercial kits.

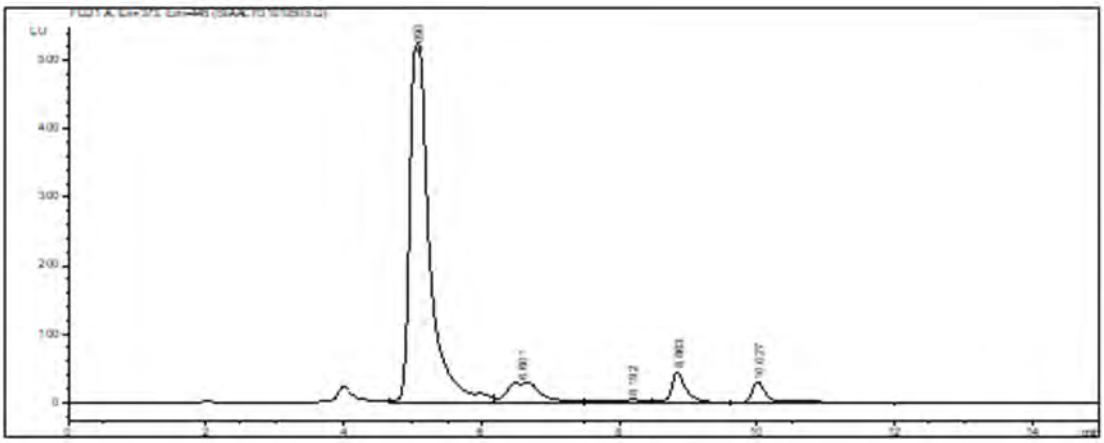
Data statistical analysis: The SPSS 13.0 program was used to analyze the study data (Özdamar, 2003). The means of the data were expressed as standard errors. The obtained data were subjected to normality tests, and the ANOVA test was used to determine statistical differences between the groups, and Duncan's test was used as a post-test. A value of $P<0.05$ was chosen for statistical significance.

RESULTS

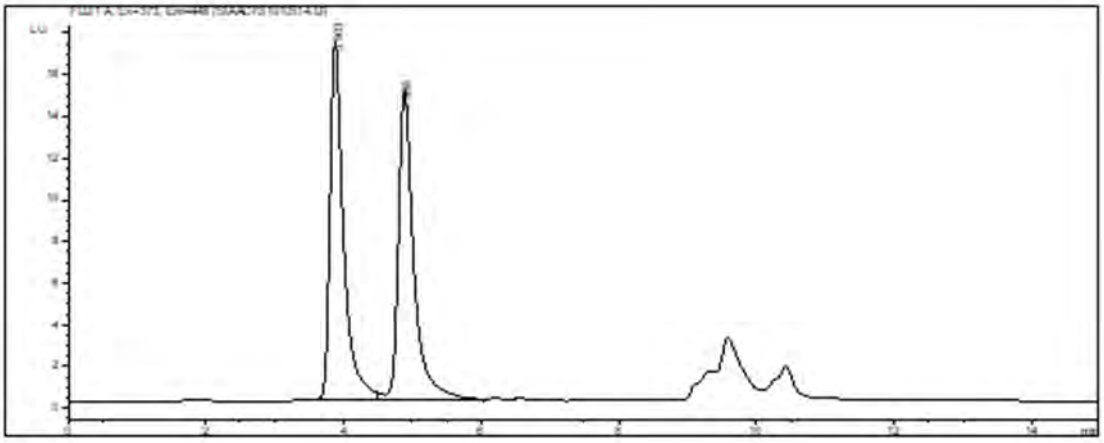
As a result of HPLC analysis of horse chestnut extract prepared as specified in the material method section, it was determined that it contained 58.28% aescin (Graphic 1, Graphic 2, Graphic 3) (Table 2).



Graphic 1. Analysis of chemicals without sialic acid and used in derivatization



Graphic 2. Sialic acid (N-Acetylneurominic acid) analysis



Graphic 3. N-Acetylneurominic acid and N-Glycolylneurominic acid analysis

Table 2. Aescin amount and aescin composition in the extract (%).

Aescin	58.28
Aescin 1a	55.25
Aescin 1b	19.21
Isoaescin 1a	20.07
Isoaescin 1b	5.14

To assess the effects of giving horse chestnut (Aesculus hippocastanum L.) seeds extract together with a high protein diet for 30 days calcium, PTH, vitamin D, calcitonin, osteocalcin, phosphorus, ALP, total protein, urea, nitrogen, and glucose levels were statistically evaluated and shown in Table 3.

Table 3. Calcium, PTH, vitamin D, calcitonin, osteocalcin, phosphorus, ALP, total protein, urea nitrogen and glucose levels in response to a high protein diet and horse chestnut extract (n=10, ± SE).

Parameter	I. C	II. HP	III. HP + HC	IV. HC	V. EA	P
Calcium mg dL^{-1}	10.34±0.17 ^{a}	10.77±0.16 ^{a}	10,77±0.10 ^{a}	10.32±0.14 ^{a}	9.62±0.11 ^{b}	<0.001
PTH pg/mL	62.22±1.46	60.00±0.00	61.42±1.42	61.11±1,11	63.00±1.52	>0.05
Vitamin D pg mL^{-1}	20.76±1.34 ^{c}	23.67±2.25 ^{bc}	29.51±0.90 ^{ab}	23.88±2.27 ^{bc}	31.62±2.84 a	<0.01
Calcitonin pg mL^{-1}	1.37±0.42	0.74±0.23	1.06±0.31	1.25±0.38	1.90±0.63	>0.05
Osteocalcin pg mL^{-1}	56.66±1,66	56.66±2.10	55.71±2.02	58.75±4,40	60.00±2.10	>0.05
Pi mg dL^{-1}	8.92±0.21 ^{b}	11.28±0.49 ^{a}	9.92±0.39 ^{b}	8.98±0.39 ^{b}	9.17±0.21 ^{b}	<0.001
ALP U L^{-1}	594.33 ± 54.40	783.25 ± 84.38	824.85 ± 83.57	736.10 ± 46.46	686.33 ± 61.31	>0.05
Total Protein g dL^{-1}	6.68 ± 0.09^{abc}	7.06 ± 0.17 ^{a}	6.96 ± 0.12 ^{ab}	6.37 ± 0.20 ^{b,c}	6.17 ± 0.34 c	<0.05
Urea nitrogen mg dL^{-1}	23.90 ± 1.29 ^{b}	31.34 ± 0.67 ^{a}	25.25 ± 1.39 ^{b}	19.61 ± 1.13 ^{c}	24.31 ± 0.95 ^{b}	<0.001
Glucose mg dL^{-1}	72.64 ± 2.18 ^{bc}	73.71± 3.57 ^{bc}	84.44 ± 6.32 ^{b}	68.63 ± 4.41 ^{c}	108.03 ± 3.33 ^{a}	<0.001

^{a,b,c}: The difference between values with different letters on the same line is significant. (P<0,05; P<0,01; P<0,001)

C: Control, HP: High protein diet group, HC: Horse chestnut extract group, EA: Ethanolgroup

PTH: Parathyroid hormone, Pi: Inorganic Phosphorus, ALP: Alkaline phosphatase

Although a high-protein diet and horse chestnut extract had no effect on serum Ca levels, ethanol application statistically decreased this parameter when tried to compare to the other groups (control and trial) ($p<0.001$) (Table 3). PTH, calcitonin, osteocalcin, and ALP levels did not change statistically significantly (Table 3). In contrast to the control group, high protein administration did not affect vitamin D levels, but high protein combined with horse chestnut extract and ethanol administration led to a significant rise in vitamin D levels. Serum phosphorus (Pi) levels in animals fed high protein diets were determined to be significantly higher ($p<0.01$) than in the control group, but no statistically significant difference existed between the other experimental groups and the control group (Table 3). Ethanol treated experimental animals' total protein levels were considerably ($p<0.05$) lower than those of high-protein diets (Table 3). Plasma urea nitrogen levels in experimental animals fed high protein were significantly higher ($p<0.001$) than in the control group. While ethanol application had no impact, it was found that horse chestnut extract application significantly lowered plasma urea nitrogen in comparison to the control and high protein diet groups (Table 3). The findings revealed that ethanol treated rats had significantly higher plasma glucose levels ($p<0.001$) than the other groups (Table 3).

DISCUSSION AND CONCLUSION

Horse chestnut extract and a high-protein diet did not alter serum Ca levels in the study, however it was found that the ethanol group had a considerable fall in this parameter. A high protein intake had no effect on serum Ca level, which is in contrast to reports that high protein consumption negatively affected Ca metabolism (Hannan et al. 2000; Ginty, 2003) and protein consumption has no effect on this metabolism (Hunt et al. 1995). This is attributed to the possibility that while the amount of Ca excreted in the urine increases due to protein consumption, the amount of Ca absorbed from the intestines may also increase (Kerstetter at al, 2003). Contrary to reports that the serum Ca level increased in studies where horse chestnut was applied, the horse chestnut extract increased the serum Ca level in this study (Kocaoğlu Güçlü, 2003; Avcı et al. 2007). This may be due to the differences in saponin sources used in the studies.

In agreement with the study's conclusions by Yoshikawa et al. (1994), the blood Ca level was lower in the ethanol - treated group in this study as compared to the control group and the saponin-treated groups. The reduced absorption of ethanol by saponins may be responsible for the lower blood Ca level in the ethanol given group. This finding suggests that by taking saponin in addition to alcohol, the negative effects of alcohol consumption on the skeletal system can be avoided. In our research, it was discovered that a high-protein diet, horse chestnut extract, and the administration of ethanol had no effect on the level of PTH, a hormone that is crucial for controlling bone formation and resorption as well as maintaining extracellular Ca and P homeostasis. Contrary to reports that alcohol consumption lowers blood PTH levels in humans (McCarty and Thomas, 2003), in this study the fact that ethanol had no effect on PTH levels most likely due to the parathyroid gland's inadequate response to blood Ca level.

Consistent with the previous research (Roughead et al., 2003; Hunt et al., 2009) that high protein consumption has no effect on vitamin D levels in this study, this parameter was not different from the control group in animals with high protein consumption. In our research, ethanol administration increased plasma vitamin D levels, contrary to reports that chronic alcohol consumption reduces plasma vitamin D levels in humans (Laitinen et al., 1990) and rats (Shankar et al., 2008). This may be due to the fact that the study period is not very long, the application dose, and possibly the effect of the liver giving more vitamin D to the circulation on the low blood Ca. According to the result of this study, horse chestnut extract had no effect on plasma vitamin D levels, which is in line with the claim made by Coulson and Evans (1960) that vitamin D levels did not change in rats fed with a high ratio of saponins. The decrease in Vitamin D level brought on by the effect of ethanol may have been prevented in the group that received saponin treatment due to aescin's impairment of ethanol absorption (Yoshikawa et al. 1994).

It was found in the study that the applications had no impact on the calcitonin and osteocalcin blood levels. This result was in line with studies showing that ethanol and a high-protein diet (Roughead, 2003; Hunt et al. 2009) had no impact on calcitonin and osteocalcin levels (Sampson et al. 1996). Similar to these findings, there was no change

in ALP activity, demonstrating that a high protein diet, saponin administration, and ethanol administration have no effect on the quantity and functionality of osteoblasts, and consequently, on bone production. Consistent with the results of the study (Graves and Wolinsky, 1980) which stated that P uptake in the body increases when the protein level is increased in the feed of rats, in this study, it was found that high protein consumption increased the serum Pi level. It was shown that administering horse chestnut extract to rats on a diet had no impact.

In the study, it was discovered that high protein intake numerically increased the level of total protein but did not achieve statistical significance. This result is in line with a study by Hunt et al. (1995), which found no differences in total protein levels in the blood of postmenopausal women who consumed high and low levels of meat. It has been suggested that amino acids are the primary source of gluconeogenesis and that amino acid catabolism has a great effect on body energy balance (Reeds et al., 1998). It has been proposed that the decrease in feed consumption caused by high protein consumption may be due to a feeling of satiety (Bensaid et al., 2003), and it has been reported that proteins are more effective than carbohydrates and fats in creating a feeling of satiety (Stubbs and Whybrow, 2004). In the study; it was observed that the administration of horse chestnut extract to both rats fed with high protein and rats fed with control food had no effect on the total protein level. According to Potter et al. (1993) that saponins inhibit the digestion of proteins in the digestive tract by forming indigestible complexes with proteins, but that this effect varies depending on the protein source in the diet, it is possible that the lack of effect of saponins on total protein levels is due to the protein source used.

High protein diet increased blood urea nitrogen levels in this study, which is consistent with studies showing that it boosts ureogenesis and gluconeogenesis in the liver (Peret et al.1984; Visek, 1984). This conclusion supports the findings of study by Hunt et al. (1995), which showed that extra proteins consumed by the body are converted to energy (Ersoy and Baysu, 1986). In our study; consistent with the reports that saponin applications reduce blood urea nitrogen levels in rats (Preston et al. 1987; Potter et al. 1993; Killeen et al. 1998), giving horse chestnut extract to rats on both a

high-protein diet and a control diet resulted in a significant decrease in the mentioned parameter. According to the study's findings, rather than the development of indigestible complexes with proteins in the digestive tract, the decrease in blood urea nitrogen level caused by saponin applications can be attributed to the inhibition of nitrogenous substances during their microbial digestion in the large intestines of rats or to their low absorption by binding ammonia formed as a result of degradation (Killeen et al. 1998; Francis et al. 2002).

In this research, it was discovered that while administering high protein and horse chestnut extract had no effect on the plasma glucose level, administering ethanol had a substantial impact on this value. This result was consistent with Yoshikawa et al. (1994), which found that ethanol raises blood glucose levels whereas horse chestnut aescin reduces the effect of ethanol.

In this study, it was found that a high protein diet and a horse chestnut ethanol extract had no effect on the hormones that control blood calcium levels or the levels of blood calcium, whereas ethanol administration decreased blood calcium levels. However, it is thought that the negative effect of alcohol on bone metabolism is not due to the secondary effect of hormones that regulate bone mineral status.

The administration of horse chestnut extract will decrease the nitrogen breakdown or ammonia absorption in the intestines, and decrease the blood urea nitrogen level and reduce the negative effect of alcohol on glucose metabolism. It was determined that administering horse chestnut extract along with a high protein diet would reduce the rate of nitrogen breakdown or ammonia absorption in the intestines, lower the level of blood urea nitrogen, and lessen the detrimental effects of alcohol on glucose metabolism.

It was concluded that the findings obtained in this study will contribute to important data and scientific interpretations for new researches to clarify the physiological and biochemical changes in bone and calcium metabolism due to high protein or alcohol consumption and the effects of saponins on these changes, and the mechanisms involved in the cause-effect relationship the mechanisms.

ACKNOWLEDGEMENT

We thank Afyon Kocatepe University Scientific Research Projects Commission for their support.

REFERENCES

Addisu S, Assefa A. Role of plant containing saponin on livestock production; a review. Advances in Biological Research, 10(5), 2016; 309-314.

Avcı G, Küçükkurt İ, Kontaş T, Eryavuz A, Fidan, A. F. Tavşanlarda rasyona ilave edilen farklı miktarlardaki Yucca Schidigera ekstraktının (de-odorase®) bazı serum makro ve mikro element düzeylerine etkisi. Fırat Üniversitesi Sağlık Bilimleri Dergisi, 2007; 21(6), 257-262.

Bensaid A, Tomé D, L'Heureux-Bourdon D, Even P, Gietzen D, Morens C et al. A high-protein diet enhances satiety without conditioned taste aversion in the rat. Physiology & behavior, 2003; 78(2), 311-320.

Cheeke PR. Toxicants of Plant Origin Volume II Glycosides, Boca Raton CRC Press: Boca Raton, FL, USA. 1998; pp. 104-126.

Chow WH, Gridley G, McLaughlin JK, Mandel JS, Wacholder S, Blot, WJ, et al. Protein intake and risk of renal cell cancer. JNCI: Journal of the National Cancer Institute, 1994; 86(15), 1131-1139.

Cigerci IH, Fidan AF, Konuk M, Yuksel H, Kucukkurt I, Eryavuz A, Baysu et al. The protective potential of Yucca schidigera (Sarsaponin 30®) against nitrite-induced oxidative stress in rats. Journal of natural medicines, 2009; 63(3), 311-317.

Coulson CB, Evans RA. The effect of saponin, sterols and linoleic acid on the weight increase of growing rats. British Journal of Nutrition, 1960; 14(2), 121-134.

Das TK, Banerjee D, Chakraborty D, Pakhira MC, Shrivastava B, Kuhad RC. Saponin: role in animal system. Veterinary World, 2012; 5(4), 248.

Desai SD, Desai DG, Kaur H. Saponins and their biological activities. Pharma Times, 2009; 41(3), 13-16.

Drăghici LR, Hădărugă, DI, Hădărugă NG. Aesculus species: a review on biologically active compounds and their possible applications. Journal of Agroalimentary Processes and Technologies, 2020; 26(4), 422-428.

Ersoy E, Bayşu N. Biyokimya, Ankara Üniversitesi Veteriner Fakültesi Yayınları No: 408 Ders Kitabı. Ankara, 1986; 520-521.

Fidan AF, Dündar Y. Yucca schidigera ve içerdiği saponinler ile fenolik bileşiklerinin, hipokolesterolemik ve antioksidan etkileri (derleme). Lalahan Hayvancılık Araştırma Enstitüsü Dergisi, 2007; 47(2), 31-39.

Francis G, Kerem Z, Makkar HP, Becker K. The biological action of saponins in animal systems: a review. British journal of Nutrition, 2002; 88(6), 587-605.

Gallelli L. Escin: A review of its anti-edematous, anti-inflammatory, and venotonic properties. Drug design, development and therapy, 2019; 13, 3425.

Ginty F. Dietary protein and bone health. Proceedings of the Nutrition Society, 2003; 62(4), 867-876.

Graves KL, Wolinsky I. Calcium and phosphorus metabolism in pregnant rats ingesting a high protein diet. The Journal of Nutrition, 1980; 110(12), 2420-2432.

Hannan MT, Tucker KL, Dawson-Hughes B, Cupples LA, Felson DT & Kiel DP. Effect of dietary protein on bone loss in elderly men and women: the Framingham Osteoporosis Study. Journal of Bone and Mineral Research, 2000; 15, 2504–2512.

Hosstettman K, Marston A. Saponins, Cambridge University Pres. 1995; pp.239-282.

Hunt JR, Gallagher SK, Johnson LK, Lykken GI. High-versus low-meat diets: effects on zinc absorption, iron status, and calcium, copper, iron, magnesium, manganese, nitrogen, phosphorus, and zinc balance in postmenopausal women. The American journal of clinical nutrition, 1995; 62(3), 621-632.

Hunt JR, Johnson LK, Fariba Roughead ZK. Dietary protein and calcium interact to influence calcium retention: a controlled feeding study. The American journal of clinical nutrition, 2009; 89(5), 1357-1365.

Kerstetter J E, O'Brien KO, Insogna KL. Dietary protein, calcium metabolism, and skeletal homeostasis revisited. The American journal of clinical nutrition, 2003; 78(3), 584S-592S.

Kerstetter JE, O'Brien KO, Caseria D M, Wall DE, Insogna, KL. The impact of dietary protein on calcium absorption and kinetic measures of bone turnover in women. The journal of clinical endocrinology & metabolism, 2005; 90(1), 26-31.

Killeen GF, Connolly CR, Walsh GA, Duffy CF, Headon DR, Power RF. The effects of dietary supplementation with Yucca schidigera extract or fractions thereof on nitrogen metabolism and gastrointestinal fermentation processes in the rat. Journal of the Science of Food and Agriculture, 1998;76(1), 91-99.

Ko GJ, Rhee, CM, Kalantar-Zadeh K, Joshi S. The effects of high-protein diets on kidney health and longevity. Journal of the American Society of Nephrology, 2020; 31(8), 1667-1679.

Kocaoğlu Güçlü B. Bıldırcın rasyonlarına katılan Yucca schidigera ekstraktının yumurta verimi ve yumurta kalitesi ile bazı kan parametrelerine etkisi. Turkish Journal of Veterinary and Animal Sciences, 2003; 27(3), 567-574.

Koçkar ÖM. At kestanesi ekstraksiyonu ve essin izolasyonu. Doktora Tezi- Anadolu Üniversitesi, Fen Bilimleri Enstitüsü, Eskişehir, 1989.

Küçükkurt İ, Fidan A F. Saponinler ve bazı biyolojik etkileri. Kocatepe Veterinary Journal, 2008; 1(1), 89-96.

Laitinen K, Välimäki M, Lamberg-Allardt C, Kivisaari L, Lalla, M, Kärkkäinen, M, et al. Deranged vitamin D metabolism but normal bone mineral density in Finnish noncirrhotic male alcoholics. Alcoholism: Clinical and Experimental Research, 1990; 14(4), 551-556.

Linn T, Geyer R, Prassek S, Laube H. Effect of dietary protein intake on insulin secretion and glucose metabolism in insulin-dependent diabetes mellitus. The Journal of Clinical Endocrinology & Metabolism, 81(11), 1996; 3938-3943.

McCarty MF. Thomas CA, PTH excess may promote weight gain by impeding catecholamine-induced lipolysis-implications for the impact of calcium, vitamin D, and alcohol on body weight. Medical hypotheses, 2003; 61(5-6), 535-542.

Neupane SP, Lien L, Hilberg T, Bramnes, JG. Vitamin D deficiency in alcohol-use disorders and its relationship to comorbid major depression: a cross-sectional study of inpatients in Nepal. Drug and alcohol dependence, 2013; 133(2), 480-485.

Özdamar K. SPSS ile Biyoistatistik. 5. Baskı. Kaan Kitap Evi, Eskişehir, 2003.

Peret J, Bach A, Delhomme B, Bois-Joyeux B, Chanez M, Schirardin H. Metabolic effects of high-protein diets in Zucker rats. Metabolism, 1984; 33(3), 200-207.

Potter SM, Jimenez-Flores R, Pollack J, Lone TA, Berber-Jimenez MD. Protein-saponin interaction and its influence on blood lipids. Journal of Agricultural and Food Chemistry, 1993; 41(8), 1287-1291.

Preston RL, Bartle J, May, T, Goodall SR. Influence of sarsaponin on growth, feed and nitrogen utilization in growing male rats fed diets with added urea or protein. Journal of Animal Science, 1987; 65(2), 481-487.

Rakhra V, Galappaththy SL, Bulchandani, S, Cabandugama, PK. Obesity and the Western diet: how we got Here. Missouri Medicine, 2020; 117(6), 536.

Reeds PJ, Burrin DG, Davis TA, Stoll B. Amino acid metabolism and the energetics of growth. Arch. Anim. Nutr., 1998; 51: 187–197.

Roughead ZK. Is the interaction between dietary protein and calcium destructive or constructive for bone?: Summary. The Journal of Nutrition, 2003; 133(3), 866S-869S.

Sağdıçoğlu AG. Aesculus hippocastanum özütlerinden aescinin mikrozomal lipid peroksidasyon üzerindeki etkileri. Yüksek Lisans Tezi- Orta Doğu Teknik Üniversitesi, 2000; Ankara.

Sampson HW, Perks N, Champney TH, DeFee B. Alcohol consumption inhibits bone growth and development in young actively growing rats. Alcoholism: clinical and experimental research, 20(8), 1996; 1375-1384.

Shankar K, Liu X, Singhal R, Chen JR, Nagarajan S, Badger TM, et al. Chronic ethanol consumption leads to disruption of vitamin D3 homeostasis associated with induction of renal 1, 25 dihydroxyvitamin D3-24-hydroxylase (CYP24A1). Endocrinology, 2008; 149(4), 1748-1756.

Sirtori CR. Aescin: pharmacology, pharmacokinetics and therapeutic profile. Pharmacological Research, 2001; 44(3), 183-193.

Stubbs RJ, Whybrow S. Energy density, diet composition and palatability: influences on overall food energy intake in humans. Physiology & behavior. 2004; 81(5), 755-764.

Tatar M. Proteinden yetersiz yemle beslenen sıçanların kan ve doku serbest radikal ve antioksidan düzeylerinin araştırılması. Doktora Tezi, Yüzüncü Yıl Üniversitesi Sağlık Bilimleri Enstitüsü, Van, 2003.

Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Science translational medicine, 2009; 1(6), 6ra14-6ra14.

Visek WJ. Ammonia: its effects on biological systems, metabolic hormones, and reproduction. Journal of Dairy Science, 67(3), 1984; 481-498.

Yadav N, Singh AP, Rana AC, Kumar S, Kaur P. Singh J et al. Aesculus indica: an updated review on its pharmacognosy, phytochemistry and pharmacological profile. Egyptian Journal of Basic and Applied Sciences, 2022; 9(1), 125-135.

Yoshikawa M, Harada E, Murakami T, Matsuda H, Wariishi N, Yamahara J, et al. Escins-Ia, Ib, IIa, IIb, and IIIa, bioactive triterpene oligoglycosides from the seeds of Aesculus hippocastanum L.: their inhibitory effects on ethanol absorption and hypoglycemic activity on glucose tolerance test. Chemical and pharmaceutical bulletin, 1994; 42(6), 1357-1359.

INVESTIGATION OF YEAST AND MOLD DETECTION AND AFLATOXIN TYPES IN RAW NATURAL NUTS IN SAKARYA REGION

Samet KORKMAZ* **Murat YILDIRIM** **Sibel KIZIL**

Kırıkkale University, Faculty of Veterinary Medicine, Department of Microbiology, Kırıkkale, Türkiye

*E-mail: *korkmazsamet@hotmail.com*

The aim of our study is to investigate the province of Sakarya Region, where hazelnut cultivation is intensive. Mold and yeast detection in raw natural hazelnuts exported to the detection and typing of the presence of aflatoxin using the method. In terms of aflatoxin, no toxin was detected in the study conducted with the HPLC method. Growth was detected in 74 (72,54%) of the 102 samples has been done. *Penicillium* spp. was statistically the most common ($p<0.05$). Colony mean value was also determined as *Penicillium* spp. It was statistically significantly higher than other species ($p<0.05$). The data obtained in the study is important in terms of showing the importance of providing suitable conditions for hazelnut production, storage and trade in Sakarya Region.

Keywords: Yeast; Mold; Aflatoxin; Hazelnut; HPLC.

INTRODUCTION

Mycotoxins are toxic metabolites synthesized by various types of pathogenic fungi that, when ingested, cause latent, acute or chronic intoxication in humans and animals. Among mycotoxins, aflatoxins are known as the most potent biological carcinogens. Aflatoxigenic molds develop and form aflatoxin when they find more suitable humidity and temperature in the products they are contaminated with. The composition of the contaminated food, the storage period, damage caused by insects, damage to the shell

are other factors affecting mold growth and toxin formation (Tunail, 2000). The interaction of these factors can lead to different results on mold growth and toxin formation. For this reason, after toxin formation, the separation and removal of contaminated grains from other grains depends on different stages. Comprehensive studies have been conducted on the effect of additional treatments on the aflatoxin risk-reducing effect in nuts, on peanuts and almonds (Tiryaki et al., 2011; Şen and Nas, 2010).

Hazelnut and its by-products, which are frequently used in the nut industry and food industry, are also used as animal food in veterinary medicine recently. The pulp remaining from the hazelnut, which is processed by removing the oil, is rich in protein (Doğan and Bircan, 2010). It is thought that hazelnut cultivation in our country creates a great added value and will have an important share in the production of healthy animal foods because of the high hazelnut harvest. There are limited number of scientific publications for our country in yeast and mold detection studies on hazelnut. In this study, information on hazelnut yeast and mold will be created and will be an important source for future studies.

MATERIAL AND METHODS

Samples: A total of 102 different samples were tested in terms of mold, yeast and aflatoxin, selected from natural raw hazelnut samples in packages in order to carry out the necessary controls in export by the members of Sakarya Commodity Exchange. Between February and July 2020, the raw hazelnut samples of 2019 crop brought to Adapazarı Commodity Exchange Special Food Control Laboratory were analyzed.

Sample Preparation for Aflatoxin Determination by HPLC Method: The conformity of the analysis sample accepted in accordance with the "Samples Acceptance, Transport and Storage Procedure (ATB.Pr.18)" was checked by the Department Officer and it was subjected to grinding process as defined in the relevant procedure and homogeneously prepared.

Yeast and Mold Detection Method: Dichloran 18 % (mass concentration) glycerol for isolation as recommended according to ISO 21527 -2, agar (DG 18) medium was used. 0.1 ml was taken from the prepared 10-1 samples and poured into petri dishes containing DG 18 medium transferred. In addition, serial dilutions were prepared at the

required levels and inoculated into DG 18 medium. The samples transferred to petri dishes were spread with the help of a drigalski spatula. It was incubated for 7 days in an incubator at 25±1 ºC. Small, round, pink colored spots were observed on petri plates after incubation. Colonies with a filamentous center were considered yeast colonies, while colonies with a filamentous center were considered mold. All petri dishes containing suspicious colonies were counted. Fewer than 150 colonies at the time of count petri dishes containing cfu/g or cfu/ml were reported.

For microscobic examination are used lactophenol cotton blue dyeing method.: In order to examine the hyphae and spores of fungi that reproduce in mold style. Since lactophenol was preferred, lactophenol blue was used in the staining of the preparations in our study we used. A drop of lactophenol on a clean slide for preparation of preparations for examination. It was placed in a cotton blue solution. The adhesive side of the cellophane tape is produced in the petri dish. Lactophenol on the slide after contact on the fungal colony cotton blue was pressed, allowing the adhesive part to adhere to the surface of the slide. Then examine it under the microscope.

Microscopic examination was performed with an Olympus CX21 light microscope. 4X, 10X, 40X and Images were taken with 100X lenses and photographed with a mobile phone lens. Records received "Species identification by comparing microscopy pictures of identified *Aspergillus* samples in the book “Atlas of Fungal Infections, Ed: C. Kauffmann, 2 nd edt, Springer’ done. Those whose species could not be definitively identified were reserved for detailed identification

HPLC: Fluorescent detector was used for mycotoxin analysis by HPLC. In the fluorescent detector, a long wavelength monochromatic beam was sent into the cell as dissolved compounds in the mobile phase passed. This radiation absorbed by the compound was then given back at another wavelength. This emission in fluorescence measurement was evaluated for analysis.

RESULTS

In terms of aflatoxin, no toxin was detected in the study conducted with the HPLC method. Growth was detected in 74 (72,54%) of the plantings made to the petri dishes for 102 samples has been done. *Penicillium* spp. (figure 1A) in 40 (54.05%) of 74 petri dishes, *Aspergillus fumigatus* (figure 1B) in 11 (14.86%), *Penicillium* spp. and

Aspergillus spp. in 7 (9.45%), and 10 (13.51%) of 74 petri dishes in microscopic examination *Aspergillus flavus* was identified by detecting growth of *Aspergillus niger*. (figure 1C) In 6 (8.01%) samples whose species could not be determined, the others were rare species evaluated as a separate group in the category.

The data obtained in the study were statistically analyzed with the help of SPSS analysis program. Accordingly, if the p value is less than 0.05, it is statistically significant. According to this, distribution statistics among species, average of colony numbers is important.

It was evaluated whether the average number of colonies differed among the species. Determining the presence of yeast-mold in raw hazelnuts by HPLC method and classical yeast-mold growing methods and efficiencies were compared. According to analysis results of classical reproduction method; *Penicillium* spp. was statistically the most common species in all. ($p<0.05$) Colony mean value was also determined as Penicillium spp..it was statistically significantly higher than other species. ($p<0.05$).

The classical medium method is statistically significantly more effective than the HPLC method in the detection of yeast and mold. ($p<0.05$).

In terms of aflatoxin, no toxin was detected in the analysis of 102 products made by HPLC method.

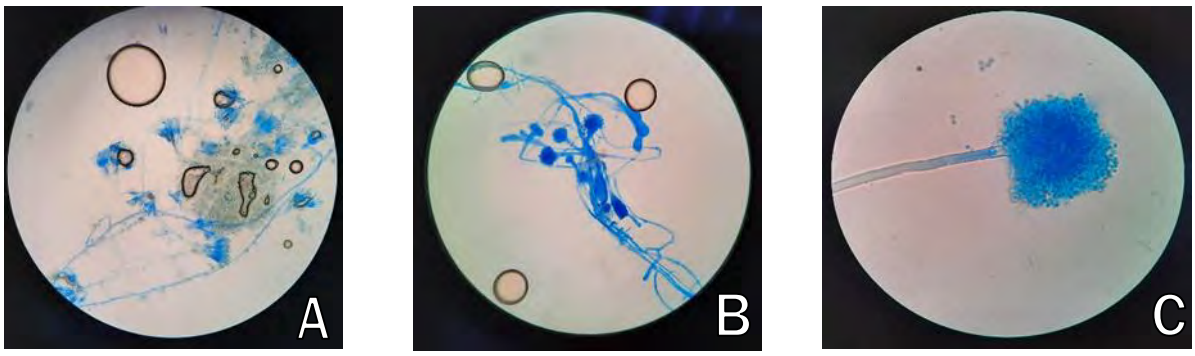


Figure 1. A. *Penicillium* spp. lactophenol blue was used in the staining and images were taken with 100X lenses, photographed with a mobile phone lens. **B.** *Aspergillus fumigatus*, lactophenol blue was used in the staining and images were taken with 100X lenses, photographed with a mobile phone lens. **C.** *Aspergillus niger*, lactophenol blue was used in the staining and images were taken with 100X lenses, photographed with a mobile phone lens.

DISCUSSION AND CONCLUSION

In our study, in terms of the presence of yeast-mold on raw hazelnuts in Sakarya Region, the most the determined species was *Penicillium* spp. Again, the highest colony reproduction average. It was found to be *Penicillium* species.

Agricultural products are contaminated with different molds depending on the environment and environmental conditions, the composition of the agricultural product and the water content, starting from the harvest and during the processing and storage stages. Contamination is important for two sides. Until recently, it was thought that the presence of mold in agricultural products only causes deterioration, loss of nutritional value of the product, and a decrease in the germination ability of the grains. The economic effects of this situation are becoming increasingly important today (Girgin et al., 2001). Another important point is the negative effects on human and animal health.

When we look at the literature, there are publications that a mysterious disease caused the death of more than 100,000 turkeys in the north and south of England in the spring and summer of 1960. This disease, which also affects ducks and pheasants, was named “Turkey X Disease”. It was realized that this disease was of nutritional origin, as changing the diet reduced the morbidity to mortality rate, and it was determined that the diet of all affected animals was Brazilian peanuts contaminated with *Aspergillus flavus* and containing the toxic substance called “aflatoxin” (Pickova et al., 2021).

In the studies conducted on this subject, it has been determined that the highest growth rate in hazelnuts is Penicillium spp. if the relative humidity in the air is above 85% and the humidity in the grains is above 18%, and if the product is stored at low temperatures (Gilbert and Anklam, 2002). In our study, we aimed to determine the amount of aflatoxin in hazelnuts around Sakarya Region. In this way, it will be possible to re-evaluate the storage conditions in this region and to provide suitable conditions.

In addition, as we found in our study, the most effective method for detecting fungi and other pathogens is the classical fattening method. Although new technologies have developed, it has shown that classical methods are more effective in this regard. This situation will be a guide in deciding the methods to be used in the examination of aflatoxin in food products. Future studies in this direction will provide more information.

In our study, the detection of pathogens responsible for aflatoxin production in hazelnuts with the classical method is important in terms of paying attention to industrial drying, processing and storage methods in hazelnut producers and processing factories. In this sense, the data obtained from the producers and the traders who prov8ide the storage conditions will be shared for the development of hazelnut farming in our region and will guide different studies to be done in the future. Continuity of yeast-mold and aflatoxin analyzes of hazelnut is important in terms of improvement of storage conditions (lycenced storage) and economic gain. The presence of *Aspergillus* species can cause serious public health problems.

ACKNOWLEDGEMENT

Sakarya Commodity Exchange Board of Directors, (ATB) Adapazarı Commodity Exchange Special Food Control Laboratory Departmant of Microbiology.

REFERENCES

Doğan, G. ve Bircan, R., Balık yemlerinde alternatif bitkisel protein kaynağı olarak fındık küspesi kullanımı. Mehmet Akif Ersoy Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 2: 49 -57, 2010

Gilbert, J., Anklam, E., Validation of analytical methods for determining mycotoxins in foodstuffs. *Trends Anal. Chem.,* 21, 468-486,2002.

Gözde Girgin, Nurşen Başaran, Gönül Şahin. Mycotoxins in Turkey and The World. Turk Hij Den Biyol Derg. 2001; 58(3): 97-118

Pickova D, Ostry V, Toman J, Malir F. Aflatoxins: History, Significant Milestones, Recent Data on Their Toxicity and Ways to Mitigation. Toxins (Basel). 2021;13(6):399. doi: 10.3390/toxins13060399.

Şen, L., ve Nas S., Fındık ve Antepfıstığının Mikotoksin Problemi. Gıda Teknolojileri Elektronik Dergisi, 5 (1), 49-56, 2010

Tunail N, Funguslar ve Mikotoksinler, Gıda Mikrobiyolojisi ve Uygulamaları, 2000. Genişletilmiş 2. Baskı; Ankara Üniversitesi Ziraat Fakültesi Gıda Mühendisliği Bölümü yayını. Sim Matbaası, Ankara 522 s

Tiryaki O, Seçer E. ve Temur C., Yemlerde mikotoksin oluşumu, toksisiteleri ve mikotoksin kalıntı analizleri, Anadolu Journal of Agricultural Sci., 21 81), 44–58, 2011.

PATHOLOGICAL FINDINGS OF UNILATERAL CHRONIC GRANULOMATOUS ORCHITIS AND ITS MANAGEMENT IN MINI POMERANIAN DOG WITH CRYPTORCHID: A CASE REPORT

Dzikri Nurma'rifah TAKARIYANTI[1] Archie Leander MASLIM[2]
Palagan Senopati SEWOYO[3] Marissa Divia DAYANTI[4] I Wayan BATAN[5]

[1] *Veterinary Practitioner, Healthy Pet Clinic, Bandung, Indonesia*
[2] *Veterinary Practitioner, Archie Veterinary Clinic, Bandung, Indonesia*
[3]*Master's Student, Postgraduate School, Faculty of Veterinary Medicine, Udayana University, Bali, Indonesia*
[4] *Veterinary Student, Faculty of Veterinary Medicine, Udayana University, Bali, Indonesia*
[5] *Laboratory of Veterinary Clinical Diagnosis, Clinical Pathology and Radiology, Faculty of Veterinary Medicine, Udayana University, Bali, Indonesia*

*E-mail: dzikritakariyanti@gmail.com

A 10-year-old male mini pomeranian dog with 3 kg body weight was brought to the Archie Veterinary Clinic, Bandung with a progressive enlargement of a lump in the right inguinal area. Clinical examination showed that the dog had bilateral cryptorchid congenitally. The lump on inguinal area was growing within two weeks. There was heat and pain on palpation of the lump. Treatment of this case was performed by orchiectomy. The testis was collected to perform histopathological examination. Based on histopathological findings, there were accumulation of the epithelioid histiocytes, lymphocytes and plasma cells in the seminiferous tubules creates an appearance of granuloma. The dog was diagnosed with chronic granulomatous orchitis involving right testes (unilateral). The dog had an uneventful recovery and skin sutures were removed on the fifth day.

Keywords: Cryptorchid; Dog; Orchitis; Orchiectomy

INTRODUCTION

Cryptorchid is the most common congenital defect found in male dogs and cats. Cryptorchid is a condition in which one or both testicles fail to descend from abdomen into the scrotum. Testis that fail to descend into the scrotum increase the risk of neoplasia. This occurrence is most common in dogs and horses. Dogs with this condition can decrease fertility rates and even become infertile (Bufalari et al., 2015). Although some dogs with cryptorchids can still mate with bitches, it is likely in similar events can occur in genetically conceived offspring (Prasad et al., 2017).

Orchitis or inflammation of the testicles, mostly followed by epididymitis. Orchitis generally results from the expansion of infection originating from the epididymis (Foster, 2014). Orchitis can occur separately from epididymitis but occurs concurrently (Kustritz, 2006). Orchitis is uncommon in dogs. One of the most common causes of orchitis is bacteria originating from the prostate or urethra via the testicular ducts. Trauma can also cause orchitis (Kinns and Nelson, 2018). Trauma that causes rupture of the capsule of the testicle can cause inflammation and destruction of testicular tissue. Inflammation of the testes and surrounding tissues will increase the temperature of the testes drastically, as a result, damage to spermatogenic cells and cause testicular atrophy which is usually irreversible (Kustritz, 2006).

The clinical manifestation of orchitis can be enlargement of the testes that resembles neoplasia, but more fluid is present in orchitis (Ober et al., 2004). Common causes of orchitis in dogs include direct trauma, possibly as a result of a fight or accident, and infection. Bacteria associated with orchitis include Escherichia coli, Staphylococcus spp., Streptococcus spp., Proteus spp., Mycoplasma, and other bacteria that are normal flora in the male reproductive tract (Kustritz, 2006). Failure of eliminating the agent causing an acute inflammation such as infectious agents that mentioned before, it may lead to chronic inflammation. One type of chronic inflammation is granulomatous inflammation. The granuloma is a focal aggregate of immune cells that forms in response to a persistent inflammatory stimulus. This article reports the pathological finding and treatment of unilateral chronic granulomatous

orchitis in a mini pomeranian dog with bilateral cryptorchids. Based on the author's knowledge, there is no report of granulomatous orchitis case in cryptorchid dogs.

CASE REPORT

Signalment and anamnesis: A 10-years-old male mini pomeranian dog, weighing 3 kg was presented to Archie Veterinary Clinic in Bandung, Indonesia for evaluation of the 2-weeks history of enlargement mass on the abdomen.

Clinical examination: clinico-physical examination revealed that the rectal temperature of the dog was 39.2℃, heart rate was 128 bpm, respiration rate 25 times/minute, and capillary refill time was under two seconds. Other findings include the absence of testicles in the scrotum and a large mass on the right inguinal region. On scrotal palpation, the dog was bilaterally cryptorchid and the inguinal mass was firm and there were signs of inflammation such as heat and pain when palpated.

Histopathological finding: Accumulation of epithelioid histiocytes, lymphocytes and plasma cells in the seminiferous tubules creates an appearance of granuloma. All seminiferous tubules are necrotic and have lost nuclear staining.

Diagnosis and Prognosis: The diagnosis was made by the histopathological finding of the testicular tissue is chronic granulomatous orchitis. Based on the animal general condition, the prognosis was good.

Figure 1. The physical condition of the lump on the right inguinal of the dog (arrow).

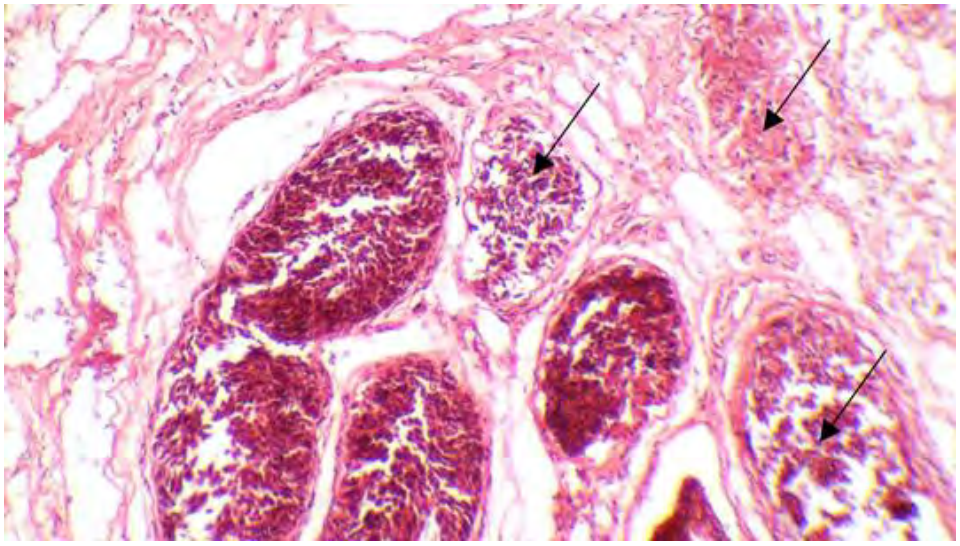


Figure 2. Granulomatous orchitis is characterized by tubular granulomas (arrow). All seminiferous tubules appeared necrotic and have lost cell nuclear staining.

TREATMENT

Treatment in this case is done by surgical removal of both testes or orchiectomy. Orchiectomy in this case was performed by incising the inguinal part of the dog because both testes did not descend into the scrotum or cryptorchid. The dog has fasted for 12 hours before the operation. Anesthesia used is general anesthesia with a combination of xylazine and ketamine. Premedication using atropine sulfate 0.02 mg/kg body weight/BW (V-tropin, Agrovetmarket, Lima, Peru) was injected subcutaneously, 10 minutes later followed by induction of general anesthesia using a combination of xylazine 2 mg/kg BW (Xyla, Interchemie, Metalweeg, Holland) and ketamine 10 mg/kg BW (Ket-A-100, Agrovetmarket, Lima, Peru) injected intramuscularly.

Pre-operatively, shaving the inguinal area to be incised and then prepared using chlorhexidine. After the animal is ready and has been anesthetized, the animal is placed on the operating table in a dorsal recumbency position and the four extremities are fixed using a simple rope knot (tomfool's knot) at each end of the operating table. Before the operation, the right inguinal area to be incised was prepared aseptically with 70% alcohol and 3% iodine tincture solution.

Orchiectomy in the treatment of cryptorchids is performed with the right inguinal incision. The incision is made at the location of the testicle, localized using a finger in order to fix the testicle to make skin and subcutaneous incisions easier. After the skin and subcutaneous incisions, tunica dartos, and spermatic fascia then proceed to incise the tunica vaginalis just above the testes, so that the testes and epididymis are no longer covered. Then the testicle that came out of the incision hole was pulled out slowly, the funiculus spermaticus was ligated using the three forceps tie method using a polyglycolic acid 3.0 suture (RTMed, Shandong Haidike Medical Product Co., Ltd Shandong, China) made between the second and third clamp arteries. If the bond is strong (no blood comes out) then the artery clamp is released and flushed using a 0.9% natrium chloride solution (Sodium Chloride 0.9%, PT. Widarta Bakti, Pasuruan, Indonesia) and the antibiotics penicillin-streptomycin (Penstrep-400, Interchemie, Metalweeg, Holland).

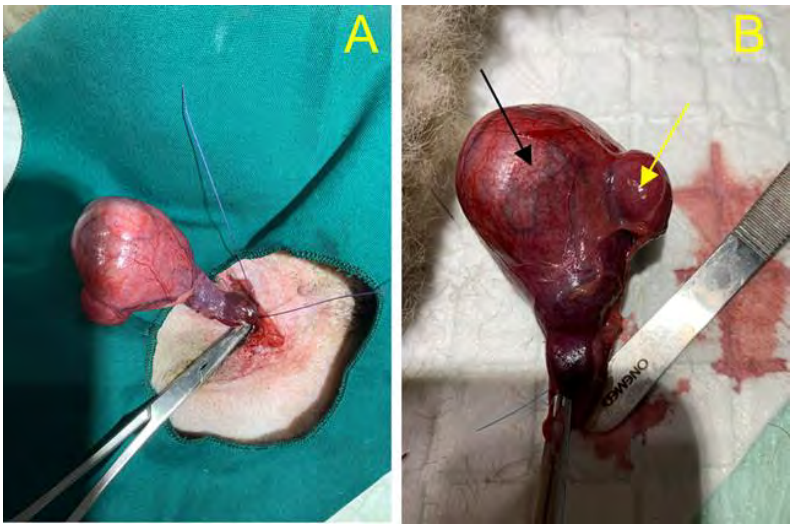


Figure 3. (A) Surgical removal of the testis with orchiectomy. (B) The orchitis testes was seen with shape abnormality (black arrow). The orchitis testes appeared large compared to the normal testes (yellow arrow).

After the testicle is removed, it can be seen that both the right and left testicles are attached with the swollen right testicle or orchitis but the left testicle still looks normal in size. Then the subcutaneous tissue was closed using a 3.0 polyglycolic acid suture (RTMed, Shandong Haidike Medical Products Co. Ltd, Shandong, China) in a subcuticular pattern. The skin was sutured using a non-absorbable suture 3.0 nylon (Dermalon, PT. Covidien Indonesia, Jakarta, Indonesia). The surgical area around the suture was smeared with povidone-iodine and given antibiotics bacitracin zinc powder and neomycin sulfate (Enbatic, Erela, Semarang, Indonesia). The postoperative treatment was given by injection of long-acting antibiotic amoxicillin 10 mg/kg BW (Intramox-150 LA, Interchemie, Metalweeg, Holland) and the analgesic tolfenamic acid (Tolfedine LA 8%, Interchemie, Metalweeg, Holland) on the first, third and fifth days. The dog had an uneventful recovery and skin sutures were removed on the fifth day.

DISCUSSION AND CONCLUSION

The case dog had a congenital defect, cryptorchidism, in which one (unilateral) or both (bilateral) testes fail to descend into the scrotum. Testes that cannot descend into the scrotal space have various anatomical positions including prescrotal, inguinal and intra-abdominal (Thipianog et al., 2019). Cryptorchid itself can be found in pure breeds, cross-breeds and domestic breeds. The most common cases of cryptorchid are found in toy dog breeds such as the Chihuahua, Miniature Schnauzer, Pomeranian, Poodle, Cocker Spaniel, and Yorkshire Terrier. Such a condition will result in a malfunction of the Leydig cell product, i.e., the decreased number of spermatozoa and increased spermatozoa abnormalities (unilateral cryptorchid). In the case of bilateral cryptorchids, the animals will become sterile/major (Memon and Tibiary, 2001).

Orchitis or testicular inflammation is an increase in the size of the testicles in male animals. Inflammation of the testes can be unilateral or bilateral in the testes, arising secondary to trauma, bacterial or viral infections, parasitic infestations, and autoimmune diseases. Observable signs of testicular inflammation are enlarged testicle size, heat, and tenderness to palpation. Bilateral orchitis may confer a poor prognosis for future fertility, localized temperature elevation may result in fibrosis and

degeneration of the testicular parenchyma. However, if one of the testes is unaffected, it may allow the return of the animal's fertility. If fertility of the animal is considered, then treatment must be started as soon as possible. The ideal systemic antibiotic is given with consideration of the culture and sensitivity of the causative organism which can be done by urine culture or semen culture after ejaculation. Administration of anti-inflammatory drugs and cold water therapy can be included in the treatment plan. If one testicle is unaffected but the other has failed therapy can be performed through unilateral castration. After unilateral castration, the unaffected testicle will return to its previous function and fertility can be restored (Turner and Ragoma, 2007).

Bilateral cryptorchid allows animals to be sterile or non-fertile (Laing et al., 1983), therefore orchiectomy or castration indicated to prevent the occurrence of tumors or inflammation that occurs in this case. Experimental study that conducted by Aldahhan et al. (2021) showed that cryptorchidism causes chronic inflammation and a progressive decline in Sertoli cell and Leydig cell function in adult rat testis. Although the causative agent of orchitis has been identified and treatment protocols were carried out, the fertility prognosis of dogs affected by orchitis is poor because of the possibility of irreversible damage to the germinal epithelium, tubular degeneration, development of immune-mediated orchitis (secondary to the blood-testicular barrier). Residual lesions or sequelae can last for months. If fertility is not important, castration is a good option for treating orchitis and epididymitis (Davidson, 2020).

Chronic inflammation occurs when (1) the acute inflammatory response fails to eliminate the inflammatory stimulus/agent, (2) after several episodes of acute inflammation causing extensive tissue damage or (3) in response to unique biochemical substances and/or virulence or microbial factors (Ackermann, 2018). The granulomatous inflammatory response is a special type of chronic inflammation characterized by the presence of macrophages, epithelioid cells, and multinucleated giant cells. This inflammatory response is common in pathology and is a manifestation of various diseases caused by infective, toxic, allergic, autoimmune, neoplastic, or idiopathic conditions. Mononuclear phagocytic cells such as macrophages and

epithelioid cells generally form an aggregate and appear as well-defined focal lesions. Such lesions are called granulomas (Williams and Williams, 1983).

ACKNOWLEDGEMENTS

The authors thank to the owner of the dog described for allowing us to share the dog's details.

FINANCIAL SUPPORT

This research received no grant from any funding agency/sector.

CONFLICT OF INTEREST

The author(s) declared no potential conflicts of interest with respect to authorship and/or publication of this article.

REFERENCES

Ackermann MR, Kinns J, Nelson N. 2018. Uterus, Ovaries, and Testes. In: Textbook Veterinary Diagnostic Radiology, th Ed. Elsevier. UK.

Aldahhan RA, Stanton PG, Ludlow H, de Kretser DM, Hedger MP. 2021. Experimental Cryptorchidism Causes Chronic Inflammation and a Progressive Decline in Sertoli Cell and Leydig Cell Function in the Adult Rat Testis. Reprod. Sci. 28(10): 2916-2928.

Bufalari A, Pronil G, Moretti A, Di Meo A. 2015. The lameness in a cryptorchid dog with intra-abdominal torsion of one of the two neoplastic testicles: a case report. Jurnal Veterinari Medicana 60(8) : 456- 459.

Davidson AP. 2020. Orchitis and Epididymitis in Dogs and Cats. https://www.msdvetmanual.com/reproductive-system/reproductive-diseases-of-the-male-small-animal/orchitis-and-epididymitis-in-dogs-and-cats [Accessed 2 September 2022].

Hayes HM dan Pendergrass TW. 1976. Canine testicular tumors: epidemiologic features of 410 dogs. Int Journal Cancer 18 (4): 482- 487

Kustritz MR. 2006. The Dog Breeder's Guide to Successful Breeding and Health Management. Cambridge Scholars Publishing, UK. Pp. 346.

Laing J, Harari J, Smith CW. 1983. Spermatic Cord Torsion and Sertoli Tumor in a Dog. Journal of American Veterinary Medical Association 183(8): 879-81.

Ober CP, Spaulding K, Breitschwerdt EB. 2004. Orchitis in two dogs with Rocky Mountain spotted fever, Vet Radiol Ultrasound 45(5):458–465.

Prasad VD, Prasad BC, Krishna NVVH, Sreenu M. 2017. Sertoli cell tumor in dogs. Indian Veterinary Journal 4(4): 394-398.

Memon M, Tibiari A. 2001. Cryptorchidism in Dogs and Cats. Advances in Small Animal Reproduction. https://www.ivis.org/library/recent-advances-small-animal-reproduction/canine-and-feline-cryptorchidism [Accessed 2 September 2022].

Turner O, Regina M. 2007. Testicular Abnormalities. In: Current Therapy in Equine Reproduction. Elsevier, UK. Pp. 195-204

Williams GT, Williams WJ. 1983. Granulomatous inflammation–a review. Journal of clinical pathology 36(7): 723-733.

VARIATION OF INTRAOCULAR PRESSURE MEASUREMENTS USING Tono-Pen Vet® AND TonoVet® TONOMETRY IN CALVES

ARTINA PRASTIWI[1*] **Mümin Gökhan ŞENOCAK[1]**

[1] *Ataturk University, Faculty of Veterinary Medicine, Department of Veterinary Surgery, Erzurum, Türkiye*

*E-mail: prastiwiartina@gmail.com

Intraocular pressure (IOP) measurement is an important component of eye assessment. Measurement of IOP is performed using a tonometer that is as part of a thorough intraocular examination. The study aimed to determine and compare the IOP data which obtained with Tono-Pen Vet® and TonoVet® devices from 500 calves. Three to five minutes after the instillation of anesthetic eye drops, IOP readings were measured randomly with Tono-Pen Vet® or TonoVet® tonometer. IOP values were taken without applying any pressure to the neck and head of the calves. Intraocular pressure measurements were always performed by the same operator. A statistical difference was found between tonometer devices ($P<0.01$). The Tono-Pen Vet® overestimated IOP values by an average of 3.8 mmHg compared to the TonoVet® tonometer. The TonoVet® yielded lower values of IOP than the Tono-Pen Vet® tonometer.

Keywords: Calves; Intraocular pressure; Tonometry; Tono-Pen Vet; TonoVet

INTRODUCTION

Based on recent literature studies, there are not currently any studies comparing applanation and rebound tonometry on a very large number calves from different breeds. Therefore, the basis of this study was carried out on large-scale animals to

determine whether there was a difference in IOP values in calves using Tono-Pen Vet® and TonoVet® tonometry randomly.

Ocular squamous cell carcinoma, orbital lymphosarcoma, and pink eye (infectious keratoconjunctivitis of cattle) are the most common eye diseases in cattle (Davidson and Pickett, 2009). Eye diseases are important that can lead to serious health problems in cattle breeding and also cause great economic losses (Potter et al., 2008). However, ocular disorders cause vision problems, pain, and blindness (Davidson and Pickett, 2009). Intraocular pressure (IOP) is a systemic eye examination (Giannetto et al., 2009; Park et al., 2011; Pereira et al., 2011), if the eye pressure is suddenly rising rapidly and there is intense eye pain, this is usually an important symptom of eye problems (Renwick and Petersen-Jones, 2009). Glaucoma is known as increased intraocular pressure (Akın and Samsar, 2005). Glaucoma (eye pressure) is a very serious eye disease that can lead to vision loss and even blindness, following increased intraocular pressure from damage to the optic nerve of the eye (Renwick and Petersen-Jones, 2009).

Intraocular pressure measurement is one of the important parameters used not only for the diagnosis of glaucoma, but also to determine the development of eye diseases (Arıtürk, 2006). Despite the low incidence of glaucoma in cattle and calves (Gum et al., 1998; Townsend, 2008; Pearce and Moore, 2014), eye diseases such as glaucoma can cause serious and advanced diseases if not treated (Gelatt et al., 2007). Treatment results may not always be positive due to the delayed of treatment of some ocular lesions. Therefore, it becomes more important to know the value of IOP and the factors affecting its measurement (Arıtürk, 2006). For this reason, abnormalities detected in the early stages of the disease can be healed by intervention without the need for treatment.

Ocular disorders in all animals need to be treated by veterinarians, and it is important that these disorders can be evaluated by measuring IOP and tear production. For this reason, information about normal IOP values of patients brought for examination should be known by veterinarians. In the last few years, normal mean values of IOP have been reported in domestic animals (Ghaffari et al., 2011;

Babrauskıenė et al., 2018; Kurt et al., 2018; Kulualp et al., 2019). However, reports of normal values of IOP in calves are rare (Townsend, 2008).

The purpose of this study was to determine and compare the values of IOP by using Tono-Pen Vet® and TonoVet® tonometry in 500 calves from different breeds. Hypothesis of study that there were IOP values differences results obtained from two different tonometers in calves.

MATERIAL AND METHODS

Animals: This study was carried out on a total of 500 calves from different breeds. Calves that received any treatment after birth were not included in the study group. This study was conducted at Aksüt and Dutpınar dairy farm, Malatya. This type of research was experimental with randomized measurement on the eye-side in calves after the administration of anesthetic eye drops.

Ethics Committee Approval: This study was completed within the framework of experimental design protocol dated 26.06.2020 and numbered 7 approved by Atatürk University Animal Experiments Local Ethics Committee (HADYEK).

Experiments: A total of 500 healthy calves was examined individually at the dairy owner's request as part of a general health check. Routine eye examinations were performed on calves for pupillary light reflex, vision and threat response tests. Following general physical evaluations, Schirmer test (Tear Flo®, Rose Stone Enterprises, India) is applied. IOP values were measured without applying pressure to the head and neck regions of the calves. Intraocular pressure measurements were always performed by the same operator.

Two drops of local anesthetic eye drops contained 2% lidocaine were applied to right and left eyes. After 3-5 minutes of eye drops instillation, intraocular pressure measurement was performed randomly by using TonoVet® (Icare, Vantaa, Finland) or Tono-Pen Vet® (Reichert, Depew, New York, USA). The mean IOP value from the right and left eye were recorded.

Statistical analysis: SPSS program (IBM Company, Version 23, SPSS Inc. USA 2015) was used for statistical analysis. Statistically significance was accepted at

P<0.05. Statistical evaluation was performed using paired t-test and simple linear regression analysis was applied to determine the differences between the two tonometers.

RESULTS

This study was the first time of IOP measurement performed in 500 calves by using rebound and applanation tonometers randomly. IOP values was recorded by measuring the right or left eye randomly with both tonometers. The figures of the tonometer devices and the method applied in our study are shown below (Figure 1-Figure 2). A significantly difference was found in IOP readings between TonoVet® and Tono-Pen Vet® devices (P<0.01). The Tono-Pen Vet® overestimated IOP values by an average of 3.8 mmHg compared to the TonoVet® tonometer (Table 1). In this study, it was found that the mean IOP values obtained with TonoVet® tonometer yielded lower than Tono-Pen Vet®.

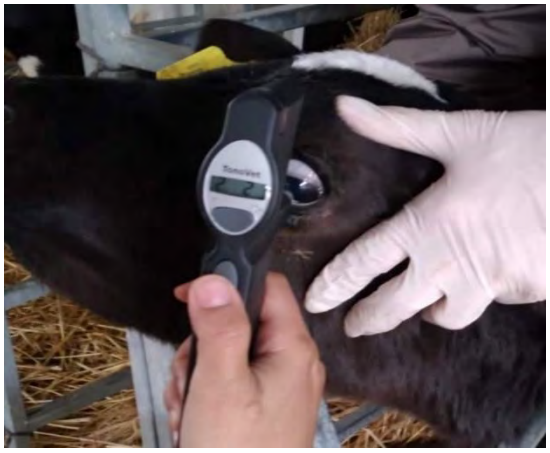


Figure 1. IOP measurement with TonoVet® tonometer

Figure 2. IOP measurement with the Tono-Pen Vet® tonometer

Table 1. Intraocular pressure values on both tonometers in calves

Tonometer	Total eye	Mean±SD (mmHg)
TonoVet®	1000	16,34±2,195
Tono-Pen Vet®	1000	20,18±2,284

*A very significant difference in IOP between the two tonometers (P<0.01).

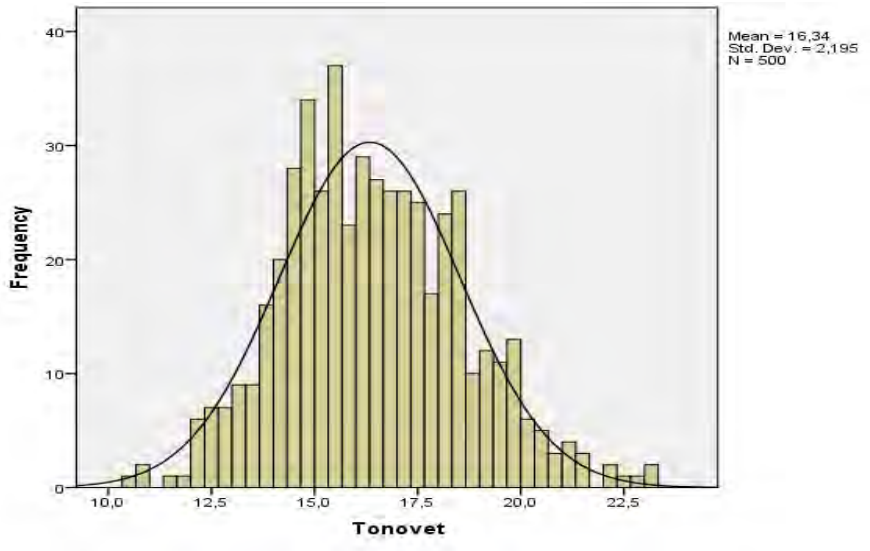


Chart 3. Histogram of IOP values with TonoVet®

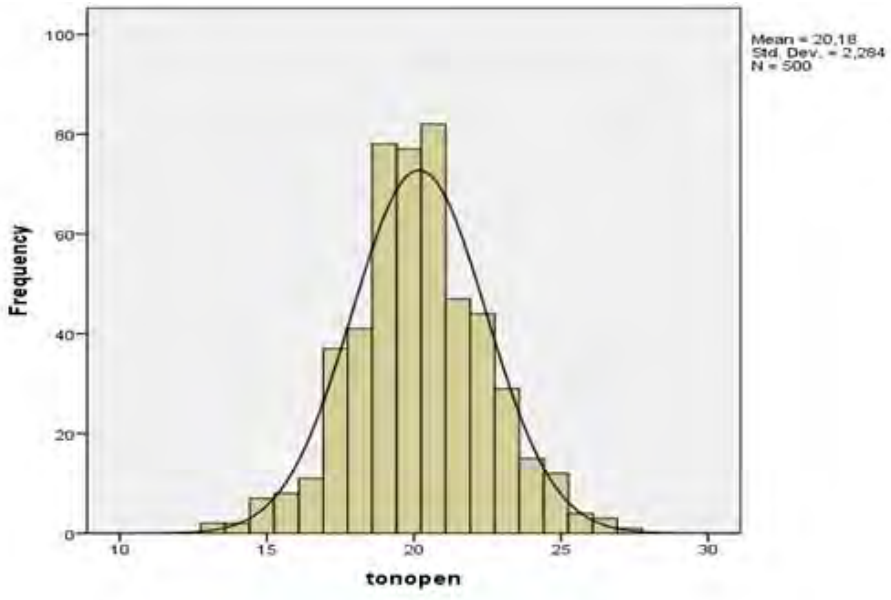


Chart 4. Histogram of IOP values with Tono-Pen Vet®

DISCUSSION AND CONCLUSION

Based on literature studies, there are only a few studies on the normal intraocular pressure in calves. Nowadays, IOP measurements have been evaluated with different tonometer devices in large animals (Pearce and Moore, 2014; Babrauskıenė et al., 2018; Kurt et al., 2018; Kulualp et al., 2019; Peche and Eule, 2018; Tektas et al., 2010). However, there have been no studies conducted on a very large number of calves and randomly measured with TonoVet® and Tono-Pen Vet® tonometer. TonoVet® offers more reliable results, but it has been determined that this device is more difficult to use in cows. In this study, IOP measurements of 1-60 day old 500 calves from different breeds conducted at Dutpınar and Aksüt dairy farms in Malatya, Turkey.

Heredity, exercise, breed, posture changes, blood pressure, eye inflammation, age, medications, eye movements, sex, seasonal changes and diurnal variation are reported as the causes of intraocular pressure changes (Gum et al., 2007). Intraocular pressure values usually increase during daylight hours, but IOP changes during the day in some species (Miller, 2008). In many studies (Giannetto et al., 2009; Pereira et al., 2011; Ribeiro et al., 2014; Garzón-Ariza et al., 2017) have been reported that IOP is not a fixed

parameter and may vary depending on different measurement times during the day. The reasons of IOP changes during the day in human have not been fully explained. However, it is may be caused by plasma cortisol levels in this condition (Tığ, 2006).

Earlier studies on various breeds have reported that IOP can show different results depending on the circadian cycle between species. In nocturnal species such as mice, cats and rabbits, the IOP level increases at night, while in diurnal species such as monkeys and humans, peak IOP is reported during the day. Our results similarly with Gelatt (Gelatt et al., 1981), IOPs showed higher in the morning hours compared to the evening, and they reported that glaucomatous beagles had slightly higher IOP values in the morning than in the evening. Some previous studies (Giannetto et al., 2009; Pereira et al., 2011; Garzón-Ariza et al., 2017) have also concluded that IOP is higher in the early hours of the day and lower in the evening.

Recent studies have shown that the mean IOP values in adult calves have been reported as 26.9±6.7 mmHg with Tono-Pen XL (Gum et al, 1998) and 18.8±1.7 mmHg with Perkins applanation tonometer (Andrade et al., 2012). The mean IOP by using Perkins applanation tonometer were 16.1±1.0 mmHg in the right eye and 16.5±1.2 mmHg in the left eye (Gerometta et al., 2009). In another study with the Mackay-Marg applanation tonometer, the mean IOP was 20.0±5.5 mmHg in healthy calves (Passaglia et al., 2004). Using the TonoVet® rebound tonometer, IOP was 15.2±5.2 mmHg (Tofflemire et al., 2014). In the latest study, Tono-Pen Vet® tonometer was used on 24 healthy calves and the mean IOP was 16.59±2.59 mmHg (Passaglia et al., 2004). The mean IOPs in humans was 16±3 (10-20) mmHg (Murgatroyd, 2008). IOP measurements using applanation tonometer in dogs and horses were 12.9±2.70 mmHg and 21.00±5.90 mmHg, respectively (Kumarasamy et al., 2006). The mean IOP value in dogs was 19.00 (11-29) mmHg (Gelatt et al., 1991). The mean normal IOPs in rabbits was 20.50±3.24 (16-25) mmHg with Schiotz tonometer and 15.13±2.14 (12-18) mmHg with Tono-Pen. The mean IOP values with Tono-Pen Vet and applanation tonometer were 20.74 and 18.40 mmHg in cats (Rusanen et al., 2010).

In our study in calves, it was found that there were significant differences in the comparison of two tonometers ($P<0.01$). The mean IOP values with the TonoVet®

yielded lower than Tono-Pen Vet®. Previous studies showed that the mean IOPs with the TonoVet® seemed higher than Tono-Pen Vet®. Many researchers such as Pereira et al., 2011 and Pigatto et al., 2011 states that even with the same working principle, the use of different methods or the use of different tonometric devices may be associated with differences in mean IOP values (Pereira et al., 2011; Pigatto et al., 2011). Moreover, although the IOP parameters use the same principle, 1-2 mmHg may vary between different tonometer devices. This variation can increase up to 3-4 mmHg when comparing devices with different working principle.

Rebound tonometer is superior to applanation tonometers because it is measured quickly, repeatable, highly reliable and can be used without topical anesthesia (Verboven et al., 2014). In this study, we faced some difficulties in measuring IOP using TonoVet®, the measurement is adversely affected by the position of the animal and sudden movements of the probe. It also needs recalibration after each use (Pereira et al., 2011). Tono-Pen XL, Tono-Pen AVIA and Tono-Pen Vet® applanation tonometers have been preferred in veterinary medicine in recent years (Rosolen et al., 2009). In this study we used Tono-Pen Vet®, it is widely used in clinics for ophthalmological examinations, quite comfortable and convenient to use in large animals. There were no difficulties in measuring the intraocular pressure value with Tono-Pen Vet. Disposable latex protector protects the tip of the Tono-Pen Vet sensor by placing it where it comes into contact with the eye and prevents diseases that can be transmitted from one eye to the other (Ollivier et al., 2007). There is no need to keep the animal's head upright, only the probe of the instrument should be applied to the corneal surface at a right angle (Maggs, 2008). The disadvantage of Tono-Pen Vet is tonometer suddenly requires recalibration and takes several minutes. This may be relation with the very cold temperature at study research conducted during the peak of winter.

In conclusion, randomized measurement of IOPs with TonoVet® and Tono-Pen Vet® in 500 calves is the first study conducted to date. Although incidence of glaucoma is rarely observed in ruminants, the tonometer is still a most important part of the ophthalmic examination. Tono-Pen Vet® and TonoVet® are hand-held devices that can be easily carried and used in applications. Taking measurements with TonoVet seems to

be more difficult due to the difficulty of keeping the head in the correct position in calves. It is necessary to keep the tip of TonoVet® in a perpendicular position to the cornea so that it is parallel to the floor. Tono-Pen Vet®, on the other hand, can be used independently of the head position.

In conclusion, our study establishes an additional reference value for IOP in ruminants. Our results demonstrate the importance of calibrating each tonometer for each animal. It should always be remembered that applanation tonometers tend to underestimate the true IOP, especially at higher pressure levels. Tono-Pen Vet® tonometer may requires recalibration suddenly and takes several time due to the cold weather. TonoVet® produces much more reliable results, but the technique is found to be more difficult to apply to a total of 500 calves.

Comparison of the Tono-Pen Vet® and TonoVet® tonometers showed a very significant difference ($P<0.01$). A comparison of the TonoVet® and Tono-Pen Vet® tonometers showed that the TonoVet® provides lower values in intraocular pressure measurements compared to Tono-Pen Vet®. The Tono-Pen Vet® overestimated IOP values by an average of 3.8 mmHg compared to the TonoVet® tonometer.

ACKNOWLEDGEMENTS

The authors would like to thank Latif Emrah Yanmaz, Serkan Yıldırım and Armağan Hayırlı for all their help and advice with this PhD thesis. The authors thank to the Scientific Research Projects Coordinator of Ataturk University who supported this research. Project Number: TDK-2021-8819. We also thank to Dutpınar and Aksüt dairy owners for the provision place, materials, and equipment during the research.

FINANCIAL SUPPORT

This research received grant from the Scientific Research Projects Coordinator of Ataturk University.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

REFERENCES

Akın F, Samsar E. 2005. Göz Hastalıkları, 3. Baskı. Ankara, Medipres, pp. 49-50, 235-254.

Andrade SF, Palozzi RJ, Giuffrida R. 2012. Comparison of intraocular pressure measurements between the Tono-Pen XL® and Perkins® applanation tonometers in dogs and cats. Veterinary Ophthalmology, 15(1): 14-20.

Arıtürk N. 2006. Glokomda Santral Korneal Kalınlık Ölçümü ve Önemi. Glokom-Katarakt, 1: 1-6.

Babrauskıenė V, Žymantıenė J, Anıulıenė A, Juozaıtıenė V, Malakauskıenė S, Alıonıenė Jl. 2018. Comparative morphological evaluation of animal corneal parameters. Med. Weter, 74(7): 452-455.

Davidson HJ and Pickett JP. 2009. Selected Eye Diseases of Cattle, in: David E. Anderson and D. Michael Rings. (Eds.), Food Animal Practice, 5th ed. Saunders Elsevier, pp. 421.

Garzón-Ariza A, Guisado A, Galán A, Martín-Suárez E. 2017. Diurnal variations in intraocular pressure and central corneal thickness and the correlation between these factors in dogs. Vet Ophthalmol, 21: 464-470.

Gelatt KN, Gum GG, Barrie KP et al. Diurnal variations in intraocular pressure in normotensive and glaucomatous Beagles. Glaucoma, 1981, 3:121-124.

Gelatt KN, Miller DN and Mackay EO. 1991. Intraocular Pressure in Normal Dairy Cattle. Veterinary Ophthalmology, (2-3): 159-161.

Gelatt KN, Brooks DE, Kallberg ME. The Canine Glaucoma. In: Gelatt KN (ed). Veterinary Ophthalmology, 4th ed. Iowa, Blackwell Publishing, 2007:762-763.

Gerometta R, Podos SM, Danias J, Candia OA. 2009. Steroid-induced ocular hypertension in normal sheep. Investigative Ophthalmology & Visual Science, 50: 669-673.

Ghaffari MS, Shojaei M, Sabzevari A, Khorami N. 2011. Reference values for intraocular pressure and Schirmer tear test in clinically normal Sanjabi sheep. Small Ruminant Research, 97: 101-103.

Giannetto C, Piccione G, Giudice E. 2009. Daytime profile of the intraocular pressure and tear production in normal dog. Vet Ophthalmol, 12: 302-305.

Gum GG, Gelatt KN, Miller DN. 1998. Intraocular pressure in normal dairy cattle. Vet Ophthalmol, 1: 159-161.

Gum GG, Gelatt KN, Esson DW. 2007. Physiology of the Eye, in: Gelatt KN. (Ed.), Veterinary Ophthalmology, 4th ed. Blackwell Publishing, Iowa, pp. 160-170.

Kulualp K, Kiliç S, Aytekin O. 2019. Effects of sex, eye-side, diurnal variation on intraocular pressure in calves. Polish Journal of Veterinary Sciences, 22(1): 67-74.

Kumarasamy NA, Lam FS, Wang AL, Theoharides TC. 2006. Glaucoma: Current and developing concepts for ınflammation, pathogenesis and treatment. European Journal of Inflammation, 4: 129-137.

Kurt B, Çağatay HH, Aksoy Ö. 2018. The effect of body position on intraocular pressure in calves. Journal of the South African Veterinary Association, 89(0), a1638: (Online) 2224-9435, (Print) 1019-9128.

Maggs DJ. 2008. Slatter's Fundamentals of Veterinary Ophthalmology, Slatter JD. (Ed.), 4th ed. Saunders Elsevier, pp. 135-150, 178-202.

Miller PE. 2008. Slatter's Fundamentals of Veterinary Ophthalmology, in: Slatter. (Ed.), 4th ed. Saunders Elsevier, pp. 1-19.

Murgatroyd H. 2008. Intraocular Pressure. Continuing Education in Anaesthesia. Critical Care & Pain J, 8(3): 100-103.

Ollivier FJ. Plummer CE, Barrie KP. Ophthalmic Examination and Diagnostics, In: Gelatt KN 4th (ed). Veterinary Ophthalmology. Blackwell Publishing, 2007:438-476.

Park YW, Jeong MB, Kim TH, Ahn JS, Ahn JT, Park SA, Kim SE, Seo K. 2011. Effect of central corneal thickness on intraocular pressure with the rebound tonometer and the applanation tonometer in normal dogs. Vet Ophthalmol, 14: 169-173.

Passaglia CL, Guo X, Chen J. 2004. Tono-Pen XL calibration curves for cats, cows and sheep. Veterinary Ophthalmology, 7(4): 261-264.

Pearce J, Moore C. 2014. Food animal ophthalmology, in: Kirk N. Gelatt. (Eds.), Veterinary Ophthalmology, 3th ed. Wiley-Blackwell, Iowa, pp. 466. Revised from "Food Animal Ophthalmology," by Jacqueline W. Pearce and Cecil P. Moore, in Kirk N.

Peche N, Eule JC. 2018. Intraocular pressure measurements in cattle, sheep, and goats with 2 different types of tonometers. The Canadian Journal of Veterinary Research, 82: 208-215.

Pereira FQ, Bercht BS, Soares MG, da Mota MG, Pigatto JA. 2011. Comparison of a rebound and an applanation tonometer for measuring intraocular pressure in normal rabbits. Veterinary Ophthalmology, 14: 321-326.

Pigatto JAT, Pereira FQ, Albuquerque L. 2011. Intraocular pressure measurement in sheep using an applanation tonometer. Revista Cere, 58: 685-689.

Potter TJ, Hallowell GD, Bowen IM. 2008. Ultrasonographic anatomy of the bovine eye. Vet. Radiol. Ultrasound, 172-177.

Renwick PW, Petersen-Jones SM. 2009. Orbital and ocular pain, in: Peiffer RL, Petersen-Jones SM. (Eds.), Small Animal Ophthalmology: A Problem-Oriented Approach, 4th ed. Saunders, Philadelphia, pp. 227-237.

Ribeiro AP, Crivelaro RM, Teixeira PP. 2014. Effects of different mydriatics on intraocular pressure, pupil diameter, and ruminal and intestinal motility in healthy sheep. Vet Ophthalmol, 17: 397-402.

Rosolen SG, Multari D, Woods M and Jongh O. 2009. Cornea, in: Robert L. Peiffer Jr, Simon M. Petersen-Jones. (Eds.), Small Animal Ophthalmology: A Problem-Oriented Approach, 4th ed. Saunders Elsevier, pp. 17.

Rusanen E, Florin M, Hässig M and Spiess BM. 2010. Evaluation of a Rebound Tonometer (TonoVet®) in Clinically Normal Cat Eyes. Vet Ophthalmol, 13(1): 31-36.

Tektas OY, Hammer CM, Danias J. 2010. Morphologic changes in the outflow pathways of bovine eyes treated with corticosteroids. Invest Ophthalmol Vis Sci, 51(8): 4060-4066.

Tığ UŞ. 2006. Göz İçi Basınç Ölçümünde Hata Kaynakları. S.D.Ü Tıp Fakültesi Dergisi, 13(3): 32-35.

Tofflemire KL, Whitley EM, Gould SA. 2014. Schirmer tear test I and rebound tonometry findings in healthy calves. Veterinary Ophthalmology, 18: 147-151.

Townsend WM. 2008. Food and Fiber-Producing Animal Ophthalmology, in: Gelatt KN. (Eds.), Essentials of Veterinary Ophthalmology, 2nd ed. Blackwel Publishing, Iowa, pp. 379-381.

Verboven CA, Djajadiningrat-Laanen SC, Teske E, Boevé MH. 2014. Development of tear production and intraocular pressure in healthy canine neonates. Vet Ophthalmol, 17: 426-431.

A SYSTEMATIC REVIEW: STEREOTYPIC BEHAVIOURS of HORSEs and THEIR EFFECTs on HORSE WELFARE

Erva ESER[1]* **Serkan ERAT[2]**

[1] *Kırıkkale University, Graduate School of Health Science Department of Animal Breeding and Husbandry and Animal Nutrition, Kırıkkale, Türkiye*

[2] *Kırıkkale University, Faculty of Veterinary Medicine, Department of Animal Breeding and Husbandry, Kırıkkale, Türkiye*

*E-mail: ervaeser-ogr@kku.edu.tr

In this study the causes of stereotypes in horses, and ways to prevent them were discuss, and various studies on the effects of horse welfare were systematically reviewed. Horses and humans bond depends for many years, and differ from another livestock animals with some basic features. After the Industrial Revolution, horse breeding started to be done for supportive purposes and hobbies. Thanks to new era for horses, housing systems and daily routine were changed quickly. Abnormal behaviours and stereotypes that arise in situations where this special care and attention level is insufficient, involve with both horses, breeders and handlers various difficulties. Abnormal behaviours that are thought to develop due to the prevention of normal behaviours are also accepted as an indicator that animal welfare is adversely affected. Stereotypes defined as repetitive movements with no definite purpose an duration. Oral stereotypes such as windsucking, crib biting, and locomotor stereotypes as pacing or ground kicking can effected the animal with clinical problems. Welfare, which is defined as animal's feeling of well-being, can present itself through behaviours. By observing the behaviour exhibited by the animal, it can be concluded that it is phychologically and physiologically health; has a good welfare. For this reason it accepted that stereotypical behaviours are also an indicator of poor welfare.

Keywords: Behaviour; Horse; Stereotype; Systematic review; Welfare.

INTRODUCTION

With domestication, many animal species and breeds began to be raised under human control; breeding has led to the emergence of new breeds one after another (Özbeyaz and Akçapınar, 2021). In human control, aquaculture includes housing and nutritional changes, which ended with some behavioural changes in domesticated animals. Animal behaviour is one of the most important indicators of animal welfare, and it can be concluded that the welfare status is good in animals that can exhibit normal behaviour. However, undesirable and abnormal behaviours are observed in situations that negatively affect well-being, such as improper care and unsuitable housing conditions (Roberts et al., 2017). Abnormal behaviours can cause difficulties, harm the animal and its environment; results in physiologically conditions. The most common abnormal behaviours appear as stereotypical behaviours (Peters et al., 2012). The most characteristic feature that distinguishes stereotypical behaviours from abnormal behaviours is that they are performed in an aimless and repetitive manner, and they are examined in two different ways as locomotor and oral stereotypes. While oral stereotypes are listed as crib biting, wind swallowing, wood gnawing, teeth grinding, caprophagia and lip biting, locomotor stereotypes can be listed as swinging, walking in a circle, and repetitive walking in boxing (Arena et al., 2021).

Horses has breeding for sportive and hobby purposes in human life following the transition to machine production. In some undeveloped countries, also bred for agriculture and transportation. In general, although the economic values of horses bred for sportive purposes and hobbies are high, it is thought that the rates of exhibiting stereotypical behaviours are higher than other animals due to their individual housing, feeding changes and weaning methods (Sarrafchi, 2012). Some researchers argue that stereotypes are exhibited as an adaptation behaviour, while others argue that certain chemicals restricted in the neuroendocrine system are effective in the development of stereotypes (Diez-Leon et al., 2019; Roberts et al., 2017; Hemmann et al., 2013; Sarrafchi, 2012). Physical damage caused by stereotypical behaviors also directly

affects welfare assessments in horses, as they cause various serious digestive and respiratory problems. The general causes of stereotypes can be counted as the limitation of movement of animals by being kept in closed and inadequate areas, the ground structure, high grain feeding, individual housing, inability to create enough resting areas after high-tempo exercises, and the release of hormones and chemicals in the neuroendocrine system such as dopamine, leptin and ghrelin.

New studies that can be created by using the data of previous studies in scientific research are prepared in the form of systematic review or meta-analysis. One of the main differences between systematic review and meta-analysis is the evaluation of the numerical data obtained in the studies to be evaluated as a single whole. In meta-analysis, the data obtained are collected and re-evaluated, while the results obtained in the systematic review are interpreted. In this study, various stereotypical behaviours on horse breeds were examined and their effects on welfare parameters were evaluated.

MATERIAL AND METHODS

In the study, the PRISMA-2020 (Page et al., 2020) selection diagram was used. A literature search was conducted with English keywords using Google Scholar and Wiley Online Library databases. Studies published between the years 2010-2021 were taken into account in the search, but the studies from 1998-2010, which made the basic definitions of the subject and were considered pioneers in their field, were also taken into account. Keywords used in the search; selected as “horse”, “behaviour”, “abnormal behaviour”, “stereotypic behaviours” and “welfare”. From the studies obtained after the screening, full text, high reliability score, 1 master's thesis, 36 published research and review articles and 6 book chapters were used. The results of the survey studies were excluded because they were regionally effective and concentrated on a single population. A summary of the path followed in the study is given in Figure 1.

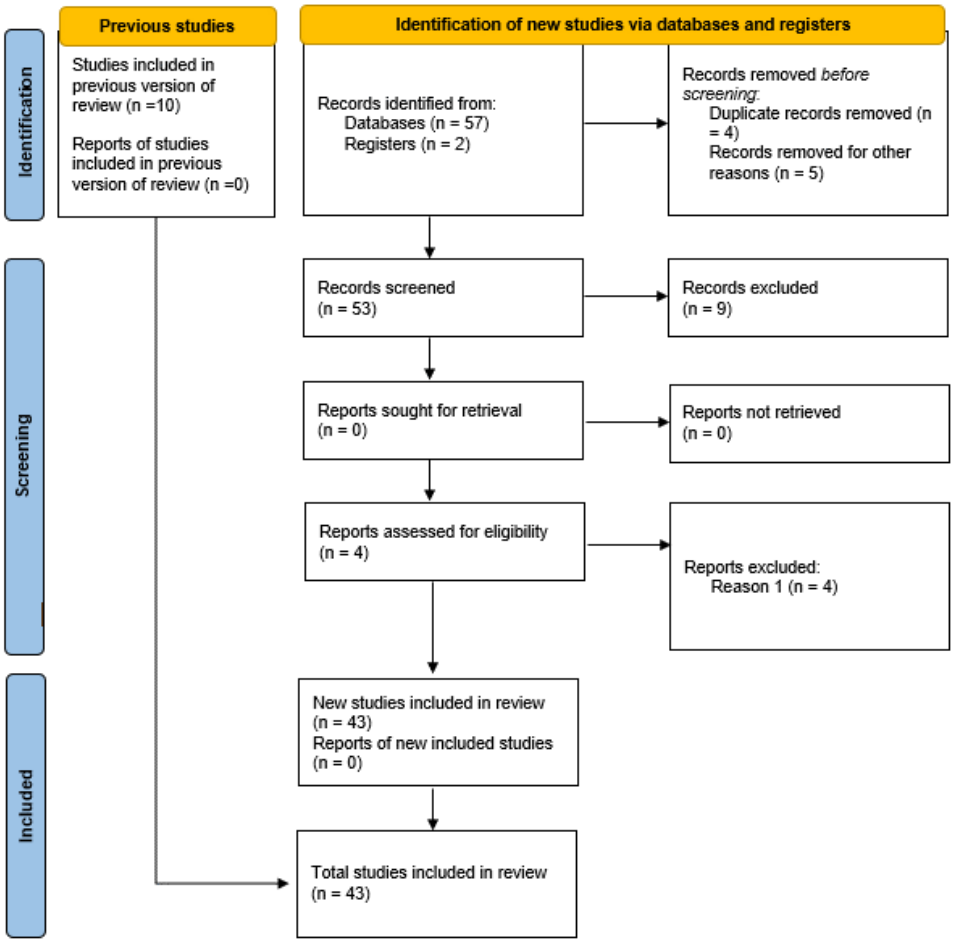


Figure 1. Preparation summary of the work with the PRISMA 2020 workflow chart used in the preparation of the systematic review (Haddaway et al., 2021).

Of the 53 sources examined in the study, 5 were excluded because they were in a language other than English, and 9 of them were not available due to the inaccessibility of the full text. Examined reports were not included in the study because they were not inclusive.

STEREOTYPIC BEHAVIORS and THEIR CAUSES

Stereotypes thought to be the result of inadequate environmental or housing conditions. It is defined as repetitive and purposeless behaviours (Mason and Latham, 2004). It is thought that stereotypes have been seen in horses since domestication, and

this is a result of the restrictive effect of domestication (McBride et al., 2009). The earliest studies on stereotypes in horses date back 400 years. Stereotypical behaviours are often compared to human psychiatric illnesses, can pose a risk to animals when exhibited intensively (McBride and Parker, 2015; Freymond et al., 2019). Although the main causes of stereotypical behaviours are not fully known, there are many hypotheses that stress in the vital area or indirectly developing chronic stress may cause stereotypical behaviours (McBride and Parker, 2015). There are many studies on stereotypical behaviour in animals; different stereotypes are observed in animals of different species and different housing conditions, such as tail biting in pigs, plucking in layer chickens, and stepping behaviours in bears in zoos (Cooper and Mason, 1998). It is thought that the neurobiological process in the development of stereotypes causes the parts of the nervous system associated with inhibited behaviours to become more sensitive, thus creating stereotypes and triggering alternative behaviour (Spruijt et al., 2001). In a study contributing to this idea, it was suggested that chronic stress increases the release of β-endorphins, stimulates dopamine release in the striatum and activates some of the basal ganglia (Dodman et al., 1987). Similarly, it has been reported that abnormal behaviours and stereotypes develop as a result of poor welfare parameters such as various injuries and feelings of pain outside of caregiving (Carroll et al., 2020).

Behaviour related clinical and care problems have become one of the biggest problems encountered in horse breeding. In a recent UK study, 91% of breeders reported that riders had problems with behaviour problems; 42.2% reported that they observed behaviour-related poor performance in dressage horses (Agar et al., 2016; Hockenhull and Creighton, 2013). Various recent studies have also revealed the relationships between well-being and stereotypical behaviours (Wickens & Heleski, 2010; Sarrafchi & Blokhuis, 2013; Escolana et al., 2014; Roberts et al., 2017; Sykes et al., 2019).

WELFARE PARAMETERS AND ASSESSMENT

With the initiation of indoor care of animals after domestication, they were partially prevented from fully expressing their natural behaviours, which led to the

development of stereotypical behaviours (Lewis et al., 2006). The Five Domain Model of Welfare, by Mellor and Reid in 1997; it was developed as a well-being assessment model that explains the effects of nutrition, environment, health and behaviour on mental state. Anxiety, frustration, and boredom used in describing the mental state are endangered in animals that do not have enough space and cannot display their natural behaviours and it negatively affects well-being (Mellor, 2017). Anticipatory Behaviour, defined in 1918, is characterized by the desire to achieve a reward or result in animals and has an important place in the development of learning behaviour (Spruijt et al., 2001; Peters et al., 2012). Observing the expectation behaviour and stimulating it with various trials provides an advantage in the evaluation of welfare parameters.

Behaviours are an important parameter in evaluating animal welfare, and they are not sufficient on their own. Non-optimal environmental and care conditions, physiological values can similarly affect the behaviour of the animal (Carenzi and Verga, 2009; Arena et al., 2021). Stress conditions and chronic stress negatively affect not only well-being parameters, but also the immune system and physiological parameters (Carenzi and Verga, 2009; Brown and Vosloo, 2017; Popescu and Diugab, 2017). Although the measurement of physiological parameters associated with well-being carries many difficulties, methods such as infrared thermography, heart rate assessments, salivary IgA examinations have been developed and adopted as established and frequently used methods in welfare studies (Whitham and Miller, 2016; Zupan, 2016; Staley et al., 2018, Arena et al., 2021). In determining whether the stress is acute or chronic, it is useful to measure the cortisol levels and investigate the activity of the neuroendocrine system (Palme, 2012). Since the increase in blood cortisol level may also develop due to the blood collection procedure, the detection of cortisol from hair samples, saliva, feces and urine by non-invasive methods is considered as frequently used methods in the evaluation of stress. Thenceforth contains cortisol during hair growth, it is considered as an indicator of long-lasting chronic stress (Mormede et al., 2007; Palme 2012, 2019; Heimbürge et al., 2019; Arena et al., 2021). Dehydroepiandrosterone (DHEA) values are used as another endocrine indicator of stress. This hormone, which has functions opposite to cortisol, gives high values after

acute stress and ACTH release in horses (Kamin and Kertes, 2017; Ayala and Martos, 2013). During welfare assessments, it is necessary to calculate the cortisol/DHEA ratio and determine which one is produced preferentially (Kamin and Kertes, 2017).

STEREOTYPES and THEIR EFFECTS on WELFARE PARAMETERS

While stereotypes are an indication of the poor welfare of animals, they also cause various physical problems and worsen poor welfare. Current care conditions make horses vulnerable to problems such as stereotypical behaviour, aggression, hyper-reactivity or anxiety disorder (Peters et al., 2012). Stomach ulcers (Casey, 2002), inflammatory respiratory system diseases (Mills and Clarke, 2002), various foot diseases (Casey, 2002), colic (Davidson and Harris, 2002) are just a few of the clinical problems encountered in stereotyped horses. In addition to all these stereotypical behaviour causes and consequences, it has been reported that stereotypic behaviours continue to increase in indoor trials when expectation behaviours result in negative or bad results (Peters et al., 2012).

In the study on anticipatory behaviour in horses, it was found that an increase in horses' mobility and heart rate occurred; it has been reported that if this situation is continued, stress symptoms are observed (Peters et al., 2012). Situations where surgical interventions are required as well as medical treatments in the health problems caused by stereotypes on animals increase the pain and stress and affect the welfare level completely negatively. It has been determined that in situations where stress increases and housing conditions are insufficient, elderly horses with orthopaedic diseases exhibit less feeding and movement behaviours compared to horses with similar conditions at other housing and stress levels (Kelemen et al., 2021).

Crib biting behaviour, which is one of the oral stereotypes, can cause abrasion on tooth surfaces and injuries in the oral cavity. This situation also affects the normal feeding behaviour of the animal. Similarly, wind swallowing, which is also defined as aerophagia, also causes digestive problems (Doodman et al., 1987; McBride and Hammings, 2009). The crib bite stereotype has also been associated with spasmodic colic and compression of the small intestine in the epiploic foramen in horses. When

this behaviour becomes part of the animal's daily behavioural routine, the incidence of satiety behaviour increases. Crib biting behaviour is observed again since the sudden intake of grains with high starch content also stimulates acidity in the digestive system and triggers the behaviour by the visceral way. It has been reported that antacids and various antibiotic substances added to the feeds to prevent this behaviour reduce the flavor of the feeds and the acidity of the digestive tract, resulting in a significant decrease in the crib biting behaviour (Johnson et al., 1998).

Considering all these examples, it is clearly observed that the definition of welfare and stereotypical behaviours are opposite processes.

CONCLUSION

At present, animal welfare debates continue at the highest level, where animal production is not only associated with agriculture, but social ethics and value judgments are developed. Welfare, which can be summarized as the feeling physically and well-being mentally of animals over basic rights and freedoms, is affected by environmental and individual variability, which adversely affects the health status of the animal. Stereotypical behaviours, known as undesirable behaviours in animals, can cause serious problems, especially in horses, which have an important place in emotional and human life. Although stereotypical behaviours cannot be fully resolved, it has been demonstrated by various studies that stress factors and care conditions are effective in the development and maintenance of stereotypes. Abnormal behaviours caused by various care and feeding errors and breeding errors in other farm animals affect the welfare criteria negatively and are effective in shaping poor and low welfare. Defined as basic freedom with care and feeding conditions for increasing animal welfare; It is important to establish systems in which animals are emotionally and physiologically healthy. For this purpose, the development of new non-invasive welfare measurement and observation methods and the measurement and evaluation of welfare measurements in animals in care will create higher standards for both animals and humanity.

FINANCIAL SUPPORT

This study was supported by Council of Higher Education 100/2000 Doctorate Bursary Programme (YÖK 100/2000).

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

REFERENCES

Agar, C., Gemmill, R., Hollands, T., Freeman, S.L. (2016). The use of nutritional supplements in dressage and eventing horses. Vet. Rec. Open 3: e000154.

Arena, I., Marliani, G., Sabioni, S., Gabai, G., Bucci, D., Accorsi, P.A. (2021). Assessment of horses' welfare: Behavioural, hormonal, and husbandry aspects. Journal of Veterinary Behaviour. 41: 82-90.

Bachmann, I., Bernasconu, P., Herrmann, R., Weishaupt, M.A., Stauffacher, M. (2003). Behavioural and physiological responses to an acute stressor in crib-biting and control horses. Appl. Anim. Behav. Sci. 82: 297–311.

Briefer Freymond, S., Bardou, D., Beuret, S., Bachmann, I., Zuberbuhler, K., Briefer, E.F. (2019). Elevated sensitivity to tactile stimuli in stereotypic horses. Front. Vet. Sci. 6:162. Doi: 10.3389/fvets.2019.00162.

Brown, E.J., & Vosloo, A. (2017). The involvement of the hypothalamo-pituitary-adrenocortical axis in stress physiology and its significance in the assessment of animal welfare in cattle. Onderst. J. Vet. Res. 84 (1): 1–9.

Carenzi, C., & Verga, M. (2009). Animal welfare: review of the scientific concept and definition. Ital. J. Anim. Sci. 8: 21–30.

Caroll, S.L., Sykes, B.W., Mills, P.C. (2020). An online survey investigating percevied prevalance and treatment options for stereotypic behaviours in horses and undesirable behaviours associated with handling and riding. Equine Veterinary Education; 32(11): 71-81.

Casey, R.A. (2002). Clinical problems in performance horses. In: Waran, N. (Ed.), The Welfare of Horses. Kluwer Academic Publishers, Netherlands, pp. 19–44.

Cooper, J.J., & Mason, G.J. (1998). The identification of abnormal behaviour and behavioural problems in stabled horses and their relationship to horse welfare: a comparative review. Equine Clinical Behaviour, Equine Vet. J. 27: 5-9.

Davidson, N., & Harris, P. (2002). Nutrition and welfare. İçinde; Waran, N. (Ed.), The Welfare of Horses. Kluwer Academic Publishers, Netherlands, pp. 45–76.

Diez-Leon, M., Kitchenham, L., Duprey, R., Bailey, C.D.C., Chleris, E., Lewis, M., et al. (2019). Neurophysiological correlates of stereotypic behaviour in a model carnivore species. Behavioral Brain Reseach; 373: 112056.

Dodman, N.H., Shuster, L., Dixon, R. (1987). Investigation into the use of narcotic antagonists in the treatment of a stereotypic behavior pattern (crib-biting) in the horse. A J Vet Res. 48, 311–9.

Escalona, E., Okell, C., Archer, D. (2014). Prevalence of and risk factors for colic in horses that display crib-biting behaviour. BMC Vet. Res. 10: S3.

Haddaway, N.R., Pritchard, C.C., McGuinness, L.A. (2021). PRISMA2020: R package and ShinyApp for producing PRISMA 2020 compliant flow diagrams. Zenodo. http://doi.org/10.5281/zenodo.4287834.

Heimbürge, S., Kanitz, E., Otten, W. (2019). The use of hair cortisol for the assessment of stress in animals. Gen. Comp. Endocrinol. 270: 10–17.

Hemmann, K.E., Koho, N.M., Vainio, O.M., Raekallio, M.R. (2013). Effects of feed on plasma leptin an ghrelin concentrations in crib-biting horses. The Veterinary Journal, 198: 122-126.

Hockenhull, J., Creighton, E. (2010). Management routine risk factors associated with handling and stabled-related behavior problems in uk leisure horses. J. Vet. Behav. 5: 57-58.

Hockenhull, J., & Creighton, E. (2012). The use of equipment and training practices and the prevalence of owner-reported ridden behaviour problems in UK leisure horses. Equine Veterinary Journal; 45: 15-19.

Johnson, K.G., Tyrell, J., Rowe, J.B., Pethick, D.W. (1998). Behavioural changes in stabled horses given nontherapeutic levels of virginiamycin. Equine Vet J. 30: 139-143.

Kamin, H.S., & Kertes, D.A. (2017). Cortisol and DHEA in development and psychopathology. Horm. Behav. 89: 69–85

Kelemen, Z., Grimm, H., Vogl, C., Long, M., Cavalleri, J.M.V., Auer, U., Jenner, F. (2021). Equine Activity Time Budgets: The Effect of Housing and Management Conditions on Geriatric Horses and Horses with Chronic Orthopaedic Disease. Animals. 11(7):1867. https://doi.org/10.3390/ani11071867.

Lesimple, C., Reverchon-Billot, L., Galloux, P., Stomp, M., Boichot, L., Coste, C., et al. (2020). Free movement: A key for welfare improvement in sport horses? Applied Animal Behaviour Science. 225: 104972.

Lewis, M.H., Presti, M.F., Lewis, J.B., Turner, C.A. (2006). The neurobiology of stereotypy i: environmental complexity. In: Mason, G., Rushen, J. (Eds.), Stereotypic Animal Behaviour: Fundamentals and Applications to Welfare. CABI, Walling- ford, pp. 190–226.

Msron, G.J. (2006). Stereotypic behaviour in captive animals: fundamentals and implications for welfare and beyond. In: Mason, G., Rushen, J. (Eds.), Stereotypic Animal Behaviour: Fundamentals and Applications to Welfare. CABI, Walling- ford, pp. 325–356.

Mason, G., & Latham, N. (2004). Can't stop, won't stop: is stereotypy a reliable animal welfare indicator? Anim Welf. 13, 57–69.

McBride, S., & Hemmings A (2009). A neurologic perspective of equine stereotypy. Journal of Equine Veterinary Science. 29(1): 10-16.

McBride, S.D., & Parker, M.O. (2015). The disrupted basal ganglia and behavioural control: an integrative cross-domain perspective of spontaneous stereotypy. Behav Brain Res. 276:45–58. doi: 10.1016/j.bbr.2014.05.057.

Mills, D.S., & Clarke, A.F. (2002). Housing, management and welfare. In: Waran, N. (Ed.), The Welfare of Horses. Kluwer Academic Publishers, Netherlands, pp. 77–97.

Mormede, P., Andanson, S., Auperin, B., Beerda, B., Guemene, D., Malmkvist, J., et al. (2007). Exploration of the hypothalamic-pituitary-adrenal function as a tool to evaluate animal welfare. Physiol. Behav. 92 (3): 317–339.

Özbeyaz, C., & Akçapınar, H. (2021). Hayvan Yetiştiriciliği Temel Bilgiler. Medisan Yayınevi, Ankara.

Page, M.J., McKenzie, J., Bossuyt, P., Hoffmann, T., et al. (2020). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. https://doi.org/10.31222/osf.io/v7gm2.

Palme, R. (2012). Monitoring stress hormone metabolites as a useful, non-invasive tool for welfare assessment in farm animals. Anim. Welf. 21: 331–337. Palme, R. (2019). Non-invasive measurement of glucocorticoids: advances and problems. Physiol. Behav. 199: 229–243.

Peters Smbelijenberg, E.H., van Dierendock, M.C., van der Harst, J., Spruijst, B.M. (2012). Characterization of anticipatory behaviour in domesticated horses (Equus caballus). Applied Animal Behaviour Science. 138: 60-69.

Popescu, S., & Diugan, E.A. (2017). The relationship between the welfare quality and stress index in working and breeding horses. Res. Vet. Sci. 115: 442–450.

Roberts, K., Hemmings, A.J., McBride, S.D., Parker, M.O. (2017). Casual factors of oral versus locomotor stereotypy in the horse. Journal of Veterinary Behaviour. 20: 37-43.

Sarrafchi, A., & Blokhuis, H.J. (2013). Equine stereotypic behaviors: causation, occurrence, and prevention. J. Vet. Behav. 8: 386–394.

Sarrafchi, A. (2012). Equine stereotypic behaviour as related to horse welfare: A review. Master Thesis Linköpings Universitet, Department of Physics, Chemistry, and Biology.

Sarrafchi, A., & Blokhuis, H.J. (2013). Equine stereotypic behaviors: causation, occurrence, and prevention. J. Vet. Behav. 8: 386-394.

Spruijt, B.M., van den Bos, R., Pijlman, F.T. (2001). A concept of welfare based on reward evaluating mechanisms in the brain: anticipatory behaviour as an indicator for the state of reward systems. Appl Anim Behav Sci. 72:145–71. doi: 10.1016/S0168-1591(00)00204-5

Staley, M., Conners, M.G, Hall, K., Miller, J.L. (2018). Linking stress and immunity: immunoglobulin A as a non-invasive physiological biomarker in animal welfare studies. Horm. Behav. 102: 55–68.

Sykes, B.W., Bowen, M., Habershon-Butcher, J.L., Green, M., Hallowell, G.D. (2019). Management factors and clinical implications of glandular and squamous gastric disease in horses. J. Vet. Intern. Med. 33: 233.

Whitham, J.C., & Miller, L.J. (2016). Using technology to monitor and improve zoo animal welfare. Anim. Welf. 25: 395–409.

Wickens, C.L., & Heleski C.R. (2010). Crib-biting behavior in horses: a review. Appl. Anim. Behav. Sci. 128: 1-9.

Zupan, M., Buskas, J., Altimiras, J., Keeling, L.J. (2016). Assessing positive emotional states in dogs using heart rate and heart rate variability. Physiol. Behav. 155: 102–111.

online

7TH INTERNATIONAL CONGRESS ON VETERINARY AND ANIMAL SCIENCES

20–22 October 2022

INTERNATIONAL CONGRESS ON VETERINARY AND ANIMAL SCIENCES
www.icvas.gen.tr

Abstracts

SUCCESSFUL TREATMENT PORTOSYSTEMIC SHUNT IN TWO DOGS

Zeynep BİLGEN **Büşra KİBAR KURT***

Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Surgery Department, Aydın, Türkiye

*E-mail:busrakibar@yandex.com

Portosystemic shunts (PSS) are one of the most common vascular anomalies in many animal species and humans. In portosystemic shunts, there is a direct connection between the portal system and the systemic venous circulation. The blood that comes with the shunt contains toxins and byproducts of the intestinal system, which have not been removed by the liver. Hepatic encephalopathy may be seen in this condition. Congenital PSS has surgical and medical treatment. The study material consisted of two dogs diagnosed with congenital extrahepatic portosystemic shunt. Both dogs in the study were male and 3 months old. One was a Maltese terrier and the other was a Yorkshire terrier. Post-meal seizures were reported in both patients. The symptoms observed in the patients were amaurosis and circling around. In biochemical blood tests, hyperuricemia, hypoglobulinemia, an increase in ALT and AST values, and a tenfold increase in TBA were observed. On ultrasonography (USG), a decrease was observed in the diameter of the portal vein at the liver entrance, while dilatation was observed in the region of the caudal vena cava (CVC) close to the liver entrance. Operative treatment was decided and partial shunt ligation with silk thread was applied in 2 cases. Yorkshire terrier up to 3 years old and Maltese terrier up to 4 years old were followed. In both cases, partial ligation with silk thread was successful in the treatment of PSS. Complications reported in total ligation cases did not occur, clinical findings improved in dogs in a short time, and postoperative laboratory findings were within reference levels.

Keywords: Amaurosis; Extrahepatic shunt; Liver; Partial shunt ligation; Seizure.

Conflict of Interest: Authors declare no conflict of interest.

PREVALENCE AND MOLECULAR CHARACTERIZATION OF *CRYPTOSPORIDIUM* SPP. IN LAMBS IN HAKKARİ REGION

Erdal BİNGÖL[1*] Abdulalim AYDIN[1] Yaşar GÖZ[2] Adnan AYAN[3] Özlem ORUNÇ KILINÇ[4] Özgür Yaşar ÇELİK[5] Fatma ERTAŞ OĞUZ[6] Gürkan AKYILDIZ[7] Burçak ASLAN ÇELİK[8] Alp ATAY[1] Özge OKTAY AYAN[9]

[1]Çölemerik Vocational School, Hakkari University, Hakkari, Türkiye

[2]Department of Nutrition and Dietetics, Faculty of Health Sciences, Van Yuzuncu Yil University, Van, Türkiye

[3]Department of Genetics, Faculty of Veterinary Medicine, Van Yuzuncu Yil University, Van, Türkiye

[4]Özalp Vocational School, Van Yuzuncu Yil University, Van, Türkiye

[5]Department of Internal Medicine, Faculty of Veterinary Medicine, Siirt University, Siirt, Türkiye

[6]Department of Medical Services and Techniques, Tuzluca Vocational School, Iğdır University, Iğdır, Türkiye

[7]Faculty of Health Sciences, Marmara University, İstanbul, Türkiye

[8]Department of Parasitology, Faculty of Veterinary Medicine, Siirt University, Siirt, Türkiye

[9]Institute of Health Sciences, Van Yuzuncu Yil University, Van, Türkiye

*E-mail: erdalbingol@hakkari.edu.tr

Cryptosporidiosis is a zoonotic coccidian protozoan that causes infections called Cryptosporidios with diarrhea and other clinical symptoms in pets and animals. Cryptosporidiosis is associated with high morbidity and mortality in farm animals. In this study, it was aimed to determine the prevalence and molecular characterization of

Cryptosporidium spp. in lambs in Hakkari Region. The animal material of the study consists of 200 lambs in the Hakkari region. Stool samples were collected from the rectum of lambs with disposable latex gloves. Stool samples were first examined microscopically for *Cryptosporidium* oocysts by Kinyoun Acid-Fast Staining method. DNA was then extracted from all 200 samples. Then, Nested PCR was performed with appropriate primers. After that, PCR products were run in agarose gel and images were obtained on a gel imaging device. Then the positive PCR products were sent to the sequence bidirectionally. Then, the sequence results were blasted and alligned and compared with the relevant reference genes in the GenBank. As a result of microscopic examination, *Cryptosporidium* spp. oocysts were found in 34 (%17) of 200 samples. As a result of Nested PCR, positivity was detected in 36 (%18) of 200 samples. *C. ryanae* and *C. parvum* were detected as a result of sequencing.

As a result, it was concluded that *Cryptosporidium* spp. is intensively observed in lambs in the Hakkari region. In addition, *Cryptosporidium parvum*, which has a zoonotic character, was obtained from the sequence results. In the light of this information, it is thought that protection control methods should be carried out and the public should be informed.

Keywords: *Cryptosporidium* spp.; Hakkari; Lamb; Molecular

Acknowledgement: This study was supported by Hakkari University Scientific Research Projects Coordination Project Code: FM22 BAP5)

GUT-HEART AXIS

Cansu BALIKÇI* **Gamze GÖKÇAY** **Songül ERDOĞAN** **Hasan ERDOĞAN** **Kerem URAL**

Aydın Adnan Menderes University, Faculty of Veterinary, Department of Internal Medicine, Aydın, Türkiye

*E-mail: balikcicansu98@gmail.com

Many interactions play a role in the gut-heart axis. These include intestinal epithelial dysfunction, dysbiosis, butyrate-producing bacteria, bile acids, and intestinal microbe-derived metabolites. In patients with heart failure (HF), mucosal malabsorption, intestinal wall edema and barrier dysfunction develop as a result of microcirculation disorders in the gut due to decreased perfusion, increased congestion and sympathetically mediated vasoconstriction. Toxic, pathogenic, immunogenic and inflammatory factors, through the increase in intestinal permeability as a result of damaged tight junctions in the intestine, pass through the mucosa and reach the systemic circulation, causing local-systemic inflammation. Studies have shown increased levels of inflammatory cytokines involved in the process of cardiomyocyte apoptosis, hypertrophy, and fibrosis in HF patients. Many factors that cause dysbiosis by changing the intestinal flora, which are frequently seen in HF, lead to bacterial overgrowth, bacterial translocation and formation of many toxic substances, including lipopolysaccharide (LPS), trimethylamine N-oxide (TMAO), p-cresylsulfate (PCS) and indoxyl sulfate (IS). Depending on the increase in intestinal permeability, these toxic substances reach the systemic circulation; It increases the risk of atherosclerosis by playing a role in thrombosis, platelet invasion, foam cell formation and inflammation processes. Decreased levels of butyrate, one of the short-chain fatty acids that have many effects on the gastrointestinal tract, including maintaining intestinal barrier

integrity; It promotes foam cell formation, exacerbates dysbiosis, and plays a role in the disruption of intestinal barrier function, causing endotoxins to reach the general circulation. With this review, it is aimed to inform about the physiopathological processes in the gut-heart axis, in the light of the current literature.

Keywords: Dysbiosis; Intestinal permeability; SCFA; TMAO.

EFFECT OF FENOKSI-2-METIL-2-PROPIONIC ACID (Hepagen®) TREATMENT ON REPRODUCTIVE PERFORMANCE IN EWES DURING THE EARLY AND LATE POSTPARTUM PERIOD

Halef DOĞAN[1]* **Metehan KUTLU[2]**

[1] *Tekirdag Namik Kemal University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology, Suleymanpasa, Tekirdag, Türkiye*

[2] *Adana Metropolitan Municipality, Department of Agriculture and Animal Services, Seyhan, Adana, Türkiye*

*E-mail: halefdogan@nku.edu.tr; halefdogan@gmail.com

The liver is a dynamic organ that plays critical roles in many physiological processes. In farm animals, supporting the liver reduces postpartum metabolic diseases and loss of reproductive and milk yield in transition period. The aim of this study to investigate the effects of fenoksi-2-metil-2-propionic acid (Hepagen) on reproductive performance of ewes in early and late pp period. Total of 89 ewes in early (n=45) and late pp period (n=44) were used in the study. In order to test the efficacy of Hepagen on reproductive outcomes, control and treatment groups were formed separately for each pp period. Following insertion of vaginal sponge containing 60 mg medroxyprogesterone acetate for 7 day, PMSG 500 IU was injected IM (day 7) to all ewes. In treatment groups of early (n=23) and late (n=22) pp period, 10 mg/kg fenoksi-2-metil-2-propionic acid was injected IM (day 7), and remaining ewes consisted of control groups. The estrus signs of the ewes were followed and they were hand-mated (ewe/ram=5/1). Pregnancies were determined with transabdominal real time B Mod ultrasonography with convex prob (3.5 MHz) on day 45 post-mating. Estrus rate, conception rate and total pregnancy rates were calculated. In early pp period, estrus rates, conception rate and total pregnancy rates were found in control and treatment groups as 81.8%, 69.5% (P>0.05); 44.4%,

43.7% (P>0.05); 63.6%, 82.6% (P>0.05), respectively and in late pp period, reproductive parameters were found in control and treatment groups as 100%, 86.3% (P>0.05); 59%, 42,1% (P>0.05); 72,7%, 63,6% (P>0.05), respectively. According to results of this study, administration of Hepagen to ewes in early and late pp period had no increasing effect on reproductive outcomes, but it was concluded that further studies should be conducted in herds applied intensive feeding program with evaluating the metabolic profile.

Keywords: Fenoksi-2-metil-2-propionic acid; Progesterone; PMSG; Postpartum; Ewes

Acknowledgement: We would like to thank Fatro GÜNEŞLİ for supporting this study about supplying of Hepagen.

TOTAL KNEE REPLACEMENT APPLICATIONS IN VETERINARY SURGERY

Ziya YURTAL[1] Kadri KULUALP[2] İbrahim ALAKUŞ[1] Halil ALAKUŞ[1]*
Ömer KIRGIZ[1] Muhammed Enes ALTUĞ[1]

[1] *1Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Surgery, Hatay, Türkiye*
[2] *Dokuz Eylül University, Faculty of Veterinary Medicine, Department of Surgery, İzmir, Türkiye*

*E-mail: halil.alakus@mku.edu.tr

The knee joint is the largest synovial joint in the musculoskeletal system. This joint consists of two different joints, the femorotibial between the femur and the tibia, and the femoropatellar between the femur and the patella. Other parts that make up the knee joint are the bone and capsuloligamentous structures, the meniscus and the muscle-tendon system. This joint is subjected to various forces and abrasions such as tension, compression, torsion. It is very difficult to restore a traumatized joint. In addition, diseases such as osteoarthritis in the knee cause joint pain, decreased physical activity and lameness. In advanced cases, desired results cannot be obtained from medical treatment methods. Surgically, techniques such as arthrodesis and amputation can be applied, but these do not provide a permanent solution. With the advancement of technological possibilities, the joints that need to be locked or cut are saved with total joint prostheses. Conditions that require a prosthesis to be applied to the knee joint can be summarized as advanced osteoarthritis cases, joint fractures that cannot be repaired by surgery, non-union fractures that do not respond to treatment, and joint dislocations that cannot be corrected by surgery. However there is not enough information and experience about total knee replacements. In the light of developing scientific techniques today, it is underlined that prosthesis applications, which were

used mostly in the hip joint in the past, can now be used in the knee joint as well. Adaptation of this technique to clinical practice is important in terms of regaining the mobility of the knee joint and preventing extremity losses. In this way, the living standard of animals, which were previously subject to limited treatment opportunities and whose welfare quality was adversely affected, will also increase. The aim of this review is to provide information about total knee replacement, which is not yet widely used, and to consider it among treatment alternatives.

Keywords: Dog; Total knee replacement; Veterinary orthopaedic surgery

PREVALENCE AND MOLECULAR CHARACTERIZATION OF *GIARDIA DUODENALIS* IN LAMBS IN HAKKARİ REGION

Erdal BİNGÖL[1*] Abdulalim AYDIN[1] Yaşar GÖZ[2] Adnan AYAN[3]
Özlem ORUNÇ KILINÇ[4] Özgür Yaşar ÇELİK[5] Fatma ERTAŞ OĞUZ[6]
Gürkan AKYILDIZ[7] Burçak ASLAN ÇELİK[8] Alp ATAY[1] Özge OKTAY AYAN[9]

[1]Çölemerik Vocational School, Hakkari University, Hakkari, Türkiye
[2]Department of Nutrition and Dietetics, Faculty of Health Sciences, Van Yuzuncu Yil University, Van, Türkiye
[3]Department of Genetics, Faculty of Veterinary Medicine, Van Yuzuncu Yil University, Van, Türkiye
[4]Özalp Vocational School, Van Yuzuncu Yil University, Van, Türkiye
[5]Department of Internal Medicine, Faculty of Veterinary Medicine, Siirt University, Siirt, Türkiye
[6]Department of Medical Services and Techniques, Tuzluca Vocational School, Iğdır University, Iğdır, Türkiye
[7]Faculty of Health Sciences, Marmara University, İstanbul, Türkiye
[8]Department of Parasitology, Faculty of Veterinary Medicine, Siirt University, Siirt, Türkiye
[9]Institute of Health Sciences, Van Yuzuncu Yil University, Van, Türkiye

*E-mail: erdalbingol@hakkari.edu.tr

Giardia duodenalis is a single-celled, flagellated protozoan parasite that lives in the intestines of various mammals, including humans. Infection is transmitted by ingestion of contaminated faeces, contaminated water, and food containing *Giardia* cysts. In this study, it was aimed to determine the prevalence and molecular characterization of Giardia duodenalis in lambs in Hakkari Region.

The animal material of the study consists of 200 lambs in the Hakkari region. Stool samples were collected from the rectum of lambs with disposable latex gloves. Stool samples were first examined microscopically for *Giardia duodenalis* cysts by native-Lugol staining method. DNA was then extracted from all 200 samples. Then, Nested PCR was performed with appropriate primers. After that, PCR products were run in agarose gel and images were obtained on a gel imaging device. Then the positive PCR products were sent to the sequence bidirectionally. Then, the sequence results were blasted and alligned and compared with the relevant reference genes in the GenBank.
As a result of microscopic examination, *Giardia duodenalis* cysts were found in 34 (%17) of 200 samples. As a result of Nested PCR, positivity was detected in 37 (%18.5) of 200 samples. Assemblage E detected as a result of sequencing.
As a result, it was concluded that *Giardia duodenalis* is intensively observed in lambs in the Hakkari region.

Keywords: *Giardia duodenalis*; Hakkari; Lamb; Molecular

Acknowledgement: This study was supported by Hakkari University Scientific Research Projects Coordination Project Code: FM22 BAP4)

A PRE-INVESTIGATION FOR THE POSITION OF KANGAL TURKISH SHEPHERD DOG IN TURKISH HAIR GOAT FLOCKS IN BURDUR PROVINCE

Aykut Asım AKBAŞ[1]* **Mustafa SAATCI[2]**

[1]Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Animal Science, Burdur, Türkiye

[2]Muğla Sıtkı Koçman University, Fethiye Faculty of Agriculture, Department of Animal Science, Muğla, Türkiye

*E-mail: icould_akbas@hotmail.com

The study was conducted to determine the position of Kangal shepherd dogs of some Turkish Hair goat farms under extensive conditions in Burdur province. Research data obtained from 40 enterprises (in total 8390 goats) at 6 villages by face to face questionnaires. While the total number of shepherd dog were 159; the average number of shepherd dog in per enterprise was 3.97 and 52,77 goats were defined for each dog. Dogs were predominantly Kangal crosses (81.13%). While the majority of the animals (85.00%) are provided from other shepherds and acquaintances, the rate of purchased from outside was 10%. Although all of shepherd dogs were kept in reserve between 7 and 15 years of age, more than half (60.00%) were between 10-12 years. While the most of the breeders (65.00%) thought that gender was important in the supply of dogs, females were more preferred with the percentage of 47.50. For feeding the animals, traditional foods (named as “Yal” and “Tepit”) prepared from flour or bran with different cooking techniques were mostly preferred (50.00%). Vaccination has a great importance in these enterprises. Therefore, all shepherd dogs are fully vaccinated. In the survey, it was determined that the puppies learned to protect goats from predators and thieves from other older shepherd dogs with the percentage of 62.50. This was followed by their owners (32.50%) and from their mothers. Almost all of goat enterprises

(97.50%) used collar (only metal) as a precaution against predators. It was detected that the number of goats perished in a year when there are shepherd dogs were between 0-5 head for large part of enterprises (72.50%) within the scope of the survey. Turkish Shepherd Dogs have a special place and importance in the protection and management of flocks in extensive Turkish Hair goat sector that have been adapted to the Anatolian geography. From this aspect, it was thought that obtained results from this study will help to characterise the usage of Turkish Shepherd Dogs and data from these results might be used for the following studies.

Keywords: Burdur, Goat flock, Kangal shepherd dog

IMPLEMENTATION OF HACCP SYSTEM: A CASE STUDY OF A CHICKEN LUNCHEON MEAT MANUFACTURING IN TÜRKİYE

İrem KARAASLAN*

[1]*Hatay Mustafa Kemal University, Technology and Research and Development Center, Hatay, Türkiye*

*E-mail: iremkaraaslan@hotmail.com

The consumption of chicken and chicken products has significantly increased in recent years in Türkiye. Although these increasing popularity, they could present several biological, chemical, and physical hazards. In the procurement process, the food safety management system is quite important and for the sake of public health, methods of detecting and controlling these hazards is neccessary. Hazard Analysis and Critical Control Point (HACCP) is a food safety management system method developed by the Codex Alimentarius Commission (CAC) which is recognized by the international food safety community as a guideline for controlling food safety hazards. The HACCP system establishes a control system that focuses more on prevention than on testing the final product. In this study, it's aimed to examine the biological, chemical, and physical hazards for all steps of the chicken luncheon meat manufacturing process, to design the prerequisite program to address some hazards prior to production. Also, the study highlighted the critical control points (CCP) by using the questions in the decision trees and the HACCP plan including hazards, detection methods, critical limits, monitoring, control measures and corrective actions for each CCP. Four CCPs and twenty-nine control points (operational prerequisite programs, CP) were identified throughout the manufacturing process. It is expected that it would assist process engineers and quality control specialists in designing and implementing control measures.

Keywords: HACCP; Chicken meat; Critical control point; Food safety and quality

Acknowledgement: The author gratefully acknowledge the top management and manufacture team of the chicken luncheon meat company who have asisted in the implementation of this research.

THE EFFECT OF ADDITION OF HEMPSEED OIL (*CANNABIS SATIVA* L.) TO BROILER RATIONS ON PERFORMANCE PARAMETERS

Mehmet DEMİRCİ[1] **Şevket EVCİ**[1]

[1] *Kırıkkale University, Vocational School, Laboratory and Veterinary Health Department, Kırıkkale, Türkiye*

*E-mail: m.demirci.tr@hotmail.com

In this study, it was aimed to investigate the effects of the addition of hempseed oil (*Cannabis sativa* L.) into the broiler diets on live weight (LW), live weight gain (LWG), feed consumption (FC), feed conversion rate (FCR), carcass yield and some internal organs weights. A total of 108 one-day-old broiler chicks (Ross 308) were used in the study, and three main groups were formed with three subgroups containing equal number of chicks. The control (C) group was fed with basal diet without any supplementation and the experimental groups were formed with addition of 1.5% (D1) and 3% (D2) hempseed oil into the basal diets and feeding was continued for 42 days. At the end of the experiment, the total average LW of C, D1 and D2 groups reached 2637.90, 2647.81 and 2665.68 g; average LWG of groups were 2595.03, 2605.16 and 2622.54 g; average FC amount were 4044.25, 3880.78 and 3900.36 g, respectively. It was determined that there was no statistical difference between the groups for LW, LWG and FC values ($P>0.05$). However, the average FCRs of groups were significantly different ($P<0.05$) between the groups and were 1.56, 1.50 and 1.49, respectively. Especially in the last two weeks of the growing period, an improvement was observed in the treatment groups compared to the control group. From the carcass parameters, average hot carcass, heart, pancreas, spleen weights and relative hot carcass rates were similar between the groups ($P>0.05$). However, there were statistically significant changes and/or trends in liver and bursa Fabricius data between the groups. It was

determined that liver weights were the lowest in the treatment groups (C>D2>D1) but bursa Fabricius weights were the lowest in the K group (C<D2<D1). In conclusion, it is possible to state that the use of hempseed oil at the rate of 1.5-3% provided increases in broiler performance, especially by improving FCRs. It is thought that the decreases in liver weights may result from decreases in hepatic steatosis rates, possibly due to the high polyunsaturated fatty acid composition of hempseed oil, while the increases in bursa Fabricius weights may be due to similar interactions of hempseed oil and increases in immune system activity specific to this organ. However, the main reasons for these changes can be clarified with histopathological examinations to be performed on these organs.

Keywords: Broiler, Ration/Diet, Hemp (*Cannabis sativa* L.), Seed, Oil, Performance

PHARMACOLOGICAL EFFECT OF *RHEUM RIBES*

Ecenur TUNÇKILIÇ[1]* **Hüsamettin EKİCİ[2]**

[1]*Kırıkkale University, Graduate School of Health Sciences, Department of Pharmacology and Toxicology, Kırıkkale, Türkiye*

[2] *Kırıkkale University, Faculty of Veterinary Medicine Department of Pharmacology and Toxicology, Kırıkkale, Türkiye*

*E-mail: ecenurakalin06@gmail.com

Rheum ribes is a member of the Polygonaceae family and is found in Iraq, Iran, Turkiye, and several neighboring countries. The sprouts and roots of this plant are widely used in the spring in Northern Iraq. Especially in Van and the surrounding provinces used in our country. Although it has a sour taste in the taste of kiwi, it is popularly called the banana of the east. It is used as a traditional treatment for diabetes diseases, urinary infections, cholesterol and stomach disorders, and hemorrhoid treatment. In the studies, its eongainstgram-positivee andgram-negativee bacteria was examined. At the same time, trial studies were conducted with HSV to determine the antiviral effect. While it has a common use for diabetes treatment, the hypoglycemic effect has not yet been discovered. The Pharmacological studies of this widely used continued from the past are not enough.

Keywords: Rheum ribes; Diabetes; Pharmacological effect; Hypoglycemic effect

PROKINETIC EFFECT OF METOCLOPRAMID

Sara Buşra EMİROĞLU*

Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Hatay, Türkiye

*E-mail: sarabusraemiroglu@gmail.com

Gastric motility is controlledby the autonomic nervous system and the enteric nervous system. Parasympathetic stimulation is provided by the vagus nerve and pelvic nerves, and the innervation here is by the effect of acetylcholine. Sympathetic stimulation is mostly due to the action of norepinephrine. Irregularities in the synthesis or disruption of these neurotransmitter substances may cause adverse effects on gastrointestinal motility (gastrointestinal dysmotility). In addition, gastrointestinal ulcers, viral, bacterial, and parasitic diseases, abdominal surgical procedures, gastric neoplasia, electrolyte irregularities, drugs, obesity, diabetes, sepsis, and foreign bodies are among the causes of gastrointestinal dysmotility. Metoclopramide antagonizes dopamine-2 and serotonin 5-HT_3 receptors. It is also an agonist of the serotonin 5-HT_4 receptor. Metoclopramide has an antiemetic effect by antagonizing the dopamine-2 and serotonin 5-HT_3 receptors in the chemoreceptor trigger zone (CTZ) in the central nervous system. It shows its prokinetic effect by affecting the 5-HT_4 receptor. 5-HT_4 receptors are reported to be located throughout the gastrointestinal tract. When this receptor is stimulated, acetylcholine is secreted in the myenteric plexus and gastrointestinal motility increases. It has been stated that metoclopramide, which is an agonist of the 5-HT_4 receptor, causes an increase in the amount of acetylcholine in the gastrointestinal tract, also sensitizes these receptors to acetylcholine. Metoclopramide stimulates the contraction of the stomach at the same timeprovides relaxation of the pylorus. Thus, it accelerates gastric emptying. It prevents esophageal reflux by increasing the tone of the lower

esophageal sphincter. In addition, it increases the peristalsis of the jejunum and reduces the transit time of nutrients from the duodenum to the ileocecal valve. Due to the prokinetic effect of metoclopramide, changes may occur in the pharmacokinetics of other drugs. Due to inhibition of dopamine-2 receptor, it can cause extrapyramidal reactions such as dystonia, dyskinesia, and Parkinson's symptoms.

Keywords: Metoclopramide; Prokinetic; Gastric dysmotility

ALTERNATIVE METHODS TO ANIMAL EXPERIMENTS IN TOXICITY TESTING

Zeyno NUHOĞLU ÖZTÜRK* **Abdurrahman AKSOY**

[1]*Ondokuz Mayıs University, Faculty of Veterinary Medicine, Department of Veterinary Pharmacology and Toxicology, Samsun, Turkiye*

*E-mail: nuhogluzeyno@gmail.com

Pharmaceutical products undergo toxicity testing to determine and evaluate their toxic effects prior to clinical use. These toxicity tests, performed on experimental animal models, are traditionally replaced by alternative methods due to ethical and social concerns, lack of compatibility between animal models, and high toxic effects. Based on the 3Rs principle, alternative toxicity methods have been developed, scientifically validated, and accepted by authorities. Regulatory agencies such as the OECD, FDA, and EPA have acknowledged alternative methods as valuable tools of modern toxicology. *In vitro* and *in silico* methods now play an essential role in defining and evaluating the toxicological profiles of drugs. Alternative methods developed for animal testing include cell and tissue culture, computational and pharmacokinetic models, microchip and omics technologies, models developed in non-mammals, and studies in human volunteers. Within the framework of alternative toxicity tests developed in recent years, proven tests for determining and studying eye, skin, reproductive, and developmental toxicity, carcinogenicity, hepatoxicity, neurotoxicity, and toxicity of biological substances are presented in databases. This presentation highlights the "Toxicity Tests and Alternative Methods to Animal Experiments" and their importance in modern toxicology.

Keywords: Replacement; Alternative toxicity tests; 3R principles

DETERMINATION OF CD AND HG LEVELS IN INFANT FORMULAS AND SUPPLEMENTARY FOODS

Hicran NURAL[1*] **İbrahim Ozan TEKELİ[2]**

[1] *Hatay Mustafa Kemal Univ., Instit. of Health Science, Depart. of Pharmacology and Toxicology, Hatay, Türkiye*

[2] *Hatay Mustafa Kemal Univ., Fac. of Veterinary Medicine, Depart. of Pharmacology and Toxicology, Hatay, Türkiye*

*E-mail: hicohuhu@gmail.com

The aim of this study was to determine the levels of Cd, Hg in infant formulas-foods and to evaluate the risks of their consumption. Breast milk is insufficient to meet the needs of babies after the 6th month and complementary or weaning formulas are recommended. The presence of contaminants such as heavy metals in infant formulas is an important risk factor. Infants consumes more food compared their body weight than adults, so they tend to be exposed to relatively higher levels of food chemicals. In the present study, 50 different brands and/or models of infant formula and foods (starting formulas, follow-on formulas, formula for special medical purposes and supplementary foods-powder form) were collected. Microwave wet digestion technique was used to remove the organic parts of samples before determining the metal levels. Cd and Hg levels in the samples were measured using inductively coupled plasma-mass spectrometry (ICP-MS). In infant formulas and foods, Cd levels were found in the range of <LOD-0.012 mg/kg. Cd was found only in supplementary infant foods. Hg was detected positive in only 6 samples. No maximum residue limit for Cd and Hg has been specified by national and international authorities (European Commission, Turkish Food Codex) for infant formulas. Due to the infants constitute the most sensitive members of

the society and may exposed to pollutants through foods, it is important to determine the concentrations of pollutants such as heavy metals and evaluate a risk assessment based on their consumption amounts.

Keywords: Infant Formulas; Cadmium; Mercury; Non-essential.

Acknowledgement: This study is summarized from a master's thesis and supported by Hatay Mustafa Kemal Univ. Coord. of Sci. Research project with project number 22.YL.004.

EVALUATION OF BISPHENOL ANALOGS: In Terms of Veterinary Toxicology

Aysun İLHAN [1*] **Mustafa YİPEL** [2]

[1] *Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, 31060 Hatay, Türkiye*

[2] *Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, 31060 Hatay, Türkiye*

*E-mail: aysunnilhann1@gmail.com

Bisphenols are one of the most widely used chemicals in the world, and they are one of the chemicals used in the production of polycarbonate and epoxy resins (since the 1950s). With the development of the food industry, the materials used in food packaging have changed, especially plastic packaging and canned food started to be used. Some plastic packaging and cans contain high amounts of BPA. Although food is the largest source of exposure to BPA in adults and children, EFSA recommended a daily tolerable intake of 5 µg/kg body weight/day for BPA in 2014. As a result of the legal regulations related to BPA, the need to find alternative substances to BPA has arisen. Substances that are structurally similar to BPA and alternatives to BPA in the production of epoxy resin and polycarbonate; Bisphenol F, Bisphenol S, Bisphenol B and Bisphenol E. BPF is a BPA analog with a wide spectrum of industrial uses. BPF is also used in food packaging, grouts, lacquers, industrial floors, plastics, liners, dental sealants, water pipes, electrical varnishes, coatings, adhesives, and tissue substitutes. BPS has also several uses, such as thermal receipt papers, epoxy glues, sulfonated poly and as an additive in dyes agents. BPAF, is used in common polymer applications, such as a cross-linker in fluoroelastomers, electronics, and optical fibers, as a high-performance monomer for polyamides, polyimides, polycarbonate copolymers, polyesters and in specialty polymer applications such as waveguides and plastic optical

fibers. Bisphenol AP (BPAP), is used as a plasticizer and flame retardant in synthesizing plastic, rubber, the chemical industry, polymer materials, and in the medical industry. Although there are fewer reports concerning BPE it has been detected in sewage sludge, aquatic organisms, and paper products. BPE also possesses potent effects on estrogen and androgen receptor activities. It is known that bisphenol A has a synthetic structure that has a very similar effect to the female sex hormone (xenoestrogen). Based on animal studies, it is possible that at high doses (100 times higher than the TDI) BPA may cause side effects on the liver and kidneys. There is also the possibility that rodents may have an effect on the mammary glands. BPA has toxicological effects on male and female reproductive, nervous, immune, metabolic and cardiovascular systems. It also has an effect on cancer formation. In addition, when exposed to BPA during pregnancy, these chemicals; It can affect both the mother and fetal development, depending on the period of exposure during pregnancy. Recently, intense studies have found that BPA affects toxic, teratogenic, carcinogenic and especially estrogenic mechanisms.

Keywords: BPA; Toxicology; Analogs; Veterinary

ANTIVIRAL EFFECTS OF FLAVONOIDS

Dilek Nur BESTİL[1]* Hamdi UYSAL[1]

[1] *Ankara. University, Faculty of Veterinary, Department of Biochemistry, Ankara, Türkiye*

*E-mail: dileknurbbestil@gmail.com

Flavonoids are polyphenolic phytochemicals commonly found in vegetables, fruits, flowers, nuts, seeds, tea, honey and propolis. They have extensive biological properties that support human and animal health and help to reduce disease risk. As a result of in vivo and in vitro studies, it has been determined that it has various biological activities. Some viral outbreaks have been confronted by humans and animals for years, including the most recent COVID-19 outbreak. Viral diseases are difficult to control because viruses consist of several structural units common to all living organisms and are equipped with powerful tools to help them spread in cells. Many researchers investigating the use of plants to cure these diseases have observed in vitro and in vivo studies that flavonoids have impressive biochemical activities such as antiviral activities, cardiovascular diseases, degenerative diseases, cancer and other age-related diseases, prevention of cardiovascular diseases, antibacterial, anti-fungal, anti-allergic, estrogenic and anti-inflammatory. For example, the anti-viral mechanism of action of flavonoids reduces virus titer by inhibiting entry of viral infection, inhibiting replication or translation of proteins. With the improvement of preparation techniques, newly developed flavonoid preparations exhibit better absorption and thus have higher bioavailability. When flavonoids are often prescribed with other drugs, understanding the compatibility of co-administered drugs is important for clinical practice.

In this review, the antiviral role of flavonoids against various viruses will be examined.

Keywords: Biological activity; Polyphenolic phytochemicals; Anti-viral mechanism.

IMMUNOLOCALIZATION OF ACTIN AND VIMENTIN PROTEINS IN FETAL BOVINE LIVER

Uğur TOPALOĞLU[1] **Nurşin AYDIN[1]***

[1] *Dicle University, Faculty of Veterinary Medicine, Department of Histology and Embriyology, Diyarbakır, Türkiye*

*E-mail:nursinaydin21@gmail.com

The liver assumes critical roles in the maintenance of life and the realization of some digestive processes. These are physiological processes such as blood production and blood volume regulation, protein synthesis, endocrine control of growth signaling pathways, and bile secretion during the embryonal period. It is known that many factors affect the regulation of these processes, especially cytoskeletal proteins, which have functions in determining cell shapes, as well as cell proliferation, division, migration, and tissue integrity. The present study was designed to determine the distribution and possibly physiological effects of the cytoskeletal proteins actin and vimentin in the fetal liver during pregnancy in bovine. For the study, 27 clinically healthy fetuses belonging to different periods of pregnancy obtained from private slaughterhouses were used. Fetuses whose ages were determined were classified into three groups belonging to the first, second, and third trimesters of pregnancy. Then, liver samples were taken from each group, passed through routine histological procedures, and subjected to immunohistochemistry staining. In the immunohistochemical staining findings, it was observed that actin and vimentin proteins were expressed very strongly in all vascular endothelial cells, kupffer cells, and smooth muscle cells around the vessels during pregnancy. In addition, it was found that vimentin also creates a moderate-intensity immunoreaction in hepatocytes in the first and second trimesters of pregnancy.

However, it was determined that actin and vimentin were negative in bile duct epithelial cells for all periods of pregnancy, and actin was negative in hepatocytes. As a result, it was thought that actin and vimentin may contribute to the formation of these cell shapes as well as the proliferation and differentiation of endothelial and smooth muscle cells by participating in the structure of the vessels in the fetal liver during pregnancy. In addition, it was suggested that actin and vimentin could positively affect physiological processes such as immunity and detoxification by participating in the structure of kupffer cells, which are responsible for defending the liver against pathogens.

Keywords: Bovine; Fetal Liver; Actin; Vimentin; Kupffer Cell

INVESTIGATION OF THE PREVALENCE OF VARROASIS AND NOSEMOSIS IN HONEY BEE (*APIS MELLIFERA*) COLONIES IN AKSARAY PROVINCE AND DETERMINATION OF RISK POTENTIALS FOR LOCAL BEEKEEPING

Neslihan SURSAL SIMSEK[1*] **Emrah SIMSEK[2]**

[1] *Mugla Sitki Kocman University, Milas Faculty of Veterinary Medicine, Department of Parasitology, Mugla, Türkiye*

[2] *Mugla Sitki Kocman University, Milas Faculty of Veterinary Medicine, Preclinical Sciences, Mugla, Türkiye*

*E-mail: neslihansursal@mu.edu.tr

Honey bee (*Apis mellifera*) colony and yield losses worldwide have been associated with many factors such as mainly parasites and other pathogens. Many studies have been conducted to detect the prevalence of parasites in honey bees in various province of Türkiye. However, there is no detailed data on honey bee parasitic diseases in Aksaray Province. In this study, it was aimed to investigate the presence and prevalence of the most important parasitic diseases such as varroasis and nosemosis in Aksaray Province by using conventional and molecular methods, to determine the risk factors for colonies in the local area, and to emphasize the necessity and importance of treatment and prevention-control programs against diseases. Survey studies were also conducted to determine the knowledge level of beekeepers on honey bee diseases and their impact, and drug apps. For this purpose, all samples were examined using the standard methods for both parasites. To detect *Varroa* mites, bees sampled from the hives were put in a container containing 70% alcohol and kept in alcohol for 5-15 minutes. Observed parasites in the bottom of the container were collected and counted. The infestation rates in bees and the number of *Varroa*-positive hives were calculated. To detect Nosema, the abdomens of 20 bees were put and homogenized in a centrifuge

tube containing 3-4 ml distilled water and examined under the light microscope. For *Nosema*, genomic DNA (gDNA) was also isolated from all microscopically examined samples and the obtained gDNAs were subjected to triplex PCR analysis. The hives examined in the Center, Ortaköy, and Güzelyurt districts of Aksaray were found to be highly (94%) contaminated by *Varroa* mites and the infestation rate in bees was determined as 22.8%. However, *Nosema* could not be detected in examined bees in both microscopic and molecular analyses. Considering the survey results, it was determined that *Varroa* mite was well known by beekeepers and they experienced colony losses due to this pathogen. However, beekeepers had limited knowledge of *Nosema*. In addition, it has been revealed that local beekeepers use various active ingredient consciously/unconsciously in the prevention or struggle of diseases. Consequently, with this project, the beekeeping potential, the status of the hives, the knowledge level of beekeepers on diseases, and the prevalence of *Varroa* mite in the Aksaray Province were determined, and also the local beekeepers/beekeepers' association was informed about parasitic diseases.

Keywords: *Varroa*; *Nosema*; Parasite; Honey bee; Aksaray

Acknowledgement: This work was supported by the Aksaray University Research Fund [grant number, Proje No: 2020/024].

CRYPTOSPORIDIOSIS IN LAMBS

Sinem AKDENİZ[1]* **Aycan Nuriye GAZYAĞCI[2]**

[1] Kırıkkale University, Graduate Scholl of Health Science, Department of Parasitology, Kırıkkale, Türkiye

[2] Kırıkkale University, Faculty of Veterinary Medicine, Department of Parasitology, Kırıkkale, Türkiye

*E-mail: sinemakdeniz72@gmail.com

Cryptosporidiosis is an acute or chronic enteric disease in humans and neonatal or immunocompromised animals caused by Cryptosporidium species in the class Apicomplexa. Infection is considered to be a serious disease of especially newborn lambs in terms of economy. Although most of the data on the prevalence of cryptosporidiosis in livestock relate to calves, cryptosporidiosis is a disease that causes economic loss in sheep worldwide. The presence of this infection in sheep was first reported in Australian lambs with diarrhea in 1974. In sheep, C. parvum, C. suis, C. andersoni, C. hominis, C. bovis, C. bovis-like genotype, new bovine B genotype, pig genotype II, marsupial genotype, new sheep genotype, Cryptosporidium xiaoin species have been reported. C. parvum is the most dominant species in sheep as in calves, and experimental lamb infections have been established with C. hominis species. Cryptosporidiosis is seen as one of the most important diseases in 4-10 days old neonatal lambs. Infected lambs have profuse watery diarrhea. Dehydration, loss of appetite, lethargy and abdominal tension are noted due to diarrhea. Diarrhea begins 3-7 days after the first oocyst ingestion and reaches its peak depending on the amount of oocysts in the stool. Even if clinical signs decrease, oocyst excretion may continue for days. In severe cases, deaths may occur within 2-3 days with diarrhea. In this presentation, it is aimed to give information about cryptosporidiosis in lambs.

Keywords: Apicomplexa, intestine, diarrhea, lambs; neonatal

HATCHLING OF INFECTIVE STAGE LARVAE OF *TOXOCARA CANIS* IN VITRO

Gözde Nur AKKUŞ[1]* **Kader YILDIZ[2]**

[1] *Kırıkkale University, Graduate School of Health Science, Department of Parasitology, Kırıkkale, Türkiye*

[2] *Kırıkkale University, Faculty of Veterinary Medicine, Department of Parasitology, Kırıkkale, Türkiye*

*E-mail: goznurak06@gmail.com

Toxocara canis is a parasite that lives in the intestines of Canidae, especially dogs. Some animal species such as mice, earthworms, chickens, sheep, pigs and poultry and also humans play a role as paratenic hosts in the life cycle of the parasite. The parasite eggs are excreted into the environment with the faeces of the dog. Infective larvae (L3) develop in the eggs at the appropriate temperature (15-35°C) and humidity in the environment for about 3-4 weeks period. Infection is formed by oral ingestion of the eggs with L3. *Toxocara canis* eggs are used for many experiments such as investigating the ovicidal effect of chemicals in vitro. Besides eggs, *T. canis* L3 is used for some studies such as obtaining excretory/secretory products. Some difficulties are encountered in the process of the hatchling of L3 from the eggs. This study aims to obtain live larvae from the egg in vitro. In this study, an adult parasite was collected from the faeces of dogs naturally infected with *T. canis*. The parasite has been diagnosed as *T.canis* based on morphological characters. The parasite eggs were removed from the *T. canis* uterus after dissected and washed with sterile distilled water. Then the eggs were incubated in 0.5% formaline solution at 28°C until development of L3. The embriogenesis into egg was observed daily using a light microscope. 28 days later, 5% sodium hypochlorite solution was used to hatch the *T. canis* larvae. The live

larva was observed microscopically. The method of the study offered the possibility to get live *T. canis* L3.

Keywords: *Toxocara canis*; Egss; Infective larvae; Hatchling; In vitro

PUBLIC AND MEDICAL USE OF *ARUM RUPICOLA* BOISS VAR. *RUPICOLA*

Tuğrul ATALAY[1,2] **Kader YILDIZ**[3]

[1]*Bozok University, Şefaatli Vocational Training School Laboratory and Veterinary Health,* [2]*Yozgat, Türkiye, Kirikkale University, Graduate School of Health Science, Department of Parasitology, Kırıkkale, Türkiye*

[3]*Kırıkkale University, Faculty of Veterinary Medicine, Department of Parasitology, Kırıkkale, Türkiye*

*E-mail: tugrul.atalay@bozok.edu.tr

In our country, *Arum rupicola* Boiss var. *rupicola* is called “dağsorsalı”. *Arum rupicola* Boiss var. *rupicola* is a poisonous and endemic plant seen in Cyprus, Israel, Iran, Iraq, Lebanon, Syria, Palestine, Georgia, Azerbaijan and Armenia, including our country. It is one of the Arum species used as herbal medicine in Turkish Traditional Medicine. Its main active ingredient is aronin. *Arum rupicola* Boiss var. *rupicola* is used to treat hemorrhoids, warts, cough, headache and diarrhea. Arum, one of the flowering plants, is a good antioxidant. Arum plant is collected fresh from nature in April-May and consumed after boiling in Güdül District of Ankara. It is believed to be medicinal by the people of the region.

Keywords: *Arum rupicola* Boiss var. *rupicola*; Dağsorsalı; Poisonous plant; Alternative medicine.

THIS PAGE INTENTIONALLY LEFT BLANK

online

7TH INTERNATIONAL CONGRESS ON VETERINARY AND ANIMAL SCIENCES

20–22 October 2022

www.icvas.gen.tr

ANT ACADEMY