Ss. CYRIL AND METHODIUS UNIVERSITY IN SKOPJE
FACULTY OF VETERINARY MEDICINE - SKOPJE

BOOK OF ABSTRACTS

9th International Scientific Meeting Days of Veterinary Medicine 2022

22 - 25 September 2022
R. of North Macedonia

Ss. CYRIL AND METHODIUS UNIVERSITY IN SKOPJE
FACULTY OF VETERINARY MEDICINE - SKOPJE

BOOK OF ABSTRACTS

9th International Scientific Meeting Days of Veterinary Medicine 2022

Skopje, 2022
R. N. Macedonia

ORGANIZATION COMMITTEES OF 9th INTERNATIONAL SCIENTIFIC MEETING DAYS OF VETERINARY MEDICINE 2022

International Scientific Committee

President

Prof. Dr. Lazo Pendovski, Ss. Cyril and Methodius University in Skopje, R. N. Macedonia

Members

Prof. Alen Slavica,
University of Zagreb, Croatia
Prof. Andrej Kirbis,
University of Ljubljana, Slovenia
Prof. Artur Niedzwiedz, University of Wroclaw, Poland
Prof. Bogdan Aminkov,
University of Forestry Sofia, Bulgaria
Prof. Breda Jakovac Strajn
University of Ljubljana, Slovenia
Prof. Danijela Horvatek Tomić,
University of Zagreb, Croatia
Prof. Danijela Kirovski,
University of Belgrade, Serbia
Prof. Dean Jankulovski,
Ss. Cyril and Methodius University in Skopje, R.N.Macedonia
Prof. Dine Mitrov,
Ss. Cyril and Methodius
University in Skopje, R. N. Macedonia
Dr. Dobrila Jakić-Dimić,
Scientific Veterinary Institute of Serbia, Serbia
Prof. Drago N. Nedić,
Veterinary Institute of the Republic of Serbia, Serbia
Dr. Elizabeta Dimitrieska-Stojkovikj, PhD,
Ss. Cyril and Methodius University in Skopje, R.N.Macedonia
Assoc. Prof. Florina Popovska-Percinikj,
Ss. Cyril and Methodius University in Skopje, R.N.Macedonia
Prof. Güven Kaşikçi,
Istanbul University, Turkey
Prof. Halil Selçuk Biricik,
University of Afyon, Turkey
Prof. Jože Starič,
University of Ljubljana, Slovenia
Prof. Jozef Laurincik, DrSc, Prof.H.C.,
Constantine the Philosopher University in Nitra, Slovakia
Prof. Kaspars Kovalenko,
Latvian University of Life Sciences and Technologies, Latvia
Prof. Marko Samardžija,
University of Zagreb, Croatia
Prof. dr. Aleksnadar Dodovski,
Ss. Cyril and Methodius University in Skopje, R.N.Macedonia
Prof. Milorad Mirilović,
University of Belgrade, Serbia
Prof. Muhamed Smajlović,
University of Sarajevo,
Bosnia and Herzegovina
Prof. Nenad Turk,
University of Zagreb, Croatia
Prof. Plamen Trojachanec,
Ss. Cyril and Methodius University in Skopje, R.N.Macedonia
Prof. Rizah Avdić,
University of Sarajevo,
Bosnia and Herzegovina
Prof. Serhat Alkan,
Istanbul University, Turkey
Prof. Tolga Kahraman,
Istanbul University, Turkey
Prof. Radmila Markovikj,
University of Belgrade, Serbia
Prof. Dragan Shefer, University of Belgrade, Serbia
Prof. Toni Dovenski,
Ss. Cyril and Methodius University in Skopje, R.N.Macedonia
Prof. Velimir Stojkovski,
Ss. Cyril and Methodius University in Skopje, R.N.Macedonia
Dr. Verica Milosevic, PhD,
University of Belgrade, Serbia
Dr. Vesna Z Djordjevic, PhD,
Institute of Meat Hygiene and Technology, Serbia
Prof. Vladimir Petkov,
Ss. Cyril and Methodius University in Skopje, R.N.Macedonia
Prof. Vlatko Ilieski, Ss. Cyril and Methodius
University in Skopje, R. N. Macedonia
Prof. Misho Hristovski,
Ss. Cyril and Methodius University in Skopje, R.N.Macedonia
Prof. Zehra Hajrulai-Musliu,
Ss. Cyril and Methodius University in Skopje, R.N.Macedonia

Local Organizing Committee

President

Asst. Prof. Dr. Miroslav Kjosevski, Ss. Cyril and Methodius University in Skopje, R. N. Macedonia

Members

Prof. Igor Ulchar
Assoc. Prof. Igor Djadjovski
Assoc. Prof. Irena Celeska
Assoc. Prof. Iskra Cvetkovikj
Assoc. Prof. Kiril Krstevski
Assoc. Prof. Ksenija Ilievska
Assoc. Prof. Nikola Adamov
Assoc. Prof. Radmila Chercheva Nikolovska
Prof. Romel Velev
Assoc. Prof. Sandra Mojsova
Prof. Slavcho Mrenoshki
Asst. Prof. Elena Atanaskova Petrov
Asst. Prof. Martin Nikolovski
Asst. Prof. Mirko Prodanov
Asst. Prof. Risto Uzunov
Dr. Aleksandra Angeleska, PhD
Dr. Aleksandar Cvetkovikj, PhD
Dr. Biljana Stojanovska Dimzoska, PhD
Dr. Branko Angjelovski, PhD
Dr. Igor Esmerov, PhD
Dr. Katerina Blagoevska, PhD
Dr. Ljupcho Mickov, PhD
MSc, Viktor Denkovski

all from the Faculty of Veterinary Medicine -Skopje (Ss.Cyril and Methodius University in Skopje, R. N. Macedonia)

Clinical Workshops - Chairs

Prof. Dr. Ksenija Ilievska, Ss. Cyril and Methodius University in Skopje, R. Macedonia

Dr. Tomislav Nikolovski, DVM - Executive Director of the Veterinary Chamber of the R.N. Macedonia

Secretariat of the Meeting

Dr. Ana Angelovski, PhD; Dr. Aleksandar Janevski, PhD, DVM; Dr. Marija Ratkova, PhD, DVM; Dr. Ljupcho Angelovski, PhD, DVM; Monika Dovenska, DVM; Todor Novakov, DVM; Zagorka Popova; Ljubica Rashic, DVM; Aleksandar Trajchovski, DVM; Dimitar Bozinovski, DVM; Filip Trojachanec, DVM; Miroslav Jovanovski, DVM; Jane Vlahov, DVM; Sara Georgievska, DVM; Larisa Shakjiri, DVM; Ivan Matevski, DVM; Boris Dimitrievski, DVM; Ana Galevska, DVM; Ena Dobrikj, DVM; Gordana Arsova, DVM; Dushica Trajkovska, DVM; m-r Stefan Jovanov; Tome Nestorovski
all from the Faculty of Veterinary Medicine -Skopje (Ss.Cyril and Methodius University in Skopje, R. N. Macedonia)

Financial Secretariat

m-r Ana A. Ordanovska, MSc
m-r Milica Tosevska Apostolova, MSc
m-r Maja Menkova, MSc

IT, DTP and Web Support

m-r Viktor Denkovski, MSc

Editors

Asst. Prof. Dr. Miroslav Kjosevski
Prof. Dr. Lazo Pendovski
Prof. Dr. Florina P. Percinic
Asst. Prof. Dr. Martin Nikolovski
Monika Dovenska, DVM

Published by:

Faculty of Veterinary Medicine – Skopje,
Lazar Pop Trajkov 5/7, 1000 Skopje
Tel: ++389 2 3240 700 Fax: ++ 389 2 3114 619
www. fvm.ukim.edu.mk

CIP - Каталогизација во публикација
Национална и универзитетска библиотека "Св. Климент Охридски", Скопје

636.09(062)(048.3)

INTERNATIONAL scientific meeting (9 ; 2022 ; Skopje)
Book of abstracts : 9th International scientific meeting days of veterinary medicine 2022, Skopje, 2022 R. N. Macedonia / [editors Miroslav Kjosevski ... и др.]. - Skopje : Faculty of veterinary medicine, 2022. - 177 стр. ; 25 см

Други уредници: Lazo Pendovski, Florina P. Percinic, Martin Nikolovski, Monika Dovenska. - Регистар

ISBN 978-9989-774-38-6

а) Ветеринарна медицина -- Зборници -- Апстракти

COBISS.MK-ID 58134021

Dear colleagues,

I am pleased to welcome you to the the 9th International Scientific Meeting "Days of veterinary medicine – 2022" which will be held in Ohrid, R. of North Macedonia, from 22 to 25 September, 2022.

The event is attractive for all researchers, veterinary practitioners, official veterinarians, professors, PhD and post-doctoral students, offering an excellent opportunity to discuss the challenges and ongoing issues in the veterinary education and science.

As a previous, this year Days of veterinary medicine – 2022 retain the traditional popular format, including a number of invited plenary lectures, short presentations and poster presentations for animal health, animal welfare and reproduction, as well as public health and food safety.

It will also provide three workshops for practitioners and educators to present and discuss the most recent innovations, trends, and concerns as well as practical challenges encountered and solutions adopted in the fields of animal health, reproduction and clinical sciences.

The program for the meetings required dedicated effort of many people, in order to retain its usual popular format, including a number of plenary lectures, short presentations, student papers and poster presentations.

In this occasion I express my gratitude to the members of the International Scientific Committee and Local Committee for their diligent and professional reviewing in electing 104 papers from authors of more than 12 countries to be presented in 10 scientific sessions followed by the oral and poster sessions, 3 clinical workshops and 1 laboratory workshop.

On the behalf of the Organizing Committee, I wish you an enjoyable and pleasant stay in R. N. Macedonia.

I am look forward to welcoming you!

Prof. Dr. Lazo Pendovski,

on behalf of the Organizing Committee of
the Days of veterinary medicine – 2022

SCIENTIFIC PROGRAM

9th INTERNATIONAL SCIENTIFIC MEETING
DAYS OF VETERINARY MEDICINE – 2022

Thursday (22.09.2022)	
16:00 – 18:30	**Arrival of participants and Registration**
19:00 – 19:30	**Opening ceremony and welcome** Prof. d-r Lazo Pendovski, Dean at the Faculty of Veterinary Medicine – Skopje, UKIM Prof. d-r Nikola Jankulovski, Rector at the Ss. Cyril and Methodius University in Skopje Mr. Ljupcho Nikolovski - Minister of Agriculture, Forestry and Water Economy Dr. Nikolche Babovski, DVM, Director at the Food and Veterinary Agency Dr. Tomislav Nikolovski, DVM, Director of the Macedonian Veterinary Chamber
19:30 – 20:00	**Invited Lecture:** ***New skills and knowledge in veterinary profession*** Prof. dr. sc. Alen Slavica- Faculty of veterinary medicine, University of Zagreb
20:00	**Welcome Cocktail**

Friday (23.09.2022)		
09:00 – 09:30	**Plenary lecture I:** ***Biosecurity in animal production, what, when and how?*** Prof. dr. Jeroen Dewulf, Faculty of Veterinary Medicine, Ghent University, Belgium (Lecture room 1)	
09:30 – 11:00	**Session I:** **Farm Biosecurity and Environment** *Chairs: Jeroen Dewulf and Miroslav Kjosevski* (Lecture room 1, parallel session)	**Session II:** **Basic sciences and Veterinary education** *Chairs: Rizah Avdić and Martin Nikolovski* (Lecture room 2, parallel session)
	Biosecurity assessment and critical improvement points of dairy cattle farms - Miroslav Kjosevski*, Vanja Kondratenko, Branko Angjelovski, Aleksandar Janevski, Milenko Simovikj, Igor Djadjovski, Aleksandar Dodovski, Kiril Krstevski	***Renal microvasculature in the chicken*** - Nedžad Hadžiomerović*, Rizah Avdić, Faruk Tandir
	Comparison of the two different protocols for biosecurity assessment in commercial pig farms - Branko Angjelovski*, Vanja Kondratenko, Milenko Simovikj, Jovan Bojkovski, Miroslav Kjosevski	***Forming new collection of pharyngeal teeth from cyprinid fishes collected in Croatia and neighboring countries*** - Denis Leiner*, Srebrenka Nejedli, Snježana Ćurković, Damir Mihelić
	Assessment of biosecurity measures on the broiler farms in the region of Belgarde city - Jelena Maletic*, Ljiljana Spalević, Vesna Milićević, Bojan Milovanović, Branislav Kureljušić	***Radiological diagnosis of an ectopic ureter in a poodle – Case report*** - Boris Dimitrievski*, Aleksandar Janevski, Marko Mitrović, Dine Mitrov
	Biomonitoring of wastewaters from slaughterhouses in Macedonia Marija Ratkova Manovska, Gordana Ilievska, Snezana Dimitrovska, Ena Dobrik, Miroslav Kjosevski, Biljana Stojanovska Dimzoska, Katerina Blagoevska*	***Suppression effects of excessively expressed gene bcl-2 in cell lines of prostate cancer*** - Igor Esmerov*, Nikola Adamov, Aleksandra Angelevska, Radmila Chrcheva, Ljupco Mickov, Ljupco Angelovski, Nikolaj Markov, Branko Atanasov
	The impact of small hydro power plans on trout fish farms in North Macedonia - Aleksandar Trajchovski*, Misho Hristovski	***Digital veterinary education (devet) – an ERASMUS+ project for development/ creating of digital materials/tools in the education program of veterinary medicine (outcomes review)*** - Igor Ulchar*, Ksenija Ilievska, Lazo Pendovski, Zehra Hajruali Musliu, Alen Slavica, Lada Radin, Marko Poleto, Danijela Kirovski, Ljubomir Jovanovic, Ivan Jovanovic

09:30 – 11:00	***Initial findings of total air dust concentration in cattle and poultry houses in Macedonia*-** Ena Dobrikj*, Aleksandar Janevski, Aleksandar Dodovski, Miroslav Kjosevski	
11:00 – 11:30	**Coffee break & Poster exhibition (Part 1)**	

11:30– 12:00	**Plenary lecture II:** ***Antimicrobial resistance (AMR) problems in companion animals*** Prof. dr. Peter Damborg, University of Copenhagen, Denmark (Lecture room 1)	
12:00 – 13:30	**Session III: Antimicrobial resistance** *Chairs: Peter Damborg and Iskra Cvtekovikj* (Lecture room 1, parallel session)	**Session IV: Animal Welfare and Behaviour** *Chairs: Vlatko Ilieski* (Lecture room 2, parallel session)
	Antimicrobial resistance in staphylococci isolated from dogs in the Republic of North Macedonia - Iskra Cvetkovikj*, Ivana Arsovska, Marija Ratkova Manovska, Mirko Prodanov, Ljubica Rashikj, Zagorka Popova, Igor Djadjovski, Aleksandar Cvetkovikj	***Ultrasound – method to assess keel bone fractures in laying hens -*** Dimitar Bozinovski*, Lazo Pendovski, Miroslav Kjosevski, Martin Nikolovski, Dimitar Terzievski,Vlatko Ilieski
	The occurrence of blaCTX-M, blaSHV, blaTEM genes in extended-spectrum β-lactamase producing commensal E.coli isolates from dairy farms in the municipality of Debar - Maksud Kerluku*, Marija Ratkova Manovska, Gordana Ilievska, Dean Jankuloski, Katerina Blagoevska	***Stray dog population size trend in the city of Skopje between 2010 and 2020 -*** Dimitar Terzievski*, Miroslav Kjosevski, Dimitar Bozinovski, Vlatko Ilieski
	Antimicrobial susceptibility of Trueperella pyogenes isolates from various clinical specimens of animals - Dilek Öztürk, Sibel Yaman*	***Recognition and assessment of pain in dogs after elective surgery in order to improve animal welfare -*** Dardan Pozhegu*
	Antimicrobial resistance in commensal Escherichia coli isolated from pigs' cecal samples at slaughter - Dean Jankuloski, Marija Ratkova Manovska, Katerina Blagoevska, Gordana Ilievska, Biljana Stojanovska Dimzoska, Mirko Prodanov, Ljupco Angelovski*, Sandra Mojsova, Snezana Dimitrovska	***Welfare assessment of pigs in the time of slaughter - stunning quality as a welfare indicator -*** Emilija Murdjeva*, Branko Angjelovski, Mirko Prodanov, Miroslav Kjosevski
	Veterinarian's standpoints regarding antimicrobial stewardship in Serbia- Zorana Kovačević*, Mihajlo Erdeljan, Ivan Stančić, Dragana Tomanić, Zorana Ružić, Ivan Galić, Romel Velev	***Welfare assessment of sport horses in stables in Macedonia -*** Kristina Antovska*, Lazo Pendovski, Vlatko Ilieski, Miroslav Kjosevski
		Survey on recognition of problems of locomotion system in horses in Macedonia - Marija Solakova*, Elena Atanaskova Petrov, Miroslav Kjosevski, Ksenija Ilievska

13:30 – 14:30	**Lunch**
14:30 – 15:00	**Plenary lecture III:** ***The Practical Application of Regenerative Medicine in Canine Musculoskeletal Conditions*** Dr Russell Chandler BVSc CertSAO MSc(OrthoEng) MRCVS, Small Animal Orthopaedics Alphavet Referrals Newport, Wales, UK (Lecture room 1)
15:00 – 15:30	**Plenary lecture IV:** ***Intervertebral disc disease – a marked road or a jungle?*** Prof. dr. sc. Boris Pirkich, Faculty of veterinary medicine, University of Zagreb, Croatia (Lecture room 1)
15:30 – 16:00	**Coffee break & Poster exhibition (Part 2)**

16:00 – 17:30	**Session V:** **Veterinary Clinical Medicine** *Chairs: Russell Chandler and Ksenija Ilievska* (Lecture room 1)
	Topical application of homologous serum for treatment of corneal ulcer in dogs **-** Ksenija Ilievska*, Elena Atanaskova Petrov, Filip Trojachanec, Jane Vlahov, Plamen Trojachanec
	Disease-stage related therapeutic response of mesenchymal stromal cells after intra-articular delivery in a mouse osteoarthritic joint **-** Ana Ivanovska*, Patrizio Mancuso, Conor Hennessy, Swarna Raman, Steven McLoughlin, Jamie Reilly, Frank Barry, Mary Murphy
	Mesenchymal stromal cells lyo-secretome: preliminary clinical application in dogs and horses with naturally occurring osteoarthitis **-** Priscilla Berni*, Michela Mocchi, Virna Conti, Silvia Dotti, Dario Di Silvestre, Riccardo Villa, Roberto Ramoni, Giulia Passignani, Francesca Brambilla, Maurizio Del Bue, Gianpaolo Squassino, Laura Catenacci, Milena Sorrenti, Lorena Segale, Elia Bari, Pierluigi Mauri, Sara Perteghella, Maria Luisa Torre, Stefano Grolli
	Beneficial effect of Acepromazine on incidences of adverse effects associated with morphine premedication in dogs **-** Jane Vlahov*, Plamen Trojacanec, Todor Novakov, Ksenija Ilievska, Filip Trojacanec
	Pre-emptive multimodal analgesia with Morphine-Medetomidine-Ketamine and Acepromazine- Morphine-Ketamine for ovariohysterectomy in dogs **-** Todor Novakov*, Elena Atanaskova Petrov , Ksenija Ilievska, Irena Celeska, Jane Vlahov, Filip Trojacanec, Plamen Trojacanec
	Trilostan titration dose treatment in dogs with terminal stage of hyperadrenocorticism complicated with comorbidity disease **-** Irena Celeska*, Martin Nikolovski, Todor Novakov, Elena Atanaskova Petrov
17:30 – 20:30	**Guided Ohrid City Tour**

20:30 – 22:30	**Dinner at National Macedonian Restaurant**

Saturday (24.09.2022)		
09:00 – 09:30	**Plenary lecture V:** ***Reproductive management in goat breeding*** Prof. dr. sc. Juraj Grizelj, Faculty of veterinary medicine, University of Zagreb, Croatia (Lecture room 1, parallel session)	**Clinical Workshop 1:** (Lecture room 3, parallel session) **Orthopedic examination in dogs and cats** Prof. dr. sc. Mario Kreszinger Faculty of veterinary medicine, University of Zagreb, Croatia
09:30 – 10:30	**Session VI: Animal Reproduction** *Chairs: Juraj Grizelj and Toni Dovenski* (Lecture room 1, parallel session)	
	The effect of homologous seminal plasma and reduced glutathione on thawed ram sperm viability and motility **-** Martin Nikolovski*, Monika Dovenska, Dimitar Bozhinovski, Ljupcho Mickov, Branko Atanasov, Nikola Adamov, Toni Dovenski Vladimir Petkov	
	Kinetic parameters of chiled boar spermatozoa from different porcine breeds **-** Ljupco Mickov*, Branko Atanasov, Martin Nikolovski, Monika Dovenska, Igor Esmerov, Nikola Adamov, Vladimir Petkov, Toni Dovenski	
	Assessment of in vitro maturation rate of porcine oocytes selected by brilliant cresyl blue staining **-** Monika Dovenska*, Ljupcho Mickov, Branko Atanasov, Vladimir Petkov, Martin Nikolovski, Toni Dovenski, Florina Popovska-Perchinikj	
	Introduction of the Ovsynch-8 protocol in small dairy farms in RNM to increase the reproductive efficiency from the first AI postpartum **-** Kristina Dojchinovska*, Toni Dovenski, Ljupcho Mickov, M. Toshevska, Z. Mihaloski, I. Taleski, Lj. Andreevski, Branko Atanasov	
10:30 – 11:00	**Coffee break & Poster exhibition (Part 3)**	

<table>
<tr><td>11:00 – 11:30</td><td colspan="2">Plenary lecture VI:
Update on activities of the European Reference Laboratory for Listeria monocytogenes: focus on typing area
Sandrine Te, French Agency for Food, Environmental and Occupational Health & Safety (ANSES)
(Lecture room 1, parallel session)</td><td rowspan="4">Clinical Workshop 2:
(Lecture room 3, parallel session)

Emergency states in veterinary ophthalmology
Prof. dr. sc. Boric Pirkić
Faculty of veterinary medicine, University of Zagreb, Croatia</td></tr>
<tr><td rowspan="3">11:30 – 13:15</td><td>Session VII:
Food Safety
Chairs: Sandrine Te and Katerina Blagoevska
(Lecture room 1, parallel session)</td><td>Session VIII:
Infectious Diseases in Animals
Chair: Lejla Velić and Kiril Krstevski
(Lecture room 2, parallel session)</td></tr>
<tr><td>Prevalence of serotypes 1/2a and 4b of Listeria monocytogenes from ready-to-eat food products in Kosovo - Besart Jashari*, Katerina Blagoevska, Dafina Mehmetukaj, Dean Jankuloski</td><td>Discovery and introduction pattern of panzootic Newcastle disease virus in chickens in Macedonia, 2020 - Aleksandar Dodovski*, Renfu Yin</td></tr>
<tr><td>Enterotoxigenic staphylococcus strains isolated from raw milk and diary products from R.N. Macedonia - Marija Ratkova Manovska*, MirkoProdanov, Dean Jankuloski, Pavle Sekulovski, Katerina Blagoevska</td><td>Ovine paratuberculosis: intra-herd incidence study and assessment of commercial (ID. VET, FRANCE) indirect milk elisa test performance in dairy sheep - Anna-Rita Attili, Eleonora Bonacucina, Martina Linardi, Luciana Pacifici, Vincenzo Cuteri*</td></tr>
<tr><td>Antibacterial activity of goat whey with added kefir grains - Sandra Mojsova*, Vesna Levkov, Ljupco Angelovski</td><td>Zoonotic potential of equine-associated viruses - Mihajlo Erdeljan*, Tijana Kukurić, Ivan Stančić, Ivana Davidov, Miodrag Radinović, Annamaria Galfi Vukomanović, Nadežda Tešin</td></tr>
</table>

11:30 – 13:15	***Bacterial contamination in different stages of poultry slaughtering process*** **-** Mirko Prodanov*, Marija Ratkova Manovska, Sandra Mojsova, Ljupco Angelovski, Dean Jankuloski	***Re-emergence of rabies in Bosnia Herzegovina after the offset of oral rabies vaccination*** **-** Eterović Toni*, Lejla Velić, Oliver Stevanović, Amer Alić	**Clinical Workshop 2:** (Lecture room 3, parallel session) **Emergency states in veterinary ophthalmology** Prof. dr. sc. Boric Pirkić Faculty of veterinary medicine, University of Zagreb, Croatia
	Occurrence of aflatoxins, ochratoxin A and zearalenone in poultry feed **-** Ida Kucinoska*, Biljana Stojanovska-Dimzoska, Aleksandra Angeleska, Katerina Blagoevska, Gordana Ilievska, Dushica Koceva, Elizabeta Dimitrieska-Stojkovic	***Detection and genetic characterisation of porcine circovirus type 2 in Macedonian wild boar and domestic pig population*** **-** Zagorka Popova Hristovska, Kiril Krstevski*, Ivan Matevski, Stefanija Markozanova, Dine Mitrov, Igor Djadjovski	
	Presence of heavy metals in animal feeds of plant origin **-** Dushica Koceva*, Elizabeta Dimitrieska-Stojkovik, Biljana Stojanovska-Dimzoska, Aleksandra Angeleska	***Presence and prevalence of non-regulated infectious diseases with high economic impact on Macedonian dairy farms*** **-** Ivan Matevski*, Igor Djadjovski, Kiril Krstevski	
	Proposed chemical substances as quality parameters of chocolate **-** Stefan Jovanov*, Risto Uzunov, Tome Nestorovski, Zehra H. Musliu, Velimir Stojkovski		
13:15 – 14:00	**Lunch**		

<table>
<tr><td>14:00 – 14:30</td><td colspan="2">Plenary lecture VII:
Virtual slaughterhouse simulators as a complement, not a substitute of abattoir visits, in veterinary public health education
Prof. dr. Andrej Kirbiš, Faculty of Veterinary Medicine, University of Ljubljana Ljubljana, Slovenia
(Lecture room 1, parallel session)</td><td rowspan="4">Clinical Workshop 3:
(Lecture room 3, parallel session)

The practical application of regenerative medicine in canine musculoskeletal conditions
Dr Russell Chandler BVSc CertSAO MSc(OrthoEng) MRCVS, Small Animal Orthopaedics Alphavet Referrals Newport, Wales, UK</td></tr>
<tr><td rowspan="3">14:30 – 16:15</td><td>Session IX:
Methods and Models
Chairs: Andrej Kirbiš and Dean Jankuloski
(Lecture room 1, parallel session)</td><td>Session X:
Parasitic Diseases in Animals
Chairs: Aleksandar Cvetkovikj and Jasmin Omeragić
(Lecture room 2, parallel session)</td></tr>
<tr><td>Evaluation of the performance of a real time PCR method for detection of Salmonella spp. invA gene in chicken meat - Gordana Ilievska, Marija Ratkova Manovska, Dean Jankuloski, Katerina Blagoevska*</td><td>Clinicopathological alterations in pet dogs naturally infected with Ehrlichia canis and Leishmania infantum in Macedonia – case study - Elena Atanaskova Petrov*, Ksenija Ilievska, Todor Novakov, Ljubica Rashik, Igor Djadjovski, Miroslav Kjosevski, Irena Celeska</td></tr>
<tr><td>Identification of meat species based on DNA hybridization, real-time PCR and fatty acid profile with GC-FID - Dafina Mehmetukaj*, Vlora Zogëjani, Armend Cana, Xhavit Bytyçi, Besart Jashari, Zehra Hajrullai Musliu, Katerina Bllagoevska, Dean Jankuloski</td><td>Investigation of gastrointestinal helmint infections of horses in Thrace region, Türkiye - Fatma Nur Dal*, Şakir Pehlivan, Kerem Öter, İbrahim Kurban, Aynur Gülanber</td></tr>
</table>

14:30 – 16:15	***Diagnostic performance of eight PCR protocols and one indirect ELISA in naturally small ruminant lentiviruses infected ewes in Greece*** - Aphrodite I. Kalogianni*, Ioannis Bossis, Ilias G. Bouzalas, Athanasios I. Gelasakis	***Prevalence and genetic characterization of Echinococcus granulosus in domestic ruminants in North Macedonia*** - Ljubica Rashikj*, Aleksandar Cvetkovikj, Ivana Arsovska, Iskra Cvetkovikj, Jovana Stefanovska	**Clinical Workshop 3:** (Lecture room 3, parallel session) **The practical application of regenerative medicine in canine musculoskeletal conditions** Dr Russell Chandler BVSc CertSAO MSc(OrthoEng) MRCVS, Small Animal Orthopaedics Alphavet Referrals Newport, Wales, UK
	Suitability of using the ISO 13730:1996 method for total phosphorous quantification in milk and milk products - Tome Nestorovski*, Stefan Jovanov, Riste Uzunov, Ana Angelovska, Zehra Hajrulai Musliu, Radmila Crceva Nikolovska	***Investigation of parasitic diseases in wild animals in the federation of Bosnia and Herzegovina*** - Jasmin Omeragić, Naida Kapo*, Darinka Klarić Soldo, Vedad Škapur, Saša Kunovac, Ermin Šaljić, Šejla Goletić, Adis Softić, Sabina Šerić-Haračić, Teufik Goletić	
	Verification of a sampling method for animal feed from different origins used for chemical analysis - Ana Angelovska*, Radmila Crceva Nikolovska, Aleksandra Angeleska, Tome Nestorovski, Dean Jankuloski, Mirko Prodanov, Zehra Hajrulai Musliu	***Gastrointestinal helminths of goats breeding at moutain area of central Serbia*** - Pavlović Ivan*, Radanović Oliver, Zdravković Nemanja, Savić Božidar, Tasić Aleksandra, Pavlović Marija	
	Contribution of animal model in the development of radiopharmaceuticals and successful translational molecular imaging and therapy - Emilija Janevik-Ivanovska*, Icko Gjorgoski, Lajos Balogh	***Identification of lice species of water buffaloes in Marmara region of Türkiye*** - Şakir Pehlivan*, Fatma Nur Dal, Kerem Öter, Aynur Gülanber	

14:30 – 16:15	***Evaluation of antioxidative enzymes in rats treated with Origanum vulgare essential oil during acute intoxication with Deoxinivalenol and Fumonisin B1*** **-** Gordana Ilievska*, Katerina Blagoevska, Biljana Stojanovska-Dimzoska, Elena Rafailovska, Biljana Miova	***Pathogenicity of Triaenophorus nodulosus (cestoda: bothriocephalidea) in Northern pike (Esox lucius) from the Mrežnica river, Croatia*** **-** Valerija Benko*, Krešimir Matanović, Snježana Kužir, Zrinka Dragun, Emil Gjurčević	
16:15 – 16:30	**Coffee break & Poster exhibition (Part 4)**		
16:30 – 18:00	**Laboratory Workshop:** ***Molecular typing method validation EURL-NRL and Method implementation within NRL network*** Sandrine Te, French Agency for Food, Environmental and Occupational Health & Safety (ANSES) (Lecture room 1)		
20:00 – 23:00	**Gala Dinner**		

Sunday (25.09.2022)
Departure of participants

CONTENT

Scientific session III
ANTIMICROBIAL RESISTANCE

Plenary lecture

Oral presentations

Scientific session IV
ANIMAL WELFARE AND BEHAVIOR

Oral presentations

Scientific session V
VETERINARY CLINICAL MEDICINE

Plenary lectures

Oral presentations

Scientific session VI
ANIMAL REPRODUCTION

Plenary lecture

Oral presentations

Scientific session VIII
INFECTIOUS DISEASES IN ANIMALS

Oral presentations

Scientific session IX
METHODS AND MODELS

Plenary lecture

Oral presentations

Scientific session X
PARASITIC DISEASES IN ANIMALS

Oral presentations

POSTER PRESENTATIONS

Scientific Session I:
FARM BIOSECURITY AND ENVIRONMENT

Plenary lectures and Oral presentations

PL1

BIOSECURITY IN ANIMAL PRODUCTION, WHAT, WHEN AND HOW?

Jeroen Dewulf

Ghent University, Faculty of veterinary medicine,Department of internal medicine, reproduction and population medicine, Veterinary epidemiology unit, Campus Merelbeke Salisburylaan 133, 9820 Merelbeke, Ghent, Belgium

Biosecurity is defined as the combination of all measures implemented that reduce the risk of introduction and spread of disease agents. Biosecurity can be considered at different levels such as country, region, herd or flock, and even the individual animal. Implementing biosecurity requires the adoption of a set of attitudes and behaviors to reduce the risk in all activities involving animal production or animal care. Biosecurity is based on the prevention of and protection against infectious agents. The measures to be established should not be seen as constraints but rather as part of a process aimed at improving health of animals, people and the environment. Biosecurity can be subdivided in two main components: external biosecurity is focused at keeping pathogens out of the herd and internal biosecurity is focusing at preventing the spread of pathogens within the herd. Biosecurity is considered the basis of any disease control program. The combination of all biosecurity measures aims preventing both the introduction as well as the spread of infectious agents in a group of animals. Biosecurity is important both in controlling exotic diseases as well as endemic diseases. If biosecurity and disease prevention measures are well implemented curative treatment of diseased animals can be restricted to an absolute minimum. Improving biosecurity, and by consequence reducing infection pressure, may also result in substantial improvements of production results as well as reduced antimicrobial use. When designing biosecurity programs, there are some general principles that are of value in all settings: 1. Try to separate high and low risk animals and environments; 2. Try to reduction of the general infection pressure to a level the immunity of the animals can cope with; 3. Rank the measures from high to low importance as not every transmission route is equally important; 4. Take into account both the probability of transmission and frequency of occurrence of transmission routes; 5. Be aware that risks are increasing as farms are growing.

Key words: biosecurity, infection prevention, animal production, pigs, poultry, cattle

O1

BIOSECURITY ASSESSMENT AND CRITICAL IMPROVEMENT POINTS OF DAIRY CATTLE FARMS

Miroslav Kjosevski*[1], Vanja Kondratenko[2], Branko Angjelovski[3], Aleksandar Janevski[3], Milenko Simovikj[2], Igor Djadjovski[3], Aleksandar Dodovski[4], Kiril Krstevski[5]

[1]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Department of animal hygiene and environmental protection, Lazar Pop–Trajkov 5-7, 1000 Skopje, North Macedonia
[2]Food and veterinary agency, Department for animal health and welfare, 3-ta Makedonska Brigada 20, 1000 Skopje, North Macedonia
[3]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Department of internal diseases in farm animals, Lazar Pop–Trajkov 5-7, 1000 Skopje, North Macedonia
[4]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Department for avian diseases, Lazar Pop–Trajkov 5-7, 1000 Skopje, North Macedonia
[5]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Department for infectious diseases and epidemiology, Lazar Pop–Trajkov 5-7, 1000 Skopje, North Macedonia

Farm biosecurity represents a combination of different measures implemented to reduce the risk of introduction and spread of disease agents. The type of biosecurity measures might differ a lot between the farming systems, even though the main goal is one, i.e., accomplishing high control of disease agents. Therefore, a biosecurity assessment based on the outputs of the implemented measures should objectively estimate the farm biosecurity level. Dairy farms in Macedonia are predominantly small scale with tie stall system under individual/family holdings. The risk of outbreaks and spreading of animal diseases will be significantly reduced if these farms have functional biosecurity plan. Therefore, the objective of this study was to assess the biosecurity level of dairy farms in Macedonia and to identify the critical points from the biosecurity perspective. The BIOCHECK CATTLE®protocol for Dairy cattle, including its scoring system, was used for the biosecurity assessment of the farms. Prior to the assessment, 16 national experts in the field rated this protocol for its reliability and applicability (by using the on-line survey). Out of 1000 randomly selected dairy farms in the country, the 952 farms were visited and 723 dairy farms were fully assessed and scored. According to the national experts, the lowest median regarding the importance of the questions in the survey was 7 (on a scale from 1-10) in 2% and the median of 10 and 9 was present in 92% of all questions. Regarding the scores of the performed on-farm assessment, the median of the total biosecurity score was 47, interquartile range (IR) of 39 – 56. The External Biosecurity was scored with median 70 (IR, 58-80), average of 67.7 ± 15.4, in opposite to the Internal Biosecurity which showed lower scores 24 (IR, 18-32), average of 26.4 ± 11.7, ($p<0.0001$). Within the

external biosecurity the category with highest score was 'Purchase and reproduction' 84.0 ± 28.3 and the lowest scored category was 'Transport and carcass removal' 40.7± 28.3, ($p<0.0001$). In the internal biosecurity, the dairy farms shoved low 'Health management' 19.5± 20.0 which was significantly lower than the 'Dairy management' category 39.2± 19.1, ($p<0.0001$). In conclusion, the BIOCHECK CATTLE® survey is acceptable by the experts with some different priorities in the scores. The first conducted biosecurity assessment of dairy farms in Macedonia, indicated that the critical improvement points are the biosecurity risks related to 'transport and carcass removal' and the categories in the Internal biosecurity, especially health management.

Key words: cattle farms, prevention, biosecurity, assessment, BIOCHECK®

O2

COMPARISON OF THE TWO DIFFERENT PROTOCOLS FOR BIOSECURITY ASSESSMENT IN COMMERCIAL PIG FARMS

Branko Angjelovski[*1], Vanja Kondratenko[4], Milenko Simovikj[4], Jovan Bojkovski[3], Miroslav Kjosevski[2]

[1]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine, Department of internal diseases in farm animals, Lazar Pop Trajkov 5-7, 1000 Skopje, North Macedonia
[2]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine, Department of animal hygiene, Lazar Pop Trajkov 5-7, 1000 Skopje, North Macedonia
[3]University of Belgrade, Faculty of veterinary medicine, Department of ruminants and swine diseases, Bulevar Oslobodjenja 18, 11000 Belgrade, Serbia
[4]Food and veterinary agency, Third Macedonian Brigade 20, 1000 Skopje, North Macedonia

Biosecurity is one of the most important aspects concerning animal health in pig farms. It is associated with measures and actions taken on the farm to reduce the risk of entering and spreading infectious diseases. There are many different protocols for biosecurity assessment in pig farms. Biocheck.UGent[tm] is the most commonly used biosecurity protocol, while the biosecurity of Macedonian pig farms is assessed by the protocol implemented by the Macedonian Food Veterinary Agency (FVA). The objective of this study was to compare and identify differences between the two different protocols for biosecurity assessment in commercial pig farms. Thirteen farrow-to-finish commercial pig farms were included in the study. The mean number of sows in the herds was 364 (range 50 to 1550). The biosecurity level of the herds was assessed by both Biocheck.UGent[tm] scoring system and "score index" system for biosecurity assessment adopted by the FVA. The two assessment protocols were synchronized for score comparison. Descriptive statistical analysis (Mean, SD) was used for the data obtained by both biosecurity protocols, and mean scores were compared by non-parametric tests. Total scores on farm level gained from protocols were subjected to non-parametric correlation analyses. The level of agreement between both protocols was also calculated. The mean synchronized biosecurity score was detected by Biocheck.UGent[tm] for all farms was 44.6± 13.4 (range 19 to 64), while 32.4 ±3.8 score (range 26.4 to 40.45) was obtained by the "score index" system. A significant difference (p=0.006) between the mean scores of both protocols was found. Regarding farm categorization, according to the "score index" system developed by FVA, 92.3% of the farms were considered with low biosecurity risk and one farm had medium biosecurity risk. In contrast to the categorization based on the Biocheck.UGent[tm]38.5 % farms were classified as low biosecurity risk category and 61.5% of the farms belonged to the medium biosecurity risk category. The level of biosecurity agreement between the two scoring systems was 46%. Correlations analysis gave a low non-significant correlation

between total farm scores obtained by the two assessment protocols (Spearman R=0.36; *p*=0.23). The results of this study indicate significant differences between scores detected by two biosecurity protocols. In addition, Biocheck.UGent™ has higher and more rigorous criteria for biosecurity assessment compared to the FVA protocol due to the more detailed approach during evaluation.

Key words: biosecurity assessment, pig farm, protocol

O3

ASSESSMENT OF BIOSECURITY MEASURES ON THE BROILER FARMS IN THE REGION OF BELGARDE CITY

Jelena Maletic, Ljiljana Spalević, Vesna Milićević,
Bojan Milovanović, Branislav Kureljušić

Serbian institute of veterinary science, Janisa Janulisa 14, 11000 Belgrade, Serbia

The implementation of biosecurity measures, internal and external is one of the essential prerequisites for preventing the occurrence and spread of infectious agents in flocks. Assessment of biosecurity measures on the farm was carried out based on the application of the appropriate questionnaire where the farmer answered several questions regarding the implemented biosecurity measures. In this study, a biosecurity assessment in the broiler farms was performed by Biocheck.UGent online survey on three broiler farms (25000-100000 broilers per farm) in the region of Belgrade city. Results showed that subtotal external biosecurity scores ranged from 57% to 78 %, with an average score of 64.66%. Noteworthily, within external parameters for biosecurity, the lowest score was for the way of removal of manure and carcasses (farm 1 and farm 2 had a score of 12 %). Also, a lower score was obtained for the supply of material (56 %) that enhances pathogens' entrance on the farm, and the depopulation of broilers, a major role in introducing certain infectious agents (farm 1 had a score of 44 %, and farm 2 had a score of 51 %). The results described in the present study revealed that farms had great solutions related to infrastructure and biological agents (scores ranged from 76 % to 95 %), as well as for all measures related to the supply of feed and manipulation on the farm and drinking water (scores ranged from 78 % to 92 %). According to the results, subtotal internal biosecurity scores ranged from 48% to 73 %, with an average score of 58.66 %. Lower scores were obtained for disease management parameters on all farms (from 56 % to 64 %). Two farms had a low score for cleaning and disinfection (39 % and 47 %). Farm 2 had a very low score for the questions regarding material and measures that they apply between compartments (29 %), however, the other two farms had good scores (82 %). The total score for the biosecurity assessment ranged from 56% to 77%, with an average score of 63 %. Compared to the world scores obtained from Biocheck.UGent online survey database results of the internal biosecurity score and total score were lower. This study aimed to raise the awareness of veterinarians and farmers about the importance of management and biosecurity measures.

Key words: biosecurity, questionnaire, broilers

O4

BIOMONITORING OF WASTEWATERS FROM SLAUGHTERHOUSES IN MACEDONIA

Marija Ratkova Manovska, Gordana Ilievska, Snezana Dimitrovska, Ena Dobrik, Miroslav Kjosevski, Biljana Stojanovska Dimzoska, Katerina Blagoevska*

University Ss Cyril and Methodius, Faculty of veterinary medicine, Food institute, st. Lazar Pop – Trajkov 5-7, 1000 Skopje, Macedonia

According to the World Health Organization (WHO), the environmental health is defined as factors in the environment affecting human health. Among all factors, wastewaters are on the top of the list, taking into consideration the daily discharge of billions of liters of wastewaters from households, farms, factories and institutions. Despite their origin, wastewaters pose a serious threat to public health, both locally and globally. They represents a pool of microorganisms, both pathogenic and infectious and solid waste dominated by toxic, inflammable and corrosive materials. In 2017, the United Nations Environmental Assembly (UNEA) recognized the environment as a crucial link to the transmission and spread of resistant pathogen and commensal bacteria, from different origin. The immediate effect on the environment is the contribution towards contamination and destruction of the natural habitats of diverse plant and animal species and subsequently coping with complex health and safety issues affecting humans, thus presenting a huge biosafety risk. Current estimates from the WHO are that approximately 24% of deaths are linked with causing agents coming from the environment. The social and economic risks that the environmental factors cause should not be neglected as well. The focus of this study is antimicrobial resistance of pathogen and commensal microorganisms isolated from wastewaters from slaughterhouses in Macedonia. To prevent and/or reduce the direct and indirect transmission of resistant microorganisms or resistance within a population of animals, as well as transmission from animals to humans, an accurate picture of real and potential sources of AMO is necessary. The current Antimicrobial Resistance Monitoring Program implemented by the Food and Drug Administration in the period 2017-2021, includes samples taken from certain populations of animals raised for food production, as well as from certain types of food. The monitoring program envisages detection of: Salmonella, Campylobacter jejuni, Campylobacter coli, indicator commensal Escherichia coli, indicator commensal Enterococcus faecalis and Enterococcus faecium. The monitoring also refers to Salmonella spp. and E. coli, which produce the following enzymes: β - lactamase broad spectrum (ESBL); AmpC β-lactamases (AmpC) and carbapenemases. However, this program does not cover the monitoring of AMO in wastewater from slaughterhouses. The aim of this project is to conduct biomonitoring of wastewaters from slaughterhouses in Macedonia, to get an insight about the presence of pathogens and commensal bacteria. All isolated strains, will be subject to antimicrobial resistance testing, detection of resistance genes and genotyping.

Key words: wastewaters, slaughterhouses, environment, biomonitoring

O5

THE IMPACT OF SMALL HYDRO POWER PLANTS ON TROUT FISH FARMS IN NORTH MACEDONIA

Aleksandar Trajchovski*, Misho Hristovski

Ss. Cyril and Methodius University, Faculty of veterinary medicine - Skopje, Department for biology and pathology of fish, honeybees, wildlife, Lazar Pop – Trajkov 5-7, 1000 Skopje, North Macedonia

Currently in North Macedonia there are 107 operational hydroelectric power plants (HPP) and 53 Trout farms. The majority of this fish farms are on the same water flow with the HPP. The aim of this study was to determine how the small (HPP) are affecting the watersheds of the nearby trout farms. We observed 2 Fish farm on river where in the last 4 years there were build 3 hydroelectric power plants and there is a plan to be build 2 more. This impact of small HPP on watershed and water characteristics were assessed and compared with reference points. In the operational HPP there were severe deterioration of habitat and from the analyzed points, the strongest statistically significant changes were observed in NH_4 and dissolved oxygen. As calculated using designated indices below the hydropower plant the NH_4-N content was higher by 8.17%. In the fish farms HPP are causing significant cumulative loses in all categories from 25-75 % due the stressogenic factors which are closely related with the emerging of opportunistic pathogens (Yersinia ruckeri). In the long term, this research may help HPP planers manage the water resources more efficiently. This research will contribute to the rational management of HPP and fish farm on same watersheds considering sustainable water management principles.

Key words: Small hydro power plant, Trout farms, Yersinia ruckeri

O6

INITIAL FINDINGS OF TOTAL AIR DUST CONCENTRATION IN CATTLE AND POULTRY HOUSES IN MACEDONIA

Ena Dobrikj[1], Aleksandar Janevski[2], Aleksandar Dodovski[3], Miroslav Kjosevski[1]

[1]*Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Department of animal hygiene and environmental protection, Lazar Pop – Trajkov 5-7, 1000 Skopje, North Macedonia*
[2]*Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Department of farm animal internal medicine, Lazar Pop – Trajkov 5-7, 1000 Skopje, North Macedonia*
[3]*Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Department for avian diseases, Lazar Pop – Trajkov 5-7, 1000 Skopje, North Macedonia*

Increased air dust concentration (ADC) on farms can cause health problems for both the animals and workers on the farm. According to the available literature, cattle houses have lower levels of ADC in comparison to houses of laying hens. Determining ADC in animal houses in Macedonia has never been conducted and no records are available. Therefore, the aim of this study was to make an initial assessment of total ADC in cattle and poultry houses while taking the management on the farm into consideration. Five houses for dairy cattle and five houses for laying hens from different farms were included. Four dairy houses had a tied housed system, and one was a free-stall barn. Three poultry houses had a conventional and two an enriched cage system. The dairy cattle houses had a natural ventilation system while the poultry houses were equipped with a mechanical ventilation system based on negative pressure. Total ADC was determined by using the [Met-One, USA] Benchtop Particle Counter BT-645. Measurements were taken on two measurement points in one house on each farm. The sampling duration was 30 minutes for each measurement point and an average was estimated for every 10 minutes of sampling, resulting in three samples at each location. The volume of the house, the number of animals inside the house, as well as the number and area of inlets and outlets were correlated with total ADC. Likewise, the remaining management practices were descriptively analysed in context with the total ADC. The total ADC in dairy and poultry houses was 48 ±47 μg/m^3 (range 109.5-7.2 μg/m^3) and 71 ±65 μg/m^3 (223.7-22.5 μg/m^3), respectively (U_A=14, p>0.05). A negative correlation was observed between the ratio of house volume/ventilation openings and the total ADC in both dairy (r_s=-0.4) and poultry houses (r_s=-0.1), but it wasn't statistically significant (p>0.05). A similar correlation was observed between house volume per individual and total ADC for both types of houses (dairy r_s=-0.7, poultry r_s=-0.5, p>0.05). The lowest total ADC in dairy houses was measured in the free-stall barn, while in poultry houses it was measured in the house with the highest ratio of inlets and outlets area/total house volume, which indicates that the increased available air volume decreases the total ADC. This study represents the initial findings in animal houses from a small sample size, and further research needs to be conducted for more representative results.

Key words: air dust concentration, ventilation, livestock management, poultry houses, cattle houses

Scientific session II
BASIC SCIENCES AND VETERINARY EDUCATION

Oral presentations

O7

RENAL MICROVASCULATURE IN THE CHICKEN

Nedžad Hadžiomerović*, Rizah Avdić, Faruk Tandir

Department of basic sciences of veterinary medicine, Veterinary faculty, University of Sarajevo, Zmaja od Bosne 90, 71000 Sarajevo, Bosnia and Herzegovina

The bird vascular system is unique among other animals and has some anatomical characteristics that affect their physiological function. One of the most important characteristics is the presence of the two types of nephrons which have different position and morphological features. The nephrons located deep in the cortical region do not have loop of Henle and they are so called „reptilian“, while other nephrons are located deeper in the cortex near the medullary areas with the loop of Henle – „mammalian“ nephrons. The scanning electron microscopy (SEM) with the injection corrosion casting technique allows the examination of the micro vessels. In the combination with the 3D software morphometry, this technique is useful in studying normal vascularization of the organ or tissue, both in the adult and juvenile individuals, as well as in pathological conditions. The aim of this study was to describe morphological features of the reptilian and mammalian type of the glomerulus, by measuring vascular parameters of both types of the nephrons. The results show significant difference in the size and shape between mammalian and reptilian type of the glomerulus. The mammalian type of glomerulus has typical round shape and lots of capillary loops which arose after branching of the afferent arteriole at the vascular pole of the glomerulus. The reptilian types of the glomeruli are quite simple with few loops and without classic round shape. By means of 3D software the quantitative analysis of the vascular parameters of the both glomeruli were conducted. The results show significant difference in the internal diameter between mammalian and reptilian glomerulus ($p<0,001$). Likewise, the diameter was bigger in the females compared to males in all three kidney divisions.

Key words: kidneys, mammalian type of nephron, reptilian type of nephron, glomerulus, vascularization

O8

FORMING NEW COLLECTION OF PHARYNGEAL TEETH FROM CYPRINID FISHES COLLECTED IN CROATIA AND NEIGHBORING COUNTRIES

Denis Leiner*[1], Srebrenka Nejedli[1], Snježana Ćurković[1], Damir Mihelić[1]

[1]Department of anatomy, histology and embryology, Faculty of veterinary medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia

The fish order Cypriniformes is one of the most diverse ray-finned fish groups in the world with more than 3,000 recognized species. Cypriniformes are characterized by a striking distribution of their dentition, namely the absence of oral teeth and presence of pharyngeal teeth on the last gill arch (fifth ceratobranchial). These teeth assist in transport of food that, in these fishes, is the main function of the pharyngeal apparatus, accomplished mainly by anteroposterior movements of the lower pharyngeal jaws. Length, shape, number, and especially the number of rows of pharyngeal teeth in cyprinids is of great importance for distinguishing genera and species. The collection of pharyngeal teeth was donated by the Croatian Natural History Museum. Fish specimens were collected between the years 1979 and 1986, on 32 different locations in Croatia, Slovenia, Serbia and Bosnia and Hercegovina. So far, pharyngeal teeth of 31 species from 107 individuals were collected. Among the collected specimens there were seven species that are endemic for Danube basin and Adriatic basin: *Telestes ukliva, Squalius tenellus, Telestes polylepis, Squalius svallize, Rutilus pigus virgo, Chondrostoma phoxinus* and *Telestes croaticus*.. The aim of the research was to present the collection to the wider scientific community and make it available to scientists and students of veterinary medicine who are interested in the anatomy and morphology of fish. Pharyngeal teeth can be used in courses for comparative anatomy and fish morphology as well as for archaeozoology.

Key words: pharyngeal teeth, Cypriniformes, collection, endemic species

O9

RADIOLOGICAL DIAGNOSIS OF AN ECTOPIC URETER IN A POODLE – CASE REPORT

Boris Dimitrievski*[1], Aleksandar Janevski[1], Marko Mitrović[2], Dine Mitrov[1]

[1]Department of visual diagnostics, Faculty of veterinary medicine - Skopje, Ss. Cyril and Methodius University in Skopje, Lazar Pop Trajkov 5-7, 1000 Skopje, North Macedonia
[2]Department of radiology and radiation hygiene, Faculty of veterinary medicine, University of Belgrade, Bulevar oslobođenja 18, 11000 Belgrade, Serbia

Ectopic ureter (EU) is a rare, congenital malformation of the urinary tract in which one or both ureters do not enter the bladder in the correct anatomic location. The incidence of this disease is 0.016% where female dogs make up 89–95% of the cases, and bilateral disease makes up 32–92% of the cases. The exact cause of this anomaly is not known yet, but it is thought to have a genetic basis. Depending on the position, EU can be extramural or intramural where intramural EU make up more than 95% of the cases. The median age of bitches with EU diagnosis is 10 months and in male dogs 24 months. In addition to dogs, ectopic ureters have been reported in cats, horses, rats and humans. Breeds with a greater risk include Labrador retriever, Golden retriever, Siberian Husky, West Highland White Terrier, Newfoundland, Wire Fox Terrier, English Bulldog, and Miniature and Toy Poodles. A young, unsterilized female Poodle at the age of 4 months, was admitted to the Department for Visual Diagnostics at the Faculty of Veterinary Medicine – Skopje with a history of continuous urinary incontinence, but with good general health, adequate body weight, normal appetite, preserved reflexes and normal behavior. Laboratory examinations revealed normal complete blood count (CBC), serum biochemical profile and urinalysis values. Initially, two plain X-ray images of the abdomen were taken on which no radiologically visible changes were detected. Correspondingly to the negative radiological findings and the clinical signs of the patient, intravenous urography was performed. Three minutes after the application of the contrast, several consecutive images of the bitch's abdomen were taken in laterolateral (LL) and ventrodorsal (VD) projection. On the images, the left ureter was almost invisible due to the speed at which the contrast passes through it and enters the bladder at the correct anatomical location. In contrast, the right ureter was dilated along its entire length, clearly visible and it completely bypassed the bladder without anatomically attaching to it opening distally, directly into the urethra. Continuous urinary incontinence accompanied with orderly laboratory results and normal general health in young dogs can be an indication for EU. Intravenous urography has been considered as the "gold standard" for the diagnosis of EU and allows determination of the disease (unilateral or bilateral) as well as the location, size, and morphology of the ureter and ureterovesical junction.

Key words: dog, ectopic ureter, urinary incontinence, urography

O10

SUPPRESSION EFFECTS OF EXCESSIVELY EXPRESSED GENE BCL-2 IN CELL LINES OF PROSTATE CANCER

Igor Esmerov*[1], Nikola Adamov[1], Aleksandra Angelevska [1], Radmila Chrcheva[1], Ljupco Mickov[1], Ljupco Angelovski[1], Nikolaj Markov[2], Branko Atanasov[1]

[1]Faculty of veterinary medicine – Skopje, Lazar Pop Trajkov 5-7 1000 Skopje, Ss. Cyril and Methodius University in Skopje, North Macedonia
[2]Institute of mountain animal husbandry and agriculture, Agricultural Academy - Sofia, 5600 Troyan, Bulgaria

We constructed two plasmid-specific shRNA transcripts of the bcl-2 gene. The plasmid was designed according to a previously published sequence of interfering RNA according to an appropriate reference, using appropriate software (siRNA Wizard, InvivoGen). Designed single-stranded DNA oligonucleotides are ordered with the highest degree of purity (PAGE) from a suitable manufacturer (SIGMA-Genosys). By annulling complementary oligonucleotides, double-stranded inserts are formed. Recombinant shRNA-encoding plasmids were constructed by digestion of psiRNA-x7SKGFPzeo plasmid (psiRNA-x7SKGFPzeo, InvivoGen) with restrictive endonuclease BbsI (Fermentas International) electrophoresis in ultra-pure agarose with low melting point (LMP-Agarose, SIGMA). For each of the constructs, a suitable double-stranded insert downstream of x7SK (strong RNA III promoter) with T4 DNA ligase (Rapid Ligation Kit, Fermentas InteRNAtional) was cloned. The control plasmid psiRNAScr (InvivoGen) is ready for use and will be used directly for transformation. Cell culture procedures are performed in accordance with generally accepted measures and according to laboratory manuals. We used representative continuous cell lines from several human cancers. The cells are subcultured in a suitable medium (RPMI 1640. MEM or DMEM) supplemented with 10% veal fetal serum (FBS), 1x non-essential amino acids, 2 mM L-glutamine and a mixture of antibiotics and antifungals (all listed reagents from SIG) (Heraeus) at 37 °C with an atmosphere saturated with moisture and 5% CO_2. Special T-25 Falcon dishes and 35-mm Petri plates are used as well as all other plastic, sterile, apirogenic accessories certified for cell culture (Corning). Subcultivation was done with trypsin-EDTA treatment and cell division into an appropriate number of new vessels and fresh medium. All procedures are performed aseptically in a laminar cabinet with sterile air. The efficacy of transfection with each transfection agent and each cell line is determined by fluorescence microscopy. The efficacy of bcl-2 gene suppression constructs has been evaluated by preliminary experiments with RT-PCR for relative quantification of transcripts from each of the three genes. The most efficient plasmids have been selected for each of the three genes.

Key words: shRNA, plasmid, Bcl-2, apoptosis, cytotoxicity, prostate cancer

O11

DIGITAL VETERINARY EDUCATION (DEVET) – AN ERASMUS+ PROJECT FOR DEVELOPMENT/CREATING OF DIGITAL MATERIALS/ TOOLS IN THE EDUCATION PROGRAM OF VETERINARY MEDICINE (OUTCOMES REVIEW)

Igor Ulchar[1]*, Ksenija Ilievska[1], Lazo Pendovski[1], Zehra Hajruali Musliu[1], Alen Slavica[2], Lada Radin[2], Marko Poleto[2], Danijela Kirovski[3], Ljubomir Jovanovic[3], Ivan Jovanovic[3]

[1]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine – Skopje, Lazar Pop Trajkov 5-7 1000 Skopje,North Macedonia
[2]University of Zagreb, Faculty of veterinary medicine, Heinzelova 55, 10000 Zagreb, Croatia
[3]University of Belgrade, Faculty of veterinary medicine, Bulevar oslobođenja 18, 11000 Belgrade, Serbia

One of the biggest challenges during the Covid 19 pandemic period was how to continue with veterinary education online, without compromising the educational process. Due to the pandemic, all face-to-face learning activities were minimized and online teaching process has begun. Each hands-on training of students (laboratory, microscopy, necropsy, clinical teaching etc.), were seriously affected due to lack of suitable teaching materials to compensate some of the off-site rising problems. Therefore, many of veterinary schools developed a learning software for uploading teaching materials for students (pre-recorded lectures, presentations, video material etc.). In order to be more inventive, we decided to go one step further and propose a joint project entitled Digital Veterinary Education (DEVet), under ERASMUS+ program platform. Different digital education tools were implemented, and storage-cloud platform was developed where all teaching materials were uploaded, so veterinary students from Belgrade, Skopje and Zagreb could have unlimited access to the platform. Following our objectives, a joint production of high quality veterinary digital educational materials was made and uploaded on the platform: 116 videos, 44 micrograph collections, 31 photo collections, 95 animations / presentations, 36 digital art collections (drawings, schemes), 35 datasets, and 58 handouts / brochures (a total of 415 intellectual outputs). The Covid-19 pandemic despite that it changed our lives, it inevitably changed the way that we teach veterinary students and offered a great possibility in developing didactic skills in teachers and students towards digital era.

Key words*:* digital education, platform, Covid 19

Scientific Session III
ANTIMICROBIAL RESISTANCE

Plenary lecture and Oral presentations

PL2

ANTIMICROBIAL RESISTANCE (AMR) PROBLEMS IN COMPANION ANIMALS

Peter Panduro Damborg

Department of veterinary and animal sciences,
University of Copenhagen, Grønnegårdsvej 15,
DK-1870 Frederiksberg C, Denmark

There is increasing awareness of antimicrobial resistance (AMR) occurrence in companion animals worldwide. Such AMR threats can affect animal health and may also be transferred to humans cohabitating or working in close contact to the animal carriers. In this lecture, important AMR threats in horses, dogs and cats will be introduced, focusing on methicillin-resistant staphylococci (*S. aureus*, MRSA, and *S. pseudintermedius*, MRSP) and extended-spectrum beta-lactamase -producing *Escherichia coli* (ESBL *E. coli*). These resistant pathogens have emerged in companion animals during the last two decades, and pose significant problems for treatment due to the resistance to beta-lactams and, occasionally, several other antibiotics used in veterinary practice. After a basic introduction to microbial pheno- and genotypic characteristics, a historic view on the temporal and spatial dissemination of these pathogens will be presented. Particular attention will be paid to their ability to spread in the veterinary hospital environments ("nosocomial infections"). Finally, recommendations on prevention and treatment strategies will be presented.

Key words: companion animals, antimicrobial resistance (AMR), methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), ESBL *E. coli*

O12

ANTIMICROBIAL RESISTANCE IN STAPHYLOCOCCI ISOLATED FROM DOGS IN THE REPUBLIC OF NORTH MACEDONIA

Iskra Cvetkovikj*, Ivana Arsovska, Marija Ratkova Manovska, Mirko Prodanov, Ljubica Rashikj, Zagorka Popova, Igor Djadjovski, Aleksandar Cvetkovikj

Faculty of Veterinary Medicine-Skopje, Ss. Cyril and Methodius University in Skopje, Lazar Pop Trajkov 5-7, 1000 Skopje, Republic of North Macedonia

The emergence and global spread of antimicrobial resistance (AMR) is a cause of great concern being one of the important threats to animal and public health worldwide. Companion animals have been shown to have the potential to act as reservoirs for resistant bacteria for humans due to their close contact in the living and/or working environment. The aim of this work is focused on presenting the results of antimicrobial resistance in staphylococci isolated from dogs in the Republic of North Macedonia. Following isolation, species identification was performed by PCR. Antimicrobial susceptibility of the isolates was performed by disk diffusion method and the detection of methicillin resistance by disk diffusion and molecular detection of the *mecA* gene. Forty two (42) isolates were identified as *Staphylococcus pseudintermedius*, out of which 14 (33.33%) were identified as methicillin resistant *Staphylococcus pseudintermedius* (MRSP). Ten isolates were identified as *Staphylococcus aureus*, out of which 2 (20%) were identified as methicillin resistant *Staphylococcus aureus* (MRSA). Overall, 78,84% (41/52) of the staphylococcal isolates were resistant to more than three antimicrobial classes and classified as multidrug resistant (MDR) staphylococci.

Key words: *Staphylococcus*, antimicrobial resistance, multidrug resistant bacteria, dogs

O13

THE OCCURRENCE OF *bla*$_{CTX-M}$, *bla*$_{SHV}$, *bla*$_{TEM}$ GENES IN EXTENDED-SPECTRUM *β*-LACTAMASE PRODUCING COMMENSAL *E. COLI* ISOLATES FROM DAIRY FARMS IN THE MUNICIPALITY OF DEBAR

Maksud Kerluku*, Marija Ratkova Manovska, Gordana Ilievska, Dean Jankuloski, Katerina Blagoevska

Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Food institute, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia

Antibiotic resistance (AMR) has been recognized by the World Health Organization as top priority public health challenge, because of the rapid emergence and dissemination of resistant genes among humans, animals and the environment. Extended-spectrum β-lactamases (ESBLs) are the most prevalent antibiotic resistant determinants, coded by the bla genes family and being responsible for the resistance to 3rd and 4th generation of cephalosporins. Due to the ubiquity of commensal *Escherichia coli (E. coli)*, the bacteria plays an important role in the ecosystem, because it represents a potent reservoir of AMR genes, thus serving as bio indicator of AMR. Hence, these genes can be easily transferred from the commensal to the pathogenic strains. Until now, many different types of ESBLs have been discovered, but CTX-M, TEM and SHV enzymes producing strains are the most prevalent. The aim of this study was to evaluate the antibacterial susceptibility and ESBLs pattern of commensal *E. coli* strains isolated from dairy farms in the Municipality of Debar. For this purpose, 159 fecal samples from 34 farms were collected. For the detection and confirmation of the ESBL/AmpC isolates the protocol proposed by EURL-AR Denmark was followed. The antibiotic susceptibility profiles and phenotypic categorization were determined based on the microbroth dilution method according to ISO 20776-1/2 and were categorized according the ECOFF values given in the Commission Implementing Decision 2013/652/EU. AMR testing showed a high resistance to ampicillin, cefotaxime, sulphamethoxazole, ceftazidime, tetracycline and trimethoprim. Twenty-eight (72%) isolates were found to be multidrug resistant. Thirty-nine (24.5%) phenotypically confirmed ESBL producing commensal *E. coli* strains, were genotypically characterized. Conventional PCR method was used to confirm the occurrence of *bla*CTX-M, *bla*SHV, *bla*TEM, OXA1 and OXA2 genes. The results showed presence of 27 (69%), 1 (2.5%), 28 (72%), of bla-CTX-M, bla-SHV, bla-TEM genes, respectively. Additionally, the presence of OXA1 bla-gene in three isolates (8%) was detected, while no OXA2 bla-gen wasn't. Twenty isolates (51%) showed presence of both bla CTX-M and TEM genes. The strain carrying bla SHV gene was found to be positive on bla TEM gene, also. Overall, β-lactamase genes were identified in 36 (92%) isolates, while three (8%) of the strains showed another phenotype than ESBLs. The obtained results for phenotypic resistance of isolates correspond with detection of *bla*-genes. This study clearly indicates the widespread of multidrug resistant commensal *E. coli* isolated from the dairy farms in the Municipality of Debar.

Key words: commensal *E. coli*, ESBLs, bla genes, antimicrobial resistance

O14

ANTIMICROBIAL SUSCEPTIBILITY OF *TRUEPERELLA PYOGENES* ISOLATES FROM VARIOUS CLINICAL SPECIMENS OF ANIMALS

Dilek Öztürk, Sibel Yaman*

Burdur Mehmet Akif Ersoy University, Faculty of veterinary medicine, Department of microbiology, 15100 Burdur, Türkiye

Trueperella pyogenes (*T. pyogenes*) (formerly *Arcanobacterium pyogenes*) is a commensal bacterium present on the upper respiratory and urogenital tract mucosa of domestic and wild animals. It is also the most common opportunistic pathogen isolated from the infections of cattle such as mastitis, metritis, arthritis and pneumonia. This study aimed to determine the susceptibility of *T. pyogenes* isolated from farm animals to various antibiotics used in veterinary medicine by disc diffusion methods. In this study, 105 *T.pyogenes* isolates, which were isolated from the samples brought to Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Microbiology Department between 2015-2020 and found in the culture collection, were used. Of the isolates, 64 were isolated from milk, 27 from joint contents, 5 from abscess contents, 5 from internal organs, 22 from bronchoalveolar lavage (BAL), and 2 from vaginal discharge. The samples were inoculated on blood agar containing 5% sheep blood and incubated for 24-48 hours, at 37 °C in 5% CO_2. Growing colonies were examined by conventional bacteriological methods (Gram staining, colony morphology, hemolysis, catalase test, oxidase test, CAMP test). Bacteria identified as *T. pyogenes* were stored at -80°. The susceptibility of *T.pyogenes* isolates to 19 antibiotics was determined by Kirby-Bauer disk diffusion method. All *T.pyogenes* isolates were found to be sensitive to amoxicillin, ampicillin, cefoperazone, enrofloxacin, florfenicol, doxycyline, ceftiofur, cephalexin and cloxacillin. While 104 (99.04%) of the isolates were susceptible to amoxicillin+clavulanic acid and penicillin, 101 (96.19%) to ciprofloxacin, 58 (55.23%) to oxytetracycline, 71 (69.52%) to lincomycin, 73 (69.52%) of them were found to be sensitive to erythromycin. It was determined that *T. pyogenes* isolates were most resistant to neomycin (85.71%). This was followed by tetracycline (75.23%), gentamicin (55.23%), and sulfamethaxazol+trimethoprim (50.47%), respectively. *T. pyogenes* standard strain was found to be sensitive to all antibiotics except neomycin.

Key words: *Trueperella pyogenes*, antimicrobial suscepibility, Kirby-Bauer

O15

ANTIMICROBIAL RESISTANCE IN COMMENSAL ESCHERICHIA COLI ISOLATED FROM PIGS' CECAL SAMPLES AT SLAUGHTER

Dean Jankuloski, Marija Ratkova Manovska, Katerina Blagoevska, Gordana Ilievska, Biljana Stojanovska Dimzoska, Mirko Prodanov, Ljupco Angelovski, Sandra Mojsova*, Snezana Dimitrovska

Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Food institute, Lazar Pop – Trajkov 5-7, 1000 Skopje, North Macedonia

Over the recent decades, antimicrobial resistance (AMR) in commensal bacteria has been of an emerging concern for the livestock producing sector, and it has been intriguing the academic community as well. Commensal bacteria like *Escherichia coli (E. coli)* represent a potential important and inevitable reservoir of antimicrobial resistance, making it a huge concern in the whole concept of "One health." Its ubiquitous character and its autochthony to the gastrointestinal tract, play a crucial role in the resistance spread, via various modes of transmission, thus making the commensal *E. coli* a significant indicator of antimicrobial resistance (AMR) in animals and food of animal origin. It represents a reservoir of antimicrobial resistance genes, from which pathogenic bacteria can acquire resistance through horizontal gene transfer. For the purpose of this study, we analyzed 62 cecal samples, from pigs at slaughter, during the period from February – March, 2019. Isolation of indicator commensal *Escherichia coli* and testing for antimicrobial resistance were done according to the Protocol from EURL AMR and the Decision 652/2013/EU of the European Commission. The method is based on the use of microbroth dilution plates, with a series of doubling dilution antibiotic concentrations to determine the minimal inhibitory concentration (MIC). Isolates that showed resistance to FOT, FOX and/or MERO were tested for the production of extended spectrum β-lactamases (ESBL), AmpC β-lactamases (AmpC) and carbapenemases. The highest AMR from EUVSEC1 plate was determined on tetracycline, ampicillin and sulfamethoxazole with 61.9%, 53.2% and 36%, respectively. Analyses of the three isolates that showed resistance for cefotaxime and ceftazidime on EUVSEC 1, were further on tested on the antibiotic panel from the EUVSEC2 plates. No ESBL or AmpC or carbapenemase producing strains were detected. From the results, we can conclude that a strong monitoring AMR plan in commensal bacteria should be a mandatory part of the surveillance programs, because their importance in the antimicrobial resistance gene transfer should not be neglected.

Key words: AMR, commensal *E. coli*, pigs, cecal sample

Acknowledgments: This presentation is part of a project financed by Faculty of veterinary medicine-Skopje, Code FVM-IPR-01 (Decision no. 0202/2090/3, from 3.12.2019) with title "Antimicrobial resistance of commensal Escherichia coli In Republic of Macedonia".

O16

VETERINARIAN'S STANDPOINTS REGARDING ANTIMICROBIAL STEWARDSHIP IN SERBIA

Zorana Kovačević[1]*, Mihajlo Erdeljan[1], Ivan Stančić[1], Dragana Tomanić[1], Zorana Ružić[1], Ivan Galić[1], Romel Velev[2]

[1]University of Novi Sad, Faculty of agriculture, Department of veterinary medicine, Trg Dositeja Obradovica 8, 21000 Novi Sad, Serbia
[2]Ss. Cyril and Methodius University in Skopje, Faculty of Veterinary Medicine - Skopje, Department for pharmacology and toxicology, Lazar Pop Trajkov 5-7, 1000 Skopje, North Macedonia

Antimicrobial stewardship (AMS) describes measures that can help mitigate the public health crisis and preserve the effectiveness of available antimicrobial agents. AMS programs have been principally developed, implemented, and studied in human medicine. On the other hand, in veterinary medicine AMS programs are relatively new terms and veterinarians might be familiar with its principles only in theory. The questionary based online survey conducted in Serbia during 2021 among farm animal veterinarians show that only 30.9% of 110 questioned were previously familiar with the term "antimicrobial stewardship", while more than two-thirds (69.1%) had never heard about it. Additionally, only 11.2% of these respondents thought that the implementation of AMS would not lead to any significant changes, while the rest assumed that it would lead to a reduction in antimicrobial resistance (AMR) in humans and animals and an increase in the responsible antibiotics use. In addition, another survey conducted among 62 of all veterinarian specialists during 2020 from the one part of Serbia, South Bačka District showed that those who had sufficient AMR knowledge considered hygiene in hospitals, hygiene during food preparation and consumption and hygiene on farms to be the most important sectors to focus on to slow down the development of AMR. Interestingly, all these mentioned reasons are part of the AMS principals, which have to be adopted in veterinary sectors. Furthermore, AMS practices are encouraged among veterinarians to reduce the indiscriminate use of antimicrobials, as well as to improve AMU in animal health care delivery. These results show that there is still a lot of work to be done in promoting this concept and raising awareness in the veterinary sector to facilitate the implementation of AMS strategies.

Key words: antimicrobial stewardship, veterinarians, antimicrobial resistance

Scientific session IV
ANIMAL WELFARE AND BEHAVIOR

Oral presentations

O17

ULTRASOUND – METHOD TO ASSESS KEEL BONE FRACTURES IN LAYING HENS

Dimitar Bozinovski[*1], Lazo Pendovski[1], Miroslav Kjosevski[2], Martin Nikolovski[1], Dimitar Terzievski[3], Vlatko Ilieski[1]

[1]Ss. Cyril and Methodius University in Skopje, Department of functional morphology, Faculty of veterinary medicine - Skopje, Lazar Pop Trajkov 5-7, Skopje, North Macedonia
[2]Ss. Cyril and Methodius University in Skopje, Department of animal hygiene and environmental protection, Faculty of veterinary medicine - Skopje, Lazar Pop Trajkov 5-7, Skopje, North Macedonia
[3]Food and veterinary agency, 3-ta Makedonska Brigada 20, 1000 Skopje, North Macedonia

The goal of this study was to confirm the use of ultrasonography as an accurate diagnostic method for interpreting keel bone damage (KBD) with a comparison with different methods in detection of keel bone damage in laying hens in different age categories. Sixteen weeks old laying hens were used as a representative model with a high percentage of the damaged keel bone. The methods used in this study were palpation, radiography, ultrasonography, and dissected osteological model. The measurements were performed on flocks of three age categories 25, 45 and 65 weeks, with 20 chickens from each category. Method of palpation was performed in vivo with bilateral palpation along the ridge of the keel bone, following X-rayed on LL projection were performed. In order to perform comparation, same laying hens were examined by ultrasonography, where 20 non-selective laying hens were sacrificed to confirm the results found. The results were analyzed by using a scoring system and a scale for assessing fractures and deviations of the keel bone from 0 (without changes) to 4 (with major changes). These results indicate that the use of ultrasonography allows an accurate evaluation of diagnostic results for KBD, and are compatible with the results obtained by the other methods. To validate the results of the investigation osteological dissected models were prepared. Further investigations are needed to establish ultrasonography as a valid method in diagnosing keel bone damage and laying hens.

Key words: ultrasonography, palpation, KBD, X-ray, laying hens

O18

STRAY DOG POPULATION SIZE TREND IN THE CITY OF SKOPJE BETWEEN 2010 AND 2020

Dimitar Terzievski*[1], Miroslav Kjosevski [2], Dimitar Bozinovski [2], Vlatko Ilieski[2]

[1]Food and Veterinary Agency,
Third Macedonian Brigade 20, 1000 Skopje, North Macedonia
[2]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje,
Animal welfare center, Lazar Pop Trajkov 5-7, 1000 Skopje, North Macedonia

The standards for the management of free-roaming dogs (FRDs) in the Republic of Macedonia are defined by national legislation but the approaches are highly variable around the country. A dog population survey is an initial tool for data collection and assessment which can be used to establish the current size of the dog population and subpopulations, to identify the source locations, key stakeholders, welfare status, and FRDs' effect on the community. The aim of the current research was to establish the FRDs size trend throughout a specified period, demographic characteristics such as density of population in surveyed area, and public opinion in the city of Skopje. Three FRD surveys were performed in 2010, 2014, and 2020. The methodology, terminology, and procedures in this study were in accordance with the guidelines for roaming dog populations by the World Society for the Protection of Animals - WSPA guidelines. According to this result, we can conclude that due to stopping the activities on stray dog control from 2018-October 2020, the number of dogs stagnated compared to 2014 and slightly began to rise. The number of estimated dogs per year is as follows: 2010-2442 estimated dogs, upper limit 2915 and lower limit 1969, 2014-1883 estimated dogs, upper limit 2309 and lower limit 1357, 2020-1996 estimated dogs, upper limit 2535 and lower limit 1397. Following the survey study, it is indicative that the current data are of low resolution and cannot reflect the actual FRDs population size trend. It is assumed that the FRD population size would have been lower compared to 2014 if a 4-year interval survey study would have been conveyed by the city authorities. The lack of baseline data renders uncertainty in defining plans for reducing and management of FRD population in the city of Skopje. Therefore, it is suggested that the FRD population survey should be conveyed on an annual basis with an appropriate management plan. The data presented in this study could be used for an objective FRDs population trends in the city of Skopje.

Key words: stray dogs, survey, animal welfare

O19

RECOGNITION AND ASSESSMENT OF PAIN IN DOGS AFTER ELECTIVE SURGERY IN ORDER TO IMPROVE ANIMAL WELFARE

Dardan Pozhegu*

Veterinary clinic "Crazy Pet", Prishtina, Kosovo

Prevention, recognition and treatment of pain are three major postulates in veterinary medicine regarding animal welfare. All surgical procedures in one way or another are considered painful and produce an inflammatory response in organism. In order to enable adequate analgesia and to reduce the risk of postoperative pain and complications, appropriate selection of the anesthetic and analgesic agents are essentials. Elective surgeries such as ovariohysterectomy and castration in companion animals are most commonly performed surgical procedures in small animal practice. Unlike in farm animals, where welfare assessment schemes are already well developed, in companion animals, veterinarians and animal welfare experts has implemented a different approach in veterinary clinics. Despite the physiological measurements such as heart rate, blood pressure and body temperature that has been used to identify pain in animals, researches have proven that these are neither specific nor accurate enough to be used as measures of animal pain. In order to ensure appropriate welfare assessment as well as to determine adequate pain management protocols, a questionnaire for veterinarians as well for the owners are wildly use in veterinary clinics that helps to introduce a small animal welfare standard across veterinary practices. The aim of the study was to present beneficial use of small animal pain questionaries as part of pain scale recognition after elective surgery in stray dogs. Total of 20 healthy dogs at age between 1-3 years were enrolled for the study. In order to determine if any of the patient submitted for elective surgery requires additional analgesia, each patient was assessed at 2, 4, 6, 8, 12 and 24 hours after surgery by measuring the physiological parameters with combination of pain score assessment and adequate questionnaires. According to the physiological parameters and pain scoring assessment, six dogs require additional analgesia, between 6 and 8 hour after surgery. Pain scoring system along with questionnaires can be used as quick and reliable tools for recognition and treatment of pain and welfare assessment in veterinary practice.

Key words: welfare, pain, dogs, elective surgery

O20

WELFARE ASSESSMENT OF PIGS IN THE TIME OF SLAUGHTER - STUNNING QUALITY AS A WELFARE INDICATOR

Emilija Murdjeva[1]*, Branko Angjelovski[2], Mirko Prodanov[3], Miroslav Kjosevski[1]

[1]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Animal welfare center, Lazar Pop – Trajkov 5-7, 1000 Skopje, North Macedonia
[2]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Department of farm animals – Internal medicine, Lazar Pop – Trajkov 5-7, 1000 Skopje, North Macedonia
[3]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Department of food safety and veterinary public health, Lazar Pop – Trajkov 5-7, 1000 Skopje, North Macedonia

Maintaining the welfare of pigs is important during all stages of breeding, including the slaughtering process itself. This process is one of the most critical points for impairing their welfare, which further leads to changes in meat quality. In order to sustain the welfare of pigs during slaughtering there are specific welfare criteria that needs to be fulfilled. The objective of this study was to assess the welfare of pigs during slaughtering with the method of electrical stunning. The welfare assessment was performed on 215 pigs in three slaughterhouses. In order to perform the assessment, the following parameters were measured: duration (seconds, s) of application of the electric current (AD), electric current (amperes, A), electric potential (voltages, V), time (s) from AD to bleeding, corneal reflex, rhythmic breathing, vocalization and righting reflex. These measures are in line with the "Assessment pig protocol" (chapter 6.3 – Collection of data for finishing pigs at slaughterhouse), derived from the Welfare Quality® project. The results of the obtained data from all of the observed pigs show that the average AD was 7.88s (range from 2.15s – 18.89s), the average time from AD to bleeding was 5.49s ± 2.31s (range from 0.67s – 18.95s), the average electrical current was 2.07A ± 0.49A (range from 1.29A – 3.09A) and the average electrical tension was 265V ± 13V (range form 237V – 296V). Regarding the quality of stunning, 37.2% of pigs had positive corneal reflex right after stunning, 20% had rhythmic breathing, vocalization was observed in 7.4% and 2.8% of the pigs showed positive righting reflex. Differences were detected between the slaughterhouses in terms of the observed parameters, including the total sum of AD and the time from AD to bleeding. The average total sum of AD and time from AD to bleeding was 10.2s, 15.2s, and 13.9s in each of the slaughterhouses separately ($p<0.05$). These differences between slaughterhouses indicate different stunning and slaughter practices among them. Analysis of the data obtained from the welfare quality parameters for stunning, points out impairment of animal welfare during slaughtering and urges an immediate action in improving of stunning process in slaughterhouses.

Key words: electrical stunning, pigs, slaughterhouse, welfare assessment

O21

WELFARE ASSESSMENT OF SPORT HORSES IN STABLES IN MACEDONIA

Kristina Antovska*, Lazo Pendovski, Vlatko Ilieski, Miroslav Kjosevski

Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Animal welfare center, Lazar Pop Trajkov 5-7, 1000 Skopje, North Macedonia

The animal welfare is defined as a condition of complete physical and mental wellbeing, the animal being in harmony with its environment. In Macedonia, horses are usually used for work, but there are also stables where horses are kept for sport purposes. Main objective of this study was to evaluate the welfare of horses used for sport purposes and identify strengths and weaknesses from the animal welfare perspective, for the first time in Macedonia. We have conducted the research in three stables for sport horses, out of four running in the country. The assessment consisted of individual assessment of the horses, using AWIN Welfare assessment protocol for horses (2015). In the study, 35 horses were assessed, 18 of which were submitted to complete assessment and 17 to partial assessment. Maximum number of analyzed horses per stable was 13, and minimum was 9. The results has shown that horses in all stables had the possibility for visual contact, but only in certain boxes they could mutually nibble, partially groom and sniff. It was also noticed that 52% of all horses had unsatisfactory box dimensions, implicating not sufficient available space. Most frequent skin integument alterations were alopeciae in different parts of the body with 1-2.33 average number of alterations by region. Total number of horses with noticed alopeciae was 23. The parameters for searching contact and curiosity of the horses, placed in the upper fourth of the protocol's scale and confirmed by the fear test for the first latency period, showed that 94% of horses finished the test within 66 s. In 20% of the horses, negative signs of forced approaching of the man to the horse was found. One of the stables stood out by high percent (76%) of acceptable body condition score, and the other showed unsatisfactory quantity of bedding in half of the boxes (56%). Nasal discharge in horses was noticed in two farms, and only one farm had no signs of hoof neglecting. This research pointed at the priority areas for improving the welfare in horses, which suggests the need for further actualization of the welfare in sport horses among relevant stakeholders in the country.

Key words: animal welfare, horses, sports, physical and mental health

O22

SURVEY ON RECOGNITION OF PROBLEMS OF LOCOMOTION SYSTEM IN HORSES IN MACEDONIA

Marija Solakova*, Elena Atanaskova Petrov, Miroslav Kjosevski, Ksenija Ilievska

Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine – Skopje, Lazar Pop Trajkov 5-7, 1000 Skopje, North Macedonia

The constant evolution of horses is among the best documented in all paleontology that began during the early Eocen epoch until Pleistocen epoch when evolution of all members of Equs caballus gave rise. People have seen their abilities and strength over time, and began to use them not only for food, work and plowing fields, but also for riding and different type of recreation. Despite the free spirit and ability of movement on different type of pads and substrates, domestication of horses withdraw a numerous problems with locomotion system. Considering the presence of horses as companion animals, the objective of this study was to investigate the knowledge of horse breeders and owners regaridng orthopedic problems in horses in our country. The used survey was mainly based on breeding, hoof shoeing, recognition and treatment of hoof diseases, and was conducted on total 11 stud farms. According to the sublimated data, we observed that the biggest problem with the locomotion system occurs in horses that are not properly showed, raised on unsuitable grounds with inappropriate nutrition, posture and muscle weakness, premature exposure to excessive efforts and inappropriate equipment (saddle). Puncture wounds and pododermatitis are among the most common orthopedic problems in horses treated mostly by the owners due to limited availability of doctor of veterinary medicine – specialist for horses. Taking into account that the number of horses as pets or working animals is rising in the country, the need for doctors of veterinary medicine – specialists for horses and sophisticated equipment is increasing. This will enable adequate diagnostic and treatment of orthopedic diseases, and consequently will improve the horse welfare in Macedonian farms.

Key words: horse, locomotion system, welfare

Scientific Session V
VETERINARY CLINICAL MEDICINE

Plenary lectures and Oral presentations

PL3

THE PRACTICAL APPLICATION OF REGENERATIVE MEDICINE IN CANINE MUSCULOSKELETAL CONDITIONS

Russell Chandler

Advanced Practitioner in Small Animal Orthopaedics
Alphavet Referrals Newport, Wales, UK

Canine musculoskeletal conditions, such as OA have seen an ever-growing list of licensed and off-label pharmaceuticals becoming available for veterinary professionals to use. These are almost all symptom modifying therapies. In recent years regenerative medicine (RM) has entered the arena of canine musculoskeletal medicine. The aim of these RMs is to fill the gap for a genuinely disease modifying therapy. The two main modalities currently in use are platelet rich plasma (PRP) and mesenchymal stromal cell (MSC) therapy. Either of these used alone, or in combination show enormous potential for a truly regenerative approach. In this presentation, the ways in which I use PRP and MSC treatments in practice are described, and clinical cases are presented. This is aimed at those of us in clinical practice who are keen to start using RM.

Key words: regenerative medicine, dog, platelet rich plasma (PRP)

PL4

INTERVERTEBRAL DISC DISEASE – A MARKED ROAD OR A JUNGLE?

Boris Pirkić

University of Zagreb, Faculty of veterinary medicine,
Heinzelova 55, 10000 Zagreb, Croatia

Intervertebral disc disease is amongst the most common neurologic problems in dogs. Every practitioner has seen it and mostly it was a nightmare of dealing with the acutely affected patient with frightened owners. Literature is quite extensive on the subject, and it seems that everything's clear and well defined, especially regarding high frequency of the occurrence and a 200-year history of researching the disease with hundreds of papers and books published. General practitioners know bits and pieces of numerous specialities in veterinary medicine, and they are the first to meet the IVDD patient. They are also the first to examine and help the animal. So, it's known that IVDD is divided according to the type in three types, every type belongs to a certain type of disc degeneration, and every type of degeneration imply a certain position within a vertebral canal. Furthermore, all the aforementioned data can be related to a typical breed and size of a dog. Further action should be sending the dog to ancillary diagnostics and the required therapy, be it surgical or not. That way the circle is closed. But is it so? Is the clinical picture at first sight the correct and final one? Could it develop unexpectedly? Is it really that urgent, however the symptoms are severe? The base of our experience and thi s lecture are numerous patients treated at the Clinic for surgery, orthopaedics and ophthalmology In Zagreb. Big decision is to refer a patient to a most helpful ancillary diagnostic. But have we gotten ourselves carried away by the cardinal symptoms, forgetting to look for the subtle and frequently, crucial ones and thus led astray? In an ambulatory patient, have we mistaken spinal walk for paraparesis and gave a wrong prognosis? Do we think about a pain as a subject in an ambulatory patient, especially in dogs with a good response on anti-inflammatory therapy? If we think thoroughly, there is a vast number of questions that one should ask himself before deciding on definitive diagnosis, and moreover, prognosis. Owners stick to our words and therefore we shouldn't decide or give opinions hastily without checking all the hidden corners because it could cause the perspective patient to terminate the therapy, or the unpromising one to continue it, causing unnecessary pain and cost. Doing the detailed full examination will guide us to follow a well paved road and prevent us to slide into a jungle.

Key words: intervertebral disc disease, dog, surgery

O23

TOPICAL APPLICATION OF HOMOLOGOUS SERUM FOR TREATMENT OF CORNEAL ULCER IN DOGS

Ksenija Ilievska*[1], Elena Atanaskova Petrov[2], Filip Trojachanec[3], Jane Vlahov[1], Plamen Trojachanec[1]

[1]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Department of veterinary surgery, Lazar Pop Trajkov 5-7, 1000 Skopje, North Macedonia
[2]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Department of small animal internal medicine and horses, Lazar Pop Trajkov 5-7, 1000 Skopje, North Macedonia
[3]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, University veterinary hospital, Lazar Pop Trajkov 5-7, 1000 Skopje, North Macedonia

Corneal ulcer represents a significant problem in veterinary practice, characterized by loss of corneal epithelium, exposing the corneal stroma, with or without stromal loss as well as patient discomfort. Diagnosis is usually performed by fluorescein test with visible retention of exposed corneal stroma and slit-lamp biomicroscopy. Topical antibiotics, artificial tears and analgesic drugs are the first choice of treatment. If superficial corneal ulcer is left untreated, it will have the tendency to involve deeper structures requiring respective surgical treatment. Grid/superficial keratotomy, diamond or bur debridement are still the most common corneal treatment for removal of loose epithelium and epithelial growth stimulation. Regenerative medicine as new discipline aims to develop biologic, cell-based therapies for reparation or replacement of injured or eroded tissue or cartilage. Platelet-rich plasma (PRP) as autologous byproduct contains various growth factors, cytokines and platelets that contribute to the healing ability and tissues regeneration. Unlike conventional prepared PRP eye drops for topical or subconjuctival administration for treatment of corneal ulcer, a sterile homologous serum can be successfully use in veterinary practice. Canine serum unlike PRP does not contain platelets or clotting factors, but is rich with hormones, minerals, proteins that are not included in clotting process. The aim of the study is to present a successful treatment of dogs with persistent superficial corneal ulcers that did not heal within weeks of standard therapy. Owners were instructed to apply a combination of one drop of sterile homologous serum with antimicrobial eye drops 2-3 times daily, within 3 weeks. Despite the possibility for using frozen samples, in order to follow up the process of corneal healing, we decided to collect the samples every 3 days and keep at $+4^0$C during the treatment. Homologous serum as a topical treatment is the cheapest, minimally invasive and effective alternative therapeutic approach for superficial or indolent corneal ulcers due to its potential to stimulate healing of corneal epithelium, with or without adjunctive medical treatment.

Key words: corneal ulcer, prp, serum, healing, dogs

O24

DISEASE-STAGE RELATED THERAPEUTIC RESPONSE OF MESENCHYMAL STROMAL CELLS AFTER INTRA-ARTICULAR DELIVERY IN A MOUSE OSTEOARTHRITIC JOINT

Ana Ivanovska[*1,2], Patrizio Mancuso[1,2], Conor Hennessy[1,2], Swarna Raman[1], Steven McLoughlin[1,2], Jamie Reilly[1], Frank Barry[1,2], Mary Murphy[1,2]

[1]National University of Ireland Galway, Regenerative medicine institute (REMEDI), Galway, Ireland
[2]National University of Ireland Galway, Centre for research in medical devices (CÚRAM), Galway, Ireland

Osteoarthritis (OA), a chronic debilitating joint disease characterized by synovial inflammation and progressive degradation of articular cartilage, is associated with lameness, pain and low quality of life in animal patients. Current treatments for OA mainly address symptoms and management of the disease using anti-inflammatory and analgesic pharmacological treatments, with surgery performed in severe cases. Based on this, there is need for innovative efficient therapeutic solutions that will address a disease-modifying approach. Mesenchymal stromal cells (MSCs) are able to exert immunomodulatory activities and secrete trophic factors that may be beneficial for the treatment of OA. Multiple pre-clinical and clinical studies have demonstrated the safety and efficacy of MSCs for OA treatment, however the precise mechanism of action of therapeutic MSCs has not yet been defined. We sought to mimic autologous delivery of MSCs and investigate the phenotypic and secretory profile of IA-delivered MSCs exposed to an in vivo OA environment, in order to understand the biological background for clinical applications. Collagenase-induced osteoarthritis (CIOA) was induced in the knee joint of C57BL/6 mice and 2x105GFP+ syngeneic bone marrow-derived MSCs (BM-MSCs) were delivered via intra-articular (IA) injection to the CIOA and control (SHAM) mice. On days 14 and 56 corresponding to early and late OA, MSCs were retrieved 12hrs and 72hrs post-IA delivery. The retrieved therapeutically activated MSCs were characterized based on their morphology, growth, immunomodulatory abilities and transcriptome. The retrieval efficiency of surviving injected cells from CIOA joints was 1.3%, higher than that obtained from SHAM joints, indicating the role of the inflammatory environment in cell survival. In terms of the molecular profile, early retrieved MSCs expressed genes and canonical pathways related with extracellular matrix organization, while late-retrieved cells were enriched for immunomodulatory elements, which might reflect the pathological differences in early and late OA. Additionally in both groups antagonists of the Wnt superfamily and chondroprogenitor genes were expressed suggesting that Wnt pathway and elements of joint organogenesis play an important role in MSCs survival. In conclusion, MSCs are highly responsive to the micro-environment and degree of inflammation in OA resulting in different cell-mediated therapeutic responses. These findings are important as different

in vitro pre-treatment strategies can be used to enhance the therapeutic response of MSCs, whereas from a clinical perspective, disease stage needs to be considered when using MSCs and other assessments such as synovial fluid analysis might give an indication of a possible therapeutic outcome.

Key words: mesenchymal stromal cells, osteoarthritis, disease-stage, clinical use, mechanism of action

O25

MESENCHYMAL STROMAL CELLS LYO-SECRETOME: PRELIMINARY CLINICAL APPLICATION IN DOGS AND HORSES WITH NATURALLY OCCURRING OSTEOARTHRITIS

Priscilla Berni[1*], Michela Mocchi[2], Virna Conti[1], Silvia Dotti[3], Dario Di Silvestre[4], Riccardo Villa[3], Roberto Ramoni[1], Giulia Passignani[4], Francesca Brambilla[4], Maurizio Del Bue[5], Gianpaolo Squassino[6], Laura Catenacci[2], Milena Sorrenti[2], Lorena Segale[7], Elia Bari[7], Pierluigi Mauri[4], Sara Perteghella[2,8], Maria Luisa Torre[2,8], Stefano Grolli[1]

[1]University of Parma, Department of veterinary medical science, Strada del Taglio 10, 43121 Parma, Italy
[2]University of Pavia, Department of drug sciences, Viale Taramelli 12, I-27100 Pavia, Italy
[3]Istituto zooprofilattico sperimentale della Lombardia e dell'Emilia-Romagna, 25124 Brescia, Italy
[4]Institute of biomedical technologies, F.lli Cervi 93, 20054 Segrate, Italy
[5]Freelance veterinary medical doctor, 43121 Parma, Italy
[6]Studio tecnico veterinario, Via Alfredo Catalani 12/14, 14100 Asti, Italy
[7] University of Piemonte Orientale, Departemt of pharmaceuticals sciences, Largo Donegani 2/3, 28100, Novara, Italy
[8]PharmaExceed S.r.l., Piazza Castello 19, 27100 Pavia, Italy

A growing body of evidence suggests that cell secretome is responsible for many of the biological and therapeutic effects of Mesenchymal Stromal Cells. A freeze-dried secretome (Lyo-secretome) derived from equine and canine Mesenchymal Stromal Cells (MSCs) prepared from adipose tissue has been prepared and characterized in terms of protein and lipid content. In addition, a complete proteomic analysis has been performed for the equine preparations. Furthermore, the in-vitro biological effects on different cell populations (synovial fluid-derived MSCs, adipose-derived MSCs, tenocyte and chondrocytes) have been analyzed with increasing doses of canine and equine Lyo-secretome evaluating cytocompatibility and proliferative effects. The proteomic analysis of equine Lyosecretome demonstrated the presence of proteins involved in the modulation of the inflammatory process, tissue homeostasis and cartilage biology. Furthermore, the Lyo-secretome in-vitro treatment stimulated cell proliferation and metabolic activity in a dose-dependent manner for all the cell types tested, reaching 85% for the higher Lyo-secretome concentration compared to the 10%FBS positive control (100%). As a matter of fact, the proteome analysis and the in-vitro activity of the Lyo-secretome, suggest a possible use in the therapy of osteoarthritis (OA). Dogs and horses with naturally occurring OA were enrolled in an in-vivo double-blinded study and treated with a double intra-articular injection at 40-days interval to assess preliminary safety and efficacy of allogeneic Lyo-

secretome. The animals were enrolled in two groups: one group was treated with intra-articular injection of Lyo-secretome, and the other group with mannitol, both resuspended in hyaluronic acid to ensure an effective therapy in the control of clinical symptoms. A general physical examination and an orthopaedic assessment were performed at short intervals, and for the canine patients, a questionnaire containing the Helsinki Chronic Pain Index was submitted to the owners (Day 0-2-4-7-20-40). The data were elaborated with multifactorial ANOVA analysis of variance. At the time of writing, 4 horses and 11 dogs have been enrolled in the study, receiving two doses of the treatments. The clinical data suggest that intra-articular injections of allogeneic Lyo-secretome are safe and do not induce any clinically significant local or systemic adverse response in both species. This study, assessing the short and long-term safety in animals with naturally occurring diseases, provides the basis for a more extensive clinical trial and suggests that Lyo-secretome can represent an innovative OA therapy.

Key words: mesenchymal stromal cells, regenerative medicine, musculoskeletal diseases, cell secretome

O26

BENEFICIAL EFFECT OF ACEPROMAZINE ON INCIDENCES OF ADVERSE EFFECTS ASSOCIATED WITH MORPHINE PREMEDICATION IN DOGS

Jane Vlahov*, Plamen Trojacanec, Todor Novakov, Ksenija Ilievska, Filip Trojacanec

Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, University veterinary hospital, Lazar Pop Trajkov 5-7, 1000 Skopje, North Macedonia

The most usual premedication option in dogs is morphine and the most common complications associated with the use of that premedication are vomiting with greatest incidence around 90%, ptyalism and panting with less percentage. Usual anti-emetic protocols have not been established for prevention of these complications. Acepromazine administered before morphine among other drugs is used to reduce the incidences of vomiting, signs of nausea, ptyalism and increased panting. Retrospective analysis of 30 patients that were submitted for surgery was performed. The patients were assigned to 2 groups according to the pre-anesthetic protocol. All groups received 0.3 mg/kg of morphine subcutaneously (s/c). Group "Morphine" received only Morphine (s/c), and Group "MorphAce" received 0.01 mg/kg of acepromazine intramuscular (i/m) 45 minutes before application of morphine. The patients were observed by a veterinary doctor and the adverse effects were documented from the time morphine was administered until the time patents were induced for surgery preparation. Our results showed that group "Morphine" had significantly more vomiting (86.6%), greater incidence of ptyalism (93.3%) and panting (96.6%), compared to group "MorphAce" which had less vomiting (6.6%), ptyalism (13.3%) and panting (36.6%). In our study, acepromazine administered before morphine significantly decreased the incidence of vomiting, ptyalism and panting, and recommend this protocol for premedication in dogs.

Key words: acepromazine, morphine, vomiting, ptyalism, panting

O27

PRE-EMPTIVE MULTIMODAL ANALGESIA WITH MORPHINE-MEDETOMIDINE-KETAMINE AND ACEPROMAZINE- MORPHINE-KETAMINE FOR OVARIOHYSTERECTOMY IN DOGS

Todor Novakov*, Elena Atanaskova Petrov , Ksenija Ilievska, Irena Celeska, Jane Vlahov, Filip Trojacanec, Plamen Trojacanec

Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, University veterinary hospital, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia

Ovariohysterectomy is a painful procedure that often requires additional analgesia during surgery. Successful pre-emptive multimodal analgesia may increase the nociceptive threshold and minimize or block nociceptor activation. The aim of this study is to compare analgetic effects of two anesthetic protocols for ovariohysterectomy in dogs. Retrospectively we investigate two different anesthetic protocols of 50 cases of ovariohysterectomy of healthy female dogs at different age and different breeds divided in two groups. Group MMK 25 cases, prior induction intravenously receive 0.2mg/kg Morphine, 0.01mg/kg Medetomidine, 1mg/kg Ketamine and group AMK 25 cases, prior induction intravenously receive 0.03 Acepromazine, 0.3mg/kg Morphine, 1mg/kg Ketamine. Heart rate, respiration rate and needs for additional analgesia to surgical response were noted intraoperatively. Nociceptive responses to surgical stimulation were obtunded to a greater degree in AMK group compared to MMK group during the surgery. There was no need for additional analgesia during the surgery in MMK group, unlike the AMK group where 14/25 patients received 2mg/kg Tramadol i/v for additional analgesia. A multimodal approach, using different classes of analgesics resulted in superior analgesia for ovariohysterectomy in dogs.

Key words: multimodal analgesia, pre-emptive analgesia, ovariohysterectomy, dogs

O28

TRILOSTAN TITRATION DOSE TREATMENT IN DOGS WITH TERMINAL STAGE OF HYPERADRENOCORTICISM COMPLICATED WITH COMORBIDITY DISEASE

Irena Celeska*[1], Martin Nikolovski[2], Todor Novakov[3], Elena Atanaskova Petrov[4]

[1]Ss. Cyril and Methodius University, Faculty of veterinary medicine - Skopje, Department of pathophysiology, Lazar Pop – Trajkov 5-7, 1000 Skopje, North Macedonia
[2]Ss. Cyril and Methodius University, Faculty of veterinary medicine - Skopje, Department of functional morphology, Lazar Pop – Trajkov 5-7, 1000 Skopje, North Macedonia
[3]Ss. Cyril and Methodius University, Faculty of veterinary medicine, University hospital, Lazar Pop – Trajkov 5-7, 1000 Skopje, North Macedonia
[4]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Department of internal medicine of small animal and horses, Lazar Pop – Trajkov 5-7, 1000 Skopje, North Macedonia

Hyperadrenocorticism (HAC) is chronical endocrinopathy in dogs with permanent serum hypercortisolemia. Middle aged or geriatric patients are affected, especially dogs with breed predisposition. Clinical manifestation is multysistemic, however early stage diagnosis is very important for regular long-term treatment, diminishing clinical symptoms and alleviate effects of cortisol impact on metabolic shift. Although many treatment protocols recommend initial dose for treatment, adverse reaction can provoke exacerbation of clinical condition, due to pharmaco-kinetic mechanism of trilostan, especially in terminal stage, with consecutive organopathies. Mechanism of trilostan is competitive inhibition of enzyme for conversion pregnenolone to progesterone thus blocking production of cortisol. Medical treatment of recommended dose in terminal stage, can cause onset of rapid decrease of serum cortisol concentration and developing macro-adenoma of adenohypophysis with typical neurology symptoms. This clinical survey covered four clinical cases in terminal stage of HAC. The first case Doberman pinscher with anamnestic data of PU/PD, non-pruritic symmetric alopecia with dark pigmentation; laboratory results revealed marked thrombocytosis, moderate lymphopenia, increase ALKP and hypothyroidism. The second case was bison freezer with paroxysmal panting, tachyarrhythmia, blindness and muscle weakness; the dog did not presented any skin lesion. Laboratory results revealed mild anemia, thrombocytosis, increase concentration of ALKP, ALT, marked hyperlipidemia and hypothyroidism. The third case was bison freezer, with polyuria, polydipsia and polyphagia, behavioral changes, with thrombocytosis, serum enzymes increase level ALKP, ALT and hyperglycemia, with poor glycemic control, besides insulin treatment. The fourth case was Shih Tzu with symmetric non-pruritic alopecia, recurrent pyoderma with subcutaneous flegmona, comedones and

calcinosis cutis. Laboratory results presented thrombocytosis, leukocytosis and significant increase of ALKP. All clinical cases were positive of low dose dexamethasone suppression test (LDDST). Hyperadrenocorticism was confirmed according anamnestic data, clinical condition, laboratory results and ultrasound finding of hepatomegaly in terminal stages of disease. Although recommended initial dose for trilostan treatment have assess risk of adverse reaction and worsening clinical condition. Treatment protocol was started with initial dose of 0.5 mg/kg SID for 7 days, then 0.5 mg/kg BID for 7 days. The dose of trilostane was increased every 7 days for 0.5 mg/kg, at first morning dose and after 7 days evening dose achieving 4-6 mg/kg total daily dose, with follow up of clinical symptoms and laboratory parameters in regular intervals. This treatment protocol with trilostan titration dose give favorable effects in remission and resolution of clinical symptoms and good control of comorbidity disease in dogs with terminal stage of hyperadrenocorticism.

Key words: hyperadrenocorticism, trilostan, dog, titration dose, terminal stage

Scientific Session VI
ANIMAL REPRODUCTION

Plenary lecture and Oral presentations

PL5

REPRODUCTIVE MENAGEMENT IN GOAT BREEDING

Juraj Grizelj*[1], Branimira Špoljarić[1], Fernando Sánchez Dávila[2], Silvijo Vince[1]

[1]Reproduction and obstetrics clinic, Faculty of veterinary medicine, University of Zagreb, Vjekoslava Heinzela 55, 10 000 Zagreb, Croatia
[2]Universidad autónoma de Nuevo León, Faculty of agronomy, General Escobedo, Mexico

Goats belong to the „short day breeders“ group of seasonal polyoestrous animals. In the temperate climate zone, their season lasts from mid-summer till mid-autumn, being regulated by epiphysis' melatonine secretion, as well as by genetics and environment factors. Therefore, the presence of goat milk and dairy products, as well as goat meat, at the food market, is exclusively seasonal. In order to provide goat food products throughout the whole year, reproductive herd menagement should be established. This paper presents a brief overview of the methods of reproductive management in dairy goat herds (natural mating and artificial insemination, hormonal and nonhormonal synchronization methods and embryo production and transfer). Application of reproductive and breeding programs allow efficient diffusion of desirable genetic traits in a selection program.

Key words: reproductive management, goats, eCG, light treatment, male effect

O29

THE EFFECT OF HOMOLOGOUS SEMINAL PLASMA AND REDUCED GLUTATHIONE ON THAWED RAM SPERM VIABILITY AND MOTILITY

Martin Nikolovski*, Monika Dovenska, Dimitar Bozhinovski, Ljupcho Mickov, Branko Atanasov, Nikola Adamov, Toni Dovenski,Vladimir Petkov

Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine – Skopje, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia

The use of homologous seminal plasma (SP) and reduced glutathione (GSH) for reducing sperm membrane damage following thawing has been reported with conflicting results. Our aim was to assess the combined use of SP and GSH supplemented in soy-bean lecithin based-semen extender (SBLE) in achieving higher viability and motility of thawed ram sperm. Fresh ram ejaculates (≥80% motile sperm, ≥60 viability, and >2.5 billion/ml sperm concentration) were pooled and divided in two portions. The first portion was sub-divided in four groups which were extended up to 50 million cells/ml with SBLE (control C-a), SBLE and GSH 5 mM (E1-a), SBLE and SP 20 vol% (E2-a), and SBLE, GSH 5 mM and SP 20 vol% (E3-a), respectively. The second portion was extended with SBLE to 100 million cells/ml. Both portions were cryopreserved in liquid nitrogen according to a standardized protocol. Following thawing, the second portion was sub-divided and extended in the same manner as previously described up to 50 million cells/ml and was separated in the respective groups C-b, E1-b, E2-b, and E3-b. Each group was sampled in ten replicates at 0- and 3-hours following thawing. Thawed samples were analyzed for viability by using Hancock-2 stain, and velocity by using CASA method (Hamilton Thorne, USA). Each sample included at least 200 cells and the results were expressed in percent values (mean±SEM). Normality was confirmed with Kolmogorov test, whereas variances comparison was performed with factorial-ANOVA. Significantly different values were considered if $p<0.05$. E2-a-0h (57.58% ±2.40) and E3-a-0h (56.94% ±1.85) yielded significantly higher viability compared to the C-a-0h (40.73 ±1.53). C-a-3h (45.40% ±2.72) yielded significantly higher viability compared to E2-a-3h (21.85% ±1.36) and E3-a-3h (21.85% ±1.36). E2-a-3 (29.25% ±2.37) and E3-a-3h (16.63% ±1.15) had significantly higher value of acrosome-affected thawed sperm compared to C-a-3h (10.80% ±1.32). C-a-3h (47.97% ±4.50) had significantly higher number of sperm with high velocity compared to E2-a-3h (17.67% ±1.92) and E3-a-3h (16.80% ±2.10). SBLE supplemented with SP and GSH prior cryopreservation yielded significantly higher number of viable sperm cells immediately post-thawing. The SBLE yields significantly higher number of viable and acrosome-intact sperm cells following extended times post-thawing (3 hours). The effect of increased viability and acrosome stability could be attributed to SP and GSH if they are supplemented to the SBLE prior cryopreservation. This does not apply if they are added following thawing.

Key words: ram sperm, cryopreservation, seminal plasma, reduced glutathione

O30

KINETIC PARAMETERS OF CHILED BOAR SPERMATOZOA FROM DIFFERENT PORCINE BREEDS

Ljupco Mickov, Branko Atanasov, Martin Nikolovski, Monika Dovenska, Igor Esmerov, Nikola Adamov, Vladimir Petkov, Toni Dovenski

Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine – Skopje, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia

Artificial insemination, as the ultimate biotechnology in the reproduction of the farm animals, is a developing industry, based on high quality control and health standards. The semen quality is highly dependable on many factors and the sire breed is one of them. The objective of this study was to compare the kinetic parameters obtained by computer assisted semen analysis (CASA) of chilled semen from 3 different porcine breeds (Duroc, Landrace and Yorkshire). For that purpose, 713 ejaculates were assessed (Duroc n=122; Landrace n=145, and Yorkshire n=446), after the final dilution with commercially available extender (Androhep, Minitube, Germany), in standard count chamber. The following kinetic parameters were assessed: velocity of average path (VAP), velocity of straight line path (VCL), velocity of curvilinear path (VCL), amplitude of lateral head displacement (ALH), beat-cross frequency (BCF), straightness (STR) and linearity (LIN). In addition, two additional morphological parameters were assessed: elongation and area of the head. Values were expressed as means ± standard deviation. Means were compared by ANOVA. Comparisons yielding $p<0.05$ were considered significantly different. There were significant differences between the kinetic parameters of chilled spermatozoa obtained from the different boar breeds. Further studies are required to validate the current findings.

Key words: boar, semen, ejaculate, spermatozoa, motility

O31

ASSESSMENT OF *IN VITRO* MATURATION RATE OF PORCINE OOCYTES SELECTED BY BRILLIANT CRESYL BLUE STAINING

Monika Dovenska[1]*, Ljupcho Mickov[2], Branko Atanasov[2], Vladimir Petkov[1], Martin Nikolovski[1], Toni Dovenski[2], Florina Popovska-Perchinikj[1]

[1]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine – Skopje, Department of functional morphology, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia
[2]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine – Skopje, Department of reproduction, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia

Morphological classification is still the primary criterion for quality evaluation of immature porcine oocytes submitted for *in vitro* embryo production. Recently, brilliant cresyl blue (BCB) staining has been used as a non-invasive indicator for the assessment of their developmental competence. The aim of our study was to determine the *in vitro* maturation (IVM) rate of porcine oocytes based on BCB classification. Cumulus-oocytes complexes (COC) (n=560) were aspirated from fifty-nine (n=59) gilt ovaries obtained from a local slaughterhouse. Only COC (n=445) with homogenous ooplasm and at least 3 layers of cumulus cells were selected for further analysis. The control group (n=80) was immediately placed into a maturation medium, whereas the experimental group was incubated for 90 min in Dulbecco's PBS containing 0.4 % BSA and 52 μM BCB. The treated COC were then classified in three groups: BCB^+ - dark blue-colored ooplasm (n=109), $BCB^{+/-}$ - light blue-colored ooplasm (n=58), and BCB^- - color-free ooplasm (n=198) and transferred into maturation medium. After 44 h of IVM at 38.8 °C and 6% CO_2, the maturation rate was evaluated under the stereo microscope, where COC with expanded and loose cumulus mass were considered as matured. Statistical analysis was performed using 'R version 4.2.1, 2022' Software. Absolute values were compared with the Chi-square test (χ^2=47.064) with a significance level of $p<0.0001$ and df=3. The BCB^- group showed a significantly lower maturation rate (28%) in comparison to the control (63%), BCB^+ (61%), as well as the $BCB^{+/-}$ group (53%). There was no significant difference of cumulus cells expansion rate between the BCB^+ (61%) and $BCB^{+/-}$ (53%), nor in these two groups compared to the control group (63%). These preliminary findings indicate that the BCB staining might be useful for the exclusion of COC that are developmentally incompetent (BCB^-). Additionally, the obtained results demonstrated that both BCB^+ and $BCB^{+/-}$ COC show meiotic competence and can be used for *in vitro* maturation. Generally, it can be concluded that for a routine IVM protocol, the BCB staining should only be used as an additional method for selection of porcine oocytes with good quality.

Key words: porcine, oocytes, brilliant cresyl blue, *in vitro* maturation

O32

INTRODUCTION OF THE OVSYNCH-8 PROTOCOL IN SMALL DAIRY FARMS IN REPUBLIC OF NORTH MACEDONIA TO INCREASE THE REPRODUCTIVE EFFICIENCY FROM THE FIRST ARTIFICIAL INSEMINATION POSTPARTUM

Kristina Dojchinovska[*1], Toni Dovenski[1], Ljupcho Mickov[1], Marina Toshevska[2], Zharko Mihaloski[3], Igor Taleski[4], Ljupcho Andreevski[5], Branko Atanasov[1]

[1]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine – Skopje, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia
[2]Agro Lider, Marshal Tito 92 7524 Krivogashtani, Prilep, North Macedonia
[3]Veterinary clinic "Medika-Vet", Malo Konjari 7509 Prilep, North Macedonia
[4]Veterinary clinic "Vet-Maks – IT", Industriska bb 7000 Bitola, North Macedonia
[5]Veterinary clinic "Ljupco i dr.", Marshal Tito bb 97240 Demir Hisar, North Macedonia

The article aimed to present modified ovulation synchronization program (Ovsynch-8) that utilize fixed time Artificial Insemination (Timed Artificial Insemination) and its implementation in small dairy family farms in Macedonia. A total of 150 dairy cows from 32 small dairy farms, starting at day 45 ± 3 postpartum, were pre-synchronized by PG-3-G protocol. Ten days later, an Ovsynch TAI program was initiated (G1–8 days – $PGF_{2\alpha}$ – 56 hours – G2 – 16 hours – TAI) and cows were inseminated at day 66 ± 3. All treatments and inseminations were done by the local veterinarians - practitioners. Transrectal ultrasonography was conducted at G1 to map ovarian structures, and at PGF2α administration to assess ovulation in response to G1. Pregnancy diagnosis was done on day 30 after AI. Of 150 cows enrolled in the study, 24 cows (16%) were classified as acyclic, 7 cows (4.66 %) as cystic and 10 cows (6.66%) had muco-purulent vaginal discharge. All remaining cows (n=109) were inseminated. Thirty two cows were re-inseminated. The pregnancy rate from the first AI postpartum was 34.86% (38/109). The pregnancy rate from the second insemination was 17/32 or 53.1%. Cows that did not conceived from both inseminations were not further followed up. According to results, it can be concluded that the ovulation synchronization program that utilized fixed time AI might be successfully implemented in small family farms and consequently might increase the reproductive efficiency from the first AI postpartum.

Key words: ovsynch, luteal regression, dairy cows, family farms

Scientific Session VII
FOOD SAFETY

Plenary lecture and Oral presentations

PL6

UPDATE ON ACTIVITIES OF THE EUROPEAN REFERENCE LABORATORY FOR LISTERIA MONOCYTOGENES: FOCUS ON TYPING ACTIVITIES

Sandrine Te*[1], Benjamin Félix[1], Karine Capitaine[1], Arnaud Felten[2], Graziella Bourdin[3], Guillaume Gillot[4], Carole Feurer[5], Sabine Delannoy[6], Bertrand Lombard[7], Jean-Charles Leblanc[1], Sophie Roussel[1]

[1]French agency for food, environmental and occupational health and safety (ANSES), European Union reference laboratory for Listeria monocytogenes (EURL Lm), Laboratory for food safety, Salmonella and Listeria unit, France
[2]ANSES, Laboratory for food safety, University Paris-Est
[3]EURL Lm ANSES, Laboratory for food safety, bacteriology and parasitology of fishery and aquaculture products unit, Boulogne-sur-Mer
[4]ADRIA Food technology institute, Quimper, France
[5]IFIP-The French pig and pork institute, Department of fresh and processed meat, Le Rheu, France
[6]Identy path platform, Food safety laboratory, ANSES, Maisons-Alfort, France
[7]ANSES, Strategy and programs department, Research and reference division.

Listeria monocytogenes (*Lm*) is a bacterium that causes a food-borne illness, the Listeriosis. Typing studies using Multi Locus Sequence Typing (MLST) reveal that most strains are gathered into few major Clonal Complexes (CCs), that account for a majority of human cases worldwide. CC language is harmonized internationally and provides crucial information on strain virulence. To date, the CC identification is based on sequencing of the strain genome and requires between 3 and 5 days depending on labs. Thus, here is a need of a front-line typing method for rapid identification of the major circulating CCs. Such a method would aim at screening the wide amount of food strains collected for surveillance and research purposes. At European level, the EU Reference Laboratory for *Lm* (EURL *Lm*) is coordinating a network of 40 National Reference Laboratories (NRL) in charge, amongst other duties, of *Lm* monitoring in food products. The EURL *Lm* in collaboration with the NRL, has developed a new real-time PCR assay that enables analysis of 40 real-time PCR in a multiplex scheme. This test provides the accurate identification of 30 CCs, including the most prevalent CCs recently reported from human cases and food in Europe, the serogroup of the strains and subdivision among CCs. This one-day method was also developed into a high throughtput real-time PCR version. We describe here (i) its design from a wide panel of 2299 strain genomes from human, food, animal and environment (ii) its validation according to the standard EN ISO16140 on a various panel of 761 strains whole sequenced and collected from 21 European countries and (iii) its assessment for the typing of hundreds strains collected during monitoring activities. This assay will represent a key tool to assist surveillance laboratories to establish strain relatedness with human clinical strains, during outbreak investigations. Moreover, this test can assist the food sectors to adapt their microbiological management plans to remove hypervirulent strains from their production line.

Key words: *Listeria monocytogenes* (Lm), Multi Locus Sequence Typing (MLST), National Reference Laboratories (NRL)

O33

PREVALENCE OF SEROTYPES 1/2a AND 4b OF LISTERIA MONOCYTOGENES FROM READY-TO-EAT FOOD PRODUCTS IN KOSOVO

Besart Jashari*[1], Katerina Blagoevska[1], Dafina Mehmetukaj[2], Dean Jankuloski[1]

[1]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine – Skopje, Food institute, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia
[2]Food and Veterinary Agency of Kosovo, Serology and molecular diagnostic sector, Food and veterinary laboratory, Lidhja e Pejës 241, 10 000 Pristina, Kosovo

Listeria monocytogenes remains one of the pathogens that has the highest mortality rate in humans as a result of food poisoning, especially if we take into consideration the fact that serotypes 1/2a, 1/2b, and 4b, which are directly related to human health, are the most present in food. Due to huge losses in people and the world economy, the EU has issued regulations so that business operators and authorities can control any outbreak of the eventual disease. According to Regulation (EC) No. 2073:2005 on Microbiological Criteria, the presence of Listeria monocytogenes must be absent in 25g or less than 100 CFU in 1g of food until the expiration date. The purpose of this study was to evaluate the presence of serotypes 1/2a and 4b of *Listeria monocytogenes* strains at the level of production from ready-to-eat food in the Republic of Kosovo. A total of 2517 ready-to-eat food from the production level were token during the period January 2016 and March 2022. For the detection of *Listeria monocytogenes*, ISO 11290-1:2017 method was used, and for the serological identification commercial serums for somatic (O) and flagellar (H), antigens have been used. Out of 2517 samples of dairy and meat products at the production level tested, 40 showed positive results for *Listeria monocytogenes*, of which 21 samples (52.5%) from dairy products and 19 samples (47.5%) from meat products. Serogroup results of *Listeria monocytogenes* did not differ much between 1/2a and 4b groups. Serogroup 1/2a was represented with 19 out of 40 isolates (47.5%), while serogroup 4b with 21 out of 40 (52.5%). No statistically significant relationship ($P > 0.05$) was found between recorded serogroups and isolation sources. This research has demonstrated the necessity to investigate the diversity of *Listeria monocytogenes* strains, to facilitate the efficiency of detecting the source of an epidemic outbreak from food. These findings provide an overview of the presence of a significant pathogen in ready-to-eat food products in the territory of Kosovo and in the same time present a crucial issue for public health in particularly for consumers with a compromised health conditions.

Key words: *Listeria monocytogenes*, pathogen, serotypes, ready-to-eat food, ISO 11290-1:2017

O34

ENTEROTOXIGENIC STAPHYLOCOCCUS STRAINS ISOLATED FROM RAW MILK AND DAIRY PRODUCTS FROM R. N. MACEDONIA

Marija Ratkova Manovska*, MirkoProdanov, Dean Jankuloski,
Pavle Sekulovski, Katerina Blagoevska

Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Food institute, Lazar Pop – Trajkov 5-7, 1000 Skopje, North Macedonia

Staphylococci are ubiquitous microorganisms, present on various surfaces in the environment, animals, humans as well as food products. *Staphylococcus aureus* (*S. aureus*) is the most important pathogen representative of the genus. It's virulent and toxic properties are primarily due to the possession of a combination of genes that produce toxins, biofilm, invasive components and antibiotic resistance. The ability of the strains to produce one or more staphylococcal enterotoxins (SEs) in food, is associated with staphylococcal food poisoning. The occurrence of *S. aureus* food intoxications is usually listed as the third or fourth most common foodborne intoxications worldwide. For the purposes of this research, 333 samples of milk and 1160 samples of dairy products were analyzed in the period from 2016 to 2020. The strains were isolated and confirmed according to ISO 6888-1:1999 "Horizontal method for enumeration of coagulase positive staphylococci". Molecular analyzes of the isolates done with the conventional PCR method included: detection of the 23s gene of *S. aureus*, *nuc* gene, *mecA* gene and detection of 11 genes for the production of enterotoxins (*sea, seb, sec, sed, see, seg, seh, sei, ser, sej* and *sep*). 23s gene was detected in 93 (75.6%) out of 123 isolates of *Staphylococcus spp*. from milk. Out of 76 isolates from dairy products, *S. aureus* or 23s gene was detected in 49 of them (64.5%). *MecA* gene was detected in 3 isolates from raw milk and 5 isolates from cheese samples. The *nuc* gene was detected in 98.9% and 97.9% in *S. aureus* strains from milk and dairy products, respectively. Other staphylococcus strains carried the *nuc* gene in strains from milk 26.7% and dairy products 14.8%. SEs production genes were detected in 85 (69.1%) strains from milk and 38 (50%) from dairy products. In this research presence of 10 out of 11 SEs genes were detected, while no isolate carrying *see* gene was detected. The most prevalent detected genes were *seg* and *sei*, while their number per isolate ranged up to 5 genes. These results indicate the presence of enterotoxogenic strains of staphylococci in the tested samples, which imposes the need to take proper sanitation and hygienic practice, use of safe raw materials, adequate handling of the finished products and the need for further monitoring of the condition with SEs in order to have safe food and prevention of intoxication.

Key words: dairy products, milk, *Staphylococci*, enterotoxins, SE genes

O35

ANTIBACTERIAL ACTIVITY OF GOAT WHEY WITH ADDED KEFIR GRAINS

Sandra Mojsova[1]*, Vesna Levkov[2], Ljupco Angelovski[1]

[1]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine – Skopje, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia
[2]Ss. Cyril and Methodius University in Skopje, Institute of animal science, Bul. Ilinden 92A, 1000 Skopje, North Macedonia

Goat whey is a by-product from the cheese industry that has a rich nutritional content which plays important role in the organism physiology. Kefir is a unique fermented dairy product with well recognized probiotic property that contains lactic acid bacteria, acetic acid bacteria and yeasts. The professionals working in the dairy industry must ensure the food safety of the novel fermented products but also having in mind the health benefits of the final consumers. The present study aimed to analyze the antimicrobial activity in situ of the two different goat wheys (whey sample A, whey sample M) and two combinations of whey and kefir with different concentrations of kefir grains (5%, 8%). The chemical composition of the whey and the whey and kefir combinations were also analyzed because numerous research studies have shown that greater the nutrient content of the food, the lower the antibacterial activity seen. The whey and kefir combinations were inoculated (contaminated) with two different concentrations of *S. aureus, E. coli and L. monocytogenes* (10^5 and 10^8 cfu/ml). The inoculated samples were kept at 4-8°C. Antimicrobial activity was monitored at 0, 24, 48 and 72 hours of incubation by plating on selective media suitable for the pathogens inoculated. The summarized results showed promising potential, meaning that antimicrobial activity of whey and kefir combination was confirmed in both concentrations. We noted better activity towards the tested microorganisms in the combination with higher content of kefir (8%).In these samples the survival rate were lower in comparation with the samples smaller content of kefir (5%).

Key words: whey, kefir, antibacterial activity, *S. aureus*, *L. monocytogenes*, *E. coli*

O36

BACTERIAL CONTAMINATION IN DIFFERENT STAGES OF POULTRY SLAUGHTERING PROCESS

Mirko Prodanov*, Marija Ratkova Manovska, Sandra Mojsova,
Ljupco Angelovski, Dean Jankuloski

Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine – Skopje, Food institute, Department of food safety and veterinary public health, Lazar Pop Trajkov 5-7, 1000 Skopje, North Macedonia

Initial bacterial contamination on the broiler carcass can drastically influence the shelf life of the fresh poultry meat. A study was performed on the contamination of the broilers at 5 different stages in the slaughtering process: After plucking, before evisceration, after evisceration, after fine plucking (and spray washing), and after washing. From each stage 5 samples from the neck skin were taken and tested for total viable aerobic count (TVC), Enterobacteriaceae and *Salmonella* spp. For TVC the biggest contamination increase was observed after evisceration process log 5,23 or increase of log 0,71 from the previous stage. For Enterobacteriaceae there was significant increase in counts up to fine plucking (and spray washing) stage. For both TVC and Enterobacteriaceae the count dropped after the washing stage, log 0,66 and log 0,67 respectively. *Salmonella* spp. was not detected in any of the processing stages. From the obtained results, it is evident of the importance of the washing stage in the slaughtering process of broilers for maintaining low bacterial count in the fresh poultry meat. However, it is important to maintain good HACCP practices in all of the production stages, in order to deliver a safe poultry product to the market that will keep the quality throughout the predicted shelf life.

Key words: broiler, slaughterhouse, bacterial contamination, food hygiene

O37

OCCURRENCE OF AFLATOXINS, OCHRATOXIN A AND ZEARALENONE IN POULTRY FEED

Ida Kucinoska*, Biljana Stojanovska-Dimzoska, Aleksandra Angeleska, Katerina Blagoevska, Gordana Ilievska, Dushica Koceva, Elizabeta Dimitrieska-Stojkovic

Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Food institute, Lazar Pop Trajkov 5-7, 1000 Skopje, North Macedonia

Mycotoxins are toxic substances synthesized by fungi when found in specific conditions. They can be inhaled, absorbed through skin or consumed through food or feed making them a health risk for animals and humans as well. There are known to be around 400 mycotoxins. However, the most common and alarming are aflatoxins (B_1 B_2 G_1 and G_2), ochratoxin A and zearalenone because of their presence in animal feed and food. These secondary metabolites are known for their cancerogenic, teratogenic, nephrotoxic and immunotoxic effects. To prevent a possible mycotoxin poisoning, worldwide organization have established a maximum residue limit ,,MRL" from 20 μg/kg to 3000 μg/kg for all mycotoxins and a separate maximum permitted level for every mycotoxin accordingly. The most renowned method for quantitative determination of aflatoxins, ochratoxin A and zearalenone is high performance liquid chromatography with fluorescence detector ,,HPLC-FLD", using immunoaffinity column for clean-up because of its accuracy, efficiency, sensitivity and specificity. In interest of this study, poultry food was analyzed for aflatoxins, ochratoxin A and zearalenone content. Total of 86 samples were collected and studied for the presence of these particular mycotoxins. For the extraction and purification, immunoaffinity columns were used, specifically for each mycotoxin. Three different HPLC-FLD methods were performed for their quantification accordingly to the Association Of Official Analytical Chemists ,,AOAC" and International Organization For Standardization ,,ISO" standards and the results were interpreted in line with Macedonian and European Regulations. The samples showed relatively low concentrations of all the suspicious mycotoxins. For aflatoxins, 79 of the collected samples (92%) showed concentrations bellow the limit od detection ,,LOD" while the others 8% (7 samples) were within concentration range of 0.52 – 1.71 μg/kg. Ochratoxin A was found in concentrations between 0.2 - 4.15 μg/kg in 24 samples (29%), while the other 71% (62 samples) had concentrations below LOD. For zearalenone, 74 samples (86%) showed concentrations below LOD and in the other 14% (12 samples) had concentration ranged from 2.6 μg/kg to 30.31 μg/kg. Ultimately, the amount of each mycotoxin in the samples wasn't alarming and there were no positive results, however further caution is advised to ensure future food and feed safety.

Key words: aflatoxins, ochratoxin A, zearalenone, poultry food, HPLC-FLD

O38

PRESENCE OF HEAVY METALS IN ANIMAL FEEDS OF PLANT ORIGIN

Dushica Koceva*, Elizabeta Dimitrieska-Stojkovik,
Biljana Stojanovska-Dimzoska, Aleksandra Angeleska

Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Food institute, Lazar Pop Trajkov 5-7, 1000 Skopje, North Macedonia

Heavy metals can be accumulated into meat, egg, milk and their products through contaminated animal feeds, which can cause a number of hazards to animals and humans as well. Therefore, their control in the food chain is an important issue in terms of animal welfare and public health. This paper reports on the presence of four heavy metals, lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) and their levels in plant-based animal feeds used in the territory of Republic of North Macedonia. In total 121 different types of animal feeds of plant origin for pigs, lambs, cattle and poultry were collected from local wholesale and feed producers. The content of contaminants of concern was analyzed using electrothermal atomic absorption spectroscopy (ETAAS) for detection of Pb, Cd, and As, as well as cold vapor atomic absorption spectrometry (CVAAS) for determining Hg in the selected samples. The trend for average concentrations of toxic elements found in the studied feed samples was as follows: Pb>Cd>As>Hg, except in cattle feeds where Cd was found to be present in lowest average concentration. On the other hand, the Hg mean content (0.038±0.027 mg/kg) was detected to be higher corresponded to one of As (0.014±0.018 mg/kg) in lamb feeds. The results obtained in this research has showed that analyzed samples do not pose a risk to animal and human health. However, as preventive measure continuous monitoring of heavy metals in animal feeds should be conducted in order to ensure that these toxic elements are not passed into edible food products, thus providing safety in the food chain.

Key words: heavy metals, pig feeds, lamb feeds, cattle feeds, poultry feeds

O39

PROPOSED CHEMICAL SUBSTANCES AS QUALITY PARAMETERS OF CHOCOLATE

Stefan Jovanov*, Risto Uzunov, Tome Nestorovski, Zehra H. Musliu, Velimir Stojkovski

Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Lazar Pop Trajkov 5-7, 1000 Skopje, North Macedonia

Rich, unique and sweet taste is what makes chocolate one of the favorite and commonly used food. Ingredients such as cocoa, fats, milk and sugar play an important role in chocolate's quality. In this study we propose several chemical indicators as parameters for chocolate's quality. A total of 24 samples were analysed, 5 declared as dark chocolates, 16 milk chocolates and 3 composite chocolates. Naturally present substances like methylxanthines, caffeine and theobromine, are used to estimate the amount of cocoa in chocolate. These substances were determined using HPLC-DAD method with isocratic elution with PBS 12.5 mM pH=3.5: ACN (90:10 *V/V*) and RP-C18 column. Same HPLC method was also used for the analyzing the presence of artificial sweeteners, aspartame, Na-saccharin, acesulfame-K and the preservatives, Na-benzoate and K-sorbate. The results showed that none of the analyzed samples contained these substances. The concentration range of caffeine and theobromine were from 9.94 mg/L to 2129.2 mg/L and from 473.20 mg/L to 5565.6 mg/L respectively. Composite chocolates contained the lowest amount of methylxanthines, while the dark chocolates had the highest. Fatty acids composition was determined as methyl esters using GC-FID with capillary column. These results indicated the content of dairy fat, cocoa butter or other vegetable fat, like palm or coconut oil, in the total fat content of the samples. Dark chocolates contained only cocoa butter, the dairy fat in milk chocolates and composite chocolates ranged from 8.23% to 19.57% and from 5.74% to 18.55% respectively, while the rest of the fat was cocoa butter. Two milk chocolate samples were dairy fat free, while the total fat content in one of the samples consisted from 92.11% palm oil and 7.89% coconut oil, in the other sample was determined 47.23% cocoa butter and 52.77% coconut oil. Present sugars, sucrose and lactose, were examined with HPLC-RID method using a commercial amino-column. All milk and composite chocolates contained lactose from 4.98% to 13.70% which is indication for used milk or milk powder, while none of the dark chocolates contained lactose. The concentration range for saccharose for all samples was from 29.82% to 59.51%, and it is not correlated to the type of the chocolate.

Key words: chocolate, food quality, methylxanthines, fatty acids, sugars

Scientific session VIII
INFECTIOUS DISEASES IN ANIMALS

Oral presentations

O40

DISCOVERY AND INTRODUCTION PATTERN OF PANZOOTIC NEWCASTLE DISEASE VIRUS IN CHICKENS IN MACEDONIA, 2020

Aleksandar Dodovski[1*], Renfu Yin[2]

[1]Department for Avian Diseases, Faculty of Veterinary Medicine – Skopje, Ss. Cyril and Methodius University in Skopje
[2]State Key Laboratory for Zoonotic Diseases, Key Laboratory of Zoonosis Research, Ministry of Education, College of Veterinary Medicine, Jilin University

Newcastle disease virus (NDV), also designated as avian orthoavulavirus type-1 (AOAV-1), is a worldwide concern for poultry production. It is endemic in many countries and can cause high mortality even in vaccinated poultry populations. The disease it causes is Newcastle disease (ND) which is an OIE listed and category A disease in the EU. An outbreak of ND occurred in Macedonia during the period April - May 2020. One commercial holding vaccinated against NDV and two non-vaccinated backyard holdings were affected. Submitted carcasses from all three holdings were subject to a post-mortem examination and representative tissue samples were taken and tested by nucleic acid detection methods. The clinical signs in all three holdings were associated with acute septicaemic disease and vascular disturbance. Main gross pathology findings were predominantly found in the respiratory system without characteristic lesions in the digestive system. Molecular tests revealed that the virus was virulent with the polybasic cleavage site of the fusion (F) gene 113-RQKR*F-117. The complete F gene sequence from holding 1 was deposited in GenBank under accession number MT424733. Based on the phylogenetic analyses of the complete F gene virus was grouped in class II, genotype VII.2 of the AOAV-1 and groups together with viruses collected between 2013 and 2019 in Turkey, Libya, and Bulgaria (similarity range: 98.2%-98.8%). The last major outbreak of NDV in Macedonia was in 2005-2006 of genotype VII.1.1. Although, the epidemics in 2005-2006 and 2020 were of different genotypes and 15 years apart they have similar introduction patterns. In both cases, the most similar viruses were previously detected east of the country suggesting a westward movement of virulent ND viruses in the region. However, the means of introduction remain unknown which may be either via wild birds, fomites, or trade and mass vaccination of commercial poultry make the situation more complicated. Moreover, field strains of genotype VII.2 were able to cause disease in vaccinated flocks as in this outbreak. These viruses easily infect chickens and other bird species and are able to spread rapidly. The above characteristics and the consequences of the epidemics of ND caused by this genotype suggest that viruses of genotype VII.2 have panzootic potential. Considering that the Macedonian strain was a highly virulent virus, in addition to updated and multiple vaccinations, strict biosecurity measures are needed to prevent future outbreaks.

Key words: Newcastle disease, avian orthoavulavirus 1, chickens, genotype VII.2

O41

OVINE PARATUBERCULOSIS: INTRA-HERD INCIDENCE STUDY AND ASSESSMENT OF COMMERCIAL (ID.VET, FRANCE) INDIRECT MILK ELISA TEST PERFORMANCE IN DAIRY SHEEP

Anna-Rita Attili[1], Eleonora Bonacucina[2], Martina Linardi[1], Luciana Pacifici[3], Vincenzo Cuteri[1]*

[1] University of Camerino, School of biosciences and veterinary medicine, 62024 Matelica, Italy
[2]Freelance veterinary practitioner, Via Monte D'Aria 12, 62022 Castelraimondo (MC), Italy
[3]Official veterinary, ASUR, Area Vasta 3, 62100 Macerata, Italy

Ovine paratuberculosis is chronic disease affecting domestic and wild ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). The infection causes significant economic losses worldwide and was associated to several human diseases as well. Until now, no confirmatory MAP screening technique revealing the disease stages in infected animals has been developed. This is mainly due to the lack of an efficient *gold-standard* method that can properly evaluate the performance of diagnostic assays. With this aim, from October 2019 to April 2020 an intra-herd cross-sectional and prospective incidence studies were carried out in two flocks with and without history of disease. To assess (i) the blood and milk MAP individual and bulk-milk seroprevalence by using an indirect milk ELISA (ID Screen® Paratuberculosis-Indirect Screening-ELISA test; ID.vet France); (ii) the incidence rate in a 6-month period; (iii) the faecal-excretors by qPCR IS900; and (iv) the performance and the accuracy of the ELISA test used, 200 paired serum, milk and faecal samples were collected. The 150 animals from positive flock were subdivided in three groups related to their age (young: ≤6 months, adult: <24 months and elder: ≥than 24 months). Blood seroprevalence of 11.33% and 14% were detected at the start of the study (T_0) and after 6 months (T_6) on the same sheep respectively, recording an incidence rate of 3% and a significant increase in the mean serum antibody concentration in the Elders group (S/P%: $p = 0.0005$). An 8% (T_0) and 10.67% (T_6) milk seroprevalence, and an incidence rate of 7.1%, with a significant increase in the mean antibody concentration, were recorded in Elders group (S/P%: $p<0.0001$). Comparing blood and milk seroprevalences, no statistically significant differences were observed at T_0 ($p=0.3003$) nor at T_6 ($p=0.5119$). In the positive flock, 29 (19.33%) sheep was detected positive for MAP faecal excretion at T_0: 3 animals (10.34%, n=29; mean Ct=22.23) as strongly and 26 (89.65%, n=29; average Ct=36.07) as weakly positive. The strongly faecal-excretory sheep (2% at T_0 and 3.33% at T_6; n=150) were in all three age groups and resulted positive to both, blood and milk ELISAs, except one sheep at T_6. The bulk-milk analyses revealed that MAP positivity has a seasonal pattern, linked to the lactation stage. Considering the blood ELISA test and the qPCR (strong excretory) as *gold-standard* for

MAP diagnosis, the used milk ELISA obtained an '*Almost perfect agreement*' (k=82.6%) at T_0 and a '*Substantial agreement*' (k=70.0%) at T_6, suggesting a new appropriate cut-off for milk in dairy sheep (ROC curve analysis: >21.38 S/P%; Se>80% e Sp>98%). This study suggests the possible use of milk matrix for MAP screening in dairy sheep, and a new appropriate cut-off value for ovine milk testing.

Key words: ovine paratuberculosis, milk ELISA performance, qPCR IS900, cut-off value

O42

ZOONOTIC POTENTIAL OF EQUINE-ASSOCIATED VIRUSES

Mihajlo Erdeljan*, Tijana Kukurić, Ivan Stančić, Ivana Davidov, Miodrag Radinović, Annamaria Galfi Vukomanović, Nadežda Tešin

University of Novi Sad, Faculty of agriculture, Department for veterinary medicine, Tositeja Obradovica square no. 8, 21000 Novi Sad, Serbia

Many animal viruses have a zoonotic potential. Although on the first sight horses and humans have not much in common, still they are hosts to a great number of viruses that can affect both species. Recent studies suggested that horses and humans share similar receptors for the viral spike proteins, such as dipeptidyl-peptidase-4 (DPP-4) of MERS-CoV virus. Most of those viruses are transmitted by vector insects but they can also be transmitted by direct contact, particularly important for veterinarians and animal keepers. The list of equine-associated viruses with zoonotic potential is quite long, and here we presented the most important: West Nile virus (WNV), Hendra virus (HeV), Nipah virus (NeV), Eastern equine encephalitis virus (EEEV), Venezuelan Equine Encephalitis virus (VEEV), Western equine encephalitis virus (WEEV), Japanese encephalitis virus (JEV), Vesicular stomatitis virus (VSV) and Influenza virus. In this paper work we will discuss their epidemiology, prevalence, vectors and, as the most important, their zoonotic potential. Above all, it is also important to say that all this viruses have the death potential for hosts, horses and humans. Although most of them are endemic, the climate changes, world trade and commerce, and worldwide travel can easily transmit these viruses globally. The main goal of this paper work is to emphasis the potential of these viruses to infect humans in light of the one health approach.

Key words: horses, humans, zoonotic viruses

O43

RE-EMERGENCE OF RABIES IN BOSNIA HERZEGOVINA AFTER THE OFFSET OF ORAL RABIES VACCINATION

Eterović Toni*[1], Lejla Velić[1], Oliver Stevanović[2], Amer Alić[3]

[1]University of Sarajevo, Veterinary faculty, Veterinary institute, Laboratory for serology and virology, Zmaja od Bosne 90, 71000 Sarajevo, Bosnia and Herzegovina
[2]Veterinary institute of Republic of Srpska "Dr Vaso Butozan", Branka Radičevića 18, 78000, Banja Luka, Bosnia and Herzegovina
[3]University of Sarajevo, Veterinary faculty, Department for pathology, Zmaja od Bosne 90, 71000 Sarajevo, Bosnia and Herzegovina

A new case of rabies in Bosnia and Herzegovina has been detected after more than six years of absence. The case of a non-vaccinated dog with clinical signs suggestive of rabies was reported near the Serbian border in municipality of Srebrenica. The animal was euthanized on 28th May 2020 and laboratory confirmation was made one day later using FAT, and RT-PCR. Additionally, the virus has been isolated on N2a cell culture. Genetic characterization of the rabies virus strain showed the sample grouped within the sub-group WE formed by Western European RABV samples. We presume contact with infected wildlife. Due to COVID pandemic, oral rabies vaccination (ORV) stopped in 2019 and the next planned campaign was delayed and started in late 2021, while active surveillance is planned during 2022. In the same period passive surveillance was decreased as well. This case indicates that despite of large-scale ORV campaigns rabies in Bosnia and Herzegovina is not yet eliminated. In recent years the number of rabies testing was low, and the necessity of enhanced surveillance of wildlife in Bosnia and Herzegovina and neighboring Serbia is of utmost importance.

Key words: rabies virus, oral rabies vaccination, genotyping, molecular epizootiology

O44

DETECTION AND GENETIC CHARACTERISATION OF PORCINE CIRCOVIRUS TYPE 2 IN MACEDONIAN WILD BOAR AND DOMESTIC PIG POPULATION

Zagorka Popova Hristovska, Kiril Krstevski*, Ivan Matevski,Stefanija Markozanova, Dine Mitrov, Igor Djadjovski

Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia

Porcine circovirus type 2 (PCV-2), represents one of the most significant pathogens for the pig industry. First isolated in Canada in the mid-1990s, from pigs with post-weaning multisystemic wasting syndrome (PMWS), it was soon reported worldwide. PCV-2 has been associated with several syndromes including reproductive disorders, the so-called porcine respiratory disease complex (PRDC), enteritis, porcine dermatitis and nephropathy syndrome (PDNS), and the proliferative and necrotizing pneumonia (PNP). Characteristically for the ssDNA viruses, PCV-2 demonstrates a remarkable evolutionary rate resulting in the development of significant genetic heterogeneity. The aim of this study was to confirm the presence of PCV-2 using molecular diagnostic methods, to estimate the prevalence in domestic and wild boar population, as well as to determine the genetic characteristics of the Macedonian PCV-2 isolates. Spleen samples from 582 domestic pigs, and 658 wild boars collected during the 2020 and 2021, in the frame of the activities for passive and the active surveillance on African and Classical Swine Fever, were tested for the presence of the PCV-2 nucleic acid using the Real-Time PCR method. The samples from domestic pigs originated from nine commercial farms, while the wild boar samples were selected from multiple sites equally distributed over the whole territory of the country. The observed prevalence among the wild boars was 5.01% (33 out of 658), while the prevalence in domestic pigs was 11.86% (69 out of 582). Of the nine farms included in the study, the presence of the PCV-2 was confirmed on seven farms (77.77%). Phylogenetic analysis of the complete genomic sequences, from seven Macedonian PCV-2 isolates, classified them in the PCV-2d genotype. This study presents the first confirmation of the PCV-2 in the Macedonian wild boars and determines the genetic characteristics of the Macedonian PCV-2 isolates. Widespread distribution of the virus in the wild boar population indicates the endemic character of the disease and presents a constant threat for spillover transmission to domestic pigs. On the other hand, the high percentage of infected commercial farms suggests the lack of preventive, control, and eradication measures and possible weaknesses in implementation of the biosecurity protocols on the farms. The genetic characterization reveled close relationship of Macedonian PCV-2 isolates with the genotype, recently emerged in Europe. Therefore, a more in-depth analysis of a larger number of PCV-2 isolates, preferably covering a longer time span, is required to better understand the epidemiology and ecology of the PCV-2 circulating in Macedonian pig population.

Key words: porcine circovirus type 2, Macedonian, genetic characterization, wild boar, domestic pig

O45

PRESENCE AND PREVALENCE OF NON-REGULATED INFECTIOUS DISEASES WITH HIGH ECONOMIC IMPACT ON MACEDONIAN DAIRY FARMS

Ivan Matevski*, Igor Djadjovski, Kiril Krstevski

Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Veterinary institute, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia

The occurrence of infectious diseases has a negative impact on the productive and reproductive performances of dairy cattle, leading to significant economic losses for the farmers. The list of most important economically relevant diseases not covered with national legislation for animal disease control (here referenced as *non-regulated diseases*) includes infectious bovine rhinotracheitis (IBR), Q-fever, *chlamydial infections (*Chlamydiosis*)*, bovine viral diarrhea (BVD) and enzootic bovine leukosis (EBL). To better understand the presence and prevalence of these diseases, and their correlation with the biosecurity status of the holdings, a limited serological study was conducted on thirteen Macedonian dairy farms. The farms were grouped in three categories according the size (small, less than 30 animals; medium, 30-50, and large, more than 50 animals) and the biosecurity status (weak, moderate and good). In total, 368 bovine serum samples were collected and tested for the presence of specific antibodies using the ELISA method. Presence of at least one disease was confirmed on all tested farms, with Chlamydiosis as the most prevalent (71.4% of the farms infected), followed by BVD (66.7%), EBL (53.8%), IBR (50%) and Q-fever (46.2%). Within-herd prevalence of the diseases varied across the farms and was in following minimum-maximum ranges: Chlamydiosis (8.3%-42.4%), BVD (11.1%-61.9%), EBL- (1.3%-71.4%), IBR (1.3% - 44.7%), Q-fever (11.1% - 58.3%). In general, the biosecurity status correlated to the farm size, with large farms being assigned with high, mid-sized with moderate and small farms with low biosecurity status. Interestingly, the highest number of diseases (3 to 5) were observed on mid-sized farms characterized by moderate biosecurity status. . Overall, the study results revealed a wide presence and high prevalence of tested diseases on Macedonian dairy farms. None of the farms in the study (including the farms with highest biosecurity status), was free from all five tested diseases.. Moreover, the results highlighted the unfavorable health status of the mid-sized dairy farms, which were not only identified as a significant risk for maintenance and further spread of the diseases but also as a serious economic problem. All this require urgent actions in order to improve the health status and economical sustainability of the Macedonian dairy farms. Taking into account that these are non-regulated diseases, the focus should be on better organization and education of the farmers, and improved collaboration with relevant national institutions towards implementation of appropriate, scientifically proven solutions that will lead to achievement of the previously mentioned goals.

Key words: cattle, infectious bovine rhinotracheitis (IBR), Q-fever, chlamidiosis, bovine viral diarrhea (BVD), enzootic bovine leucosis (EBL)

Scientific Session IX
METHODS AND MODELS

Plenary lecture and Oral presentations

PL7

VIRTUAL SLAUGHTERHOUSE SIMULATORS AS A COMPLEMENT, NOT A SUBSTITUTE, OF ABATTOIR VISITS IN VETERINARY PUBLIC HEALTH EDUCATION

Andrej Kirbis[*1], Brian Mather[2], Alessandro Seguino[2]

[1]*University of Ljubljana, Veterinary faculty Gerbičeva ulica 60, 1000 Ljubljana, Slovenia*
[2]*University of Edinburgh, The royal (Dick) school of veterinary science, Easter Bush Campus, Midlothian EH25 9RG, United Kingdom*

The current and emerging challenges in Veterinary Public Health (VPH) resulting from globalization, climate change, industrialization of food production, and crises such as the COVID-19 pandemic and wars require a parallel evolution of the role of the veterinarian and an adaptation of the veterinary curriculum to reflect these changes. Among the many topics covered by the VPH curriculum, the tasks performed by the official veterinarian in the slaughterhouse remains very important. As such, familiarity with ante-mortem and post-mortem inspection is one of the first day competencies that the veterinary graduate must master. Due to the decreasing number of slaughterhouses in Europe, which were highly affected by the COVID-19 pandemic, and due to the inflation of students in European veterinary schools, it is necessary to develop new tools that can be instrumental in facilitating the achievement of these first day competencies. One of these tools is the virtual slaughterhouse simulator, developed at the University of Edinburgh. This is a computer-based simulation that helps students to familiarize themselves with the technological process of slaughtering cattle, as well as the associated ante-mortem and post-mortem inspection of slaughtered animals. The virtual slaughterhouse proved to be a very important tool during the COVID-19 pandemic, when visits to slaughterhouses were practically impossible. Many European veterinary faculties reported positive experiences with the use of the virtual slaughterhouse. However, it has its limitations, as it only deals with the slaughter of cattle. Therefore, it was necessary to update the platform. This was done within the European UNA Europa Seed Funding project, the result of which is the development of a virtual pig slaughterhouse. In the future, it is planned to develop a virtual slaughterhouse for all slaughtered animal species. However, is important to point out that the virtual slaughterhouse can in no way replace the practical work of the students in the slaughterhouse, but can be used as a complementary tool in the educational process and help students to achieve first day competencies in VPH.

Key words: Veterinary Public Health (VPH), virtual slaughterhouse simulator, veterinary curriculum

O46

EVALUATION OF THE PERFORMANCE OF A REAL TIME PCR METHOD FOR DETECTION OF *SALMONELLA* SPP. *inv*A GENE IN CHICKEN MEAT

Gordana Ilievska*, Marija Ratkova Manovska, Dean Jankuloski, Katerina Blagoevska

Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Food institute, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia

Salmonella spp. is considered one of the leading pathogens that is causing foodborne infections worldwide. In order to minimize the health risks for consumers, it is essential for laboratories to implement reliable food pathogen testing. Standard microbiological methods for detection of *Salmonella* spp. in food are laborious and time-consuming, contrary to the molecular techniques, based on the detection of nucleic acids that are faster, specific and more sensitive. This study describes the validation of a real-time PCR assay, based on the Taq-Man technology, which amplifies the target sequence (*invA* gene) from *Salmonella* spp. as well as an internal PCR control to monitor inhibitory effects. The limit of detection (LOD) was determined by testing artificially contaminated chicken meat with 10-fold dilutions of *Salmonella* Enteriditis. Two laboratory practitioners tested each of these samples in six replicates. Pre-enrichment in Buffered peptone water was employed before DNA extraction. The LOD of the assay was 1-10 CFU of *Salmonella* spp. in 25g chicken meat. The method has proven to be specific, with 0% false positive results from *Salmonella* negative samples contaminated with several other pathogens (*E. coli*, *L. monocitogenes*, *Y. enterocolitica*, *S. aureus*). Additionally, the selectivity of the assay was confirmed by 100% amplification of the target *invA* primer in samples contaminated with six different strains of *Salmonella* spp. (*S.* Enteritidis, *S. diarizone*, *S.* Typhimurium, *S.* Branderup, *S*. Infantis and *S*. Virchow). In general, the presented assay is a reliable, fast, sensitive and specific method that can be used in everyday laboratory practice for screening of *Salmonella* spp. in chicken meat. The overall analysis time of the presented real-time PCR method was approximately 28 hours, opposed to 4-5 days needed to perform the traditional methods of *Salmonella* spp.

Key words: *invA* gene, Salmonella spp., real-time PCR, chicken meat

O47

IDENTIFICATION OF MEAT SPECIES BASED ON DNA HYBRIDIZATION, REAL-TIME PCR AND FATTY ACID PROFILE WITH GC-FID

Dafina Mehmetukaj*[1,2], Vlora Zogëjani[2], Armend Cana[2,3], Xhavit Bytyçi[3], Besart Jashari[1,2], Zehra Hajrullai Musliu[1], Katerina Blagoevska[1], Dean Jankuloski[1]

[1]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine – Skopje, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia
[2]Food and veterinary agency, Kosovo food and veterinary laboratory, Industrial Zone, Fushe Kosove, 10000 Prishtine, Kosovo
[3]University of business and technology, Kalabria, 10000 Prishtine, Kosovo

Processed-meat products are highly susceptible targets for economically motivated fraud, as well as incidental adulteration along the food processing chain. Replacements of beef meat with cheaper ones like pork, horse, or chicken meat, are common. We aimed to investigate different aspects of adulteration and its reflection in cattle sausage and cattle salami, by using molecular methods and determination of fatty acid profiles. DNA was extracted with the DNeasy Mericon Food Kit, Qiagen®. PCR/DNA hybridization with LCD-Array Kit MEAT 5.0, Chipron®, was used for the detection of the DNA of 24 species: cattle, sheep, equine, goat, camels, buffalo, pork, kangaroo, hare, rabbit, reindeer, roe deer, red deer, springbok, canine, cat, chicken, turkey, goose, ostrich, mallard duck, Muscovy duck, pheasant. Real-time PCR for the detection of chicken, pork, and horse/donkey DNA was performed by using foodproof® SL Chicken DNA and DNA Halal Screen Eurofins®. All samples were tested with GC-FID to determine the fatty acid profile, to substantiate/ quantify the possible adulteration with chicken meat. A total of 16 sausages and 4 salamis were tested. All samples were positive for cattle DNA. Fifteen out of 20 samples were positive for chicken DNA in the LCD Array, whereas 16 were positive on real-time PCR. Sheep and turkey DNA was detected in one sample each. No other species' DNA was detected. In total, 3 sausages and 4 salamis reach the quantification limit of linoleic acid > 7%, indicating a substantial amount of chicken meat. There was a significant difference in mean % in ANOVA single factor test for stearic acid between samples that were positive (mean 17.01%) and negative for chicken DNA (mean 25.31%), $p = 0.007$. Linoleic acid, which appears to be indicative of chicken meat presence at first glance, failed to show statistical significance in the ANOVA test, $p = 0.13$, chicken positive (mean 9.9%) to negative ones (mean 2.7%). However, in only chicken positive samples that reach the quantification limit of linoleic acid, the difference is significant $p < 0{,}001$ with a mean value of 19% versus 2.7%. Current investigation shows that the adulteration of beef processed meat is with chicken mostly. Four out of 12 sausages positive for chicken DNA, had a substantial amount of chicken meat. The rest represent an incidental adulteration. In salami, the presence of a certain amount of chicken meat is declared. Moreover, we exclude the presence of more than 20 species in tested samples.

Key words: meat, hybridization, fatty acid, sausage, salamis

O48

DIAGNOSTIC PERFORMANCE OF EIGHT PCR PROTOCOLS AND ONE INDIRECT ELISA IN NATURALLY SMALL RUMINANT LENTIVIRUSES INFECTED EWES IN GREECE

Aphrodite I. Kalogianni*[1], Ioannis Bossis[2], Ilias G. Bouzalas[3], Athanasios I. Gelasakis[1]

[1]Agricultural University of Athens (AUA), School of animal biosciences, Department of animal science, GR 11855 Athens, Greece
[2]Aristotle University of Thessaloniki (AUTh), School of agriculture, forestry and natural resources, Department of agricultural sciences, GR 54124 Thessaloniki, Greece
[3]Veterinary Research Institute (VRI), Hellenic Agricultural Organization-DEMETER, GR 57001 Thermi,Thessaloniki, Greece

The objective of this study was to evaluate the diagnostic performance of eight conventional PCR protocols and one indirect ELISA in naturally Small Ruminant Lentiviruses (SRLVs) infected ewes in Greece. Hence, a total of 100 adult dairy ewes from five intensive maedi-visna positive sheep farms were randomly selected and enrolled in the study. For each individual animal, a serum and a whole blood sample were collected. The serum samples were used for the detection of SRLV-specific antibodies with a commercial indirect ELISA kit (CAEV/MVV Total Ab Test, IDEXX), while whole blood samples were used for the genomic DNA extraction from peripheral blood leucocytes pellets with a commercial kit (PureLink® Genomic DNA Kit, Life technologies corp). Afterwards, thirteen different sets of primers in five nested and three simple PCR protocols were used for the amplification of fragments in the pol, gag, env, and LTR regions of SRLVs' genome. Total seroprevalence at the animal level was 62.0%. Additionally, 16 animals were found positive exclusively with PCR methods. In total the prevalence of SRLVs infections based on PCR protocols was 74.0%. When both the results of ELISA and PCR protocols were considered, overall prevalence increased to 78.0%. Sensitivity, specificity, and kappa coefficient value (k-value) of all PCR protocols and ELISA were calculated using as "gold standard" a positive result in both ELISA and at least one of the utilized PCR protocols. A set of primers failed to produce any positive result and therefore, was considered as inappropriate and its diagnostic performance was not evaluated. Sensitivity, specificity, and k-value for the ELISA was 100.0%, 95.0% and 0.958, respectively. Sensitivity values for the seven PCR protocols ranged from 11.7% for the nested LTR protocol to 60.0% for the two nested gag and gag-pol protocols, whereas specificity values ranged from 72.5% for the nested gag protocol to 97.5% for the simple and nested LTR protocols. K-value for all PCR protocols ranged from 0.075 for the nested LTR protocol to 0.483 for the nested gag-pol protocol. When the results of all PC R protocols were jointly considered, sensitivity, specificity, and k-value were increased to 96.7%, 60.0% and 0.602, respectively. Our results confirm the demand for complementary use of ELISA and case-specific designed PCR protocols for the early and accurate detection of SRLVs infected animals.

Key words: small ruminant lentiviruses, diagnosis, ELISA, PCR, diagnostic performance

Acknowledgments: The research work was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "First Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant" (Project Number: HFRI-FM-17-1083).

O49

SUITABILITY OF THE ISO 13730:1996 METHOD FOR TOTAL PHOSPHOROUS QUANTIFICATION IN MILK AND MILK PRODUCTS

Tome Nestorovski*, Stefan Jovanov, Riste Uzunov, Ana Angelovska, Zehra Hajrulai Musliu, Radmila Crceva Nikolovska

Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Food institute, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia

Phosphorous can be found naturally in food but due to his useful properties, is also used as an additive in the meat and dairy industry as an inhibiting, stabilizing and emulsifying agent. The quantification and monitoring of total phosphorous in food is of high importance for food safety because high and unregulated intake can lead to cardiovascular and kidney diseases. Modern spectroscopic approaches yield high accuracy in total phosphorus quantification and can be divided into two methods based on the reagents used. One being the "molybdenum blue" method and the other being the "vanadate/molybdate yellow" method. The goal of this study was to investigate if the vanadate/molybdate method, described in the ISO 13730:1996 is suitable for accurate quantification of phosphorous in milk and milk products. The Proficiency test results with assigned value for Sample 1 of 213.7 mg/100 g and Z - score of - 0.46 and assigned value for Sample 2 of 198.8 mg/100 g and Z - score of - 0.59. This was followed by verification of the method by determining the standard deviation (SD) for each sample series, the repeatability (r), and expanded uncertainty (U). Results confirmed that the ISO 13730:1996 method is suitable for accurate quantification of total phosphorus content in both meat and milk matrixes. This unification of both matrixes directly contributes to lowering the costs of the analysis, the time needed to perform the analysis, and having excellent precision in quantifying the total phosphorus content, thus increasing the scope of application.

Key words: phosphorus, quantification, spectroscopy, milk and milk products, meat and meat products

O50

VERIFICATION OF A SAMPLING METHOD FOR ANIMAL FEED FROM DIFFERENT ORIGINS INTENDED FOR CHEMICAL ANALYSIS

Ana Angelovska*, Radmila Crceva Nikolovska, Aleksandra Angeleska,
Tome Nestorovski, Dean Jankuloski, Mirko Prodanov, Zehra Hajrulai Musliu

Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Food institute, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia

Sampling is one of the most important steps for obtaining accurate and reliable testing results in the feed industry. The purpose of the representative sampling is to obtain a small fraction from a sample batch in such a way that the determination of any particular characteristic of this fraction is the mean value of the characteristics of the series (LOT). The method prescribes requirements for manual sampling of animal feed from different origin. The main purpose of its verification is to prove that there are no major deviations in sampling that would significantly affect the validity of the results obtained from further testing. For that purpose, two aggregate samples from several different types of animal feed, such as mineral blocks (from 7 series), whey (from 5 series), premix and dry alfalfa (from 10 different series each), were sampled from two trained operators. From each laboratory sample, two test samples were taken and were tested for multiple chemical parameters such as: phosphorus, minerals, moisture, and protein. Verification procedure was performed in accordance with Nordtest Nt Tr 604 edt.2 2020, Uncertainty from sampling. The measurement uncertainty was estimated by determining SD - standard deviation and CV - coefficient of variance (RSD) with two samples and double analysis by using range statistics. The results shown that the expanded measurement uncertainty for the different types of samples ranges from 1.14% to 7.64%, which suggests that the method is appropriate for its purpose.

Key words: verification, sampling method, animal feed, chemical analysis, measurement uncertainty

O51

CONTRIBUTION OF ANIMAL MODEL IN THE DEVELOPMENT OF RADIOPHARMACEUTICALS AND SUCCESSFUL TRANSLATIONAL MOLECULAR IMAGING AND THERAPY

Emilija Janevik-Ivanovska[1]*, Icko Gjorgoski[2], Lajos Balogh[3]

[1]Goce Delcev University - Stip, Faculty of medical sciences, North Macedonia
[2]University St Cyril and Methodius in Skopje,Faculty of natural sciences and mathematics, Institute of biology, North Macedonia
[3]Gamma-VET Ltd, Deak Ferenc utca 20, Budapest, Hungary

The personalized approach to patients with various diseases, especially those with malignant and infectious diseases, requires specific and validated biomolecules for diagnosis and therapy, including radiopharmaceuticals. Their development and validation need translational preclinical models that reproduce human disease as accurately as possible. Experimental animal models have substantially contributed to a better understanding of mechanisms of disease. Novel approaches in imaging and image analysis are equally important to meet the challenges of analyzing complex mechanisms underlying pathophysiological processes *in vivo*. The appropriate animal models are not only helpful in proving longstanding hypotheses for the development of new imaging agents but have rather been used for the discovery of new unexpected mechanisms and causal relationships. In (radio) pharmaceutical development, compound design and synthesis need to be followed by a succession of studies in the following order: in situ (stability), in vitro (affinity, cellular uptake, early toxicity), ex vivo (specificity, signal intensity), and in vivo studies in models of disease (specificity, pharmacokinetics, dosing and timing). There are also research initiatives for the repurposing and reformulation of existing radiopharmaceuticals. Many factors, such as identifying the most appropriate animal model, defining the necessary control groups, randomly assigning animals to control/treatment groups, determining the number of animals needed per group, assessing the logistics of actually performing the animal experiments, and identifying the most appropriate statistical analyses are crucial to designing an experiment that will generate scientifically valid and reproducible data, which should be considered the ultimate goal of any scientific research. When performing biodistribution studies in appropriate animal models following application of radioactive preparations, it is of particular importance to realize: pharmacokinetic studies and calculation of the PK/PD ratio as a predictive tool for the assessment of human pharmacokinetic parameters; calculation and prediction of the dose to be applied to patients; toxicological analysis for determining the toxicity of the used radiopharmaceuticals and distinguishing it from the radiotoxic effects that are the result of irradiation; dosimetric analysis for obtaining values of exposure to radioactivity of the whole body and critical. Based on our experience, we can conclude that animal models have a role in the development of new radiopharmaceuticals. For therapeutical

purposes they will remain the only source of information about their in vivo behavior and an indispensable link between in vitro and clinical studies.They can be of high value, although we should be aware that animal results are sometimes not fully applicable to humans due to inherent biological differences between species. Therefore, it is necessary to carefully monitor the application of those results, always accompanied by pharmacokinetic studies and dosimetric calculation of the applied radioactivity.

Key words: animal model, applied radioactivity, radiopharmaceuticals

O52

EVALUATION OF ANTIOXIDATIVE ENZYMES IN RATS TREATED WITH *ORIGANUM VULGARE* ESSENTIAL OIL DURING ACUTE INTOXICATION WITH DEOXINIVALENOL AND FUMONISIN B1

Gordana Ilievska*[1], Katerina Blagoevska[1], Biljana Stojanovska-Dimzoska[1], Elena Rafailovska[2], Biljana Miova[2]

[1]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Food institute, Lazar Pop-Trajkov, 5-7, 1000, Skopje, North Macedonia
[2]Ss. Cyril and Methodius University in Skopje, Faculty of natural sciences and mathematics, Institute of biology, Department of physiology and biochemistry, Arhimedova, 3, 1000 Skopje, North Macedonia

As one of the most common contaminants of food and feed, mycotoxins pose a great risk factor for human and animal health. At a molecular level, their toxicity is manifested through oxidative stress and subsequent generation of free radicals. When ingested, Deoxynivalenol (DON) and Fumonisin B1 (FB1) produced by *Fusarium* species, mainly affect the liver and kidneys. For centuries, essential oil from *Origanum vulgare* L. was used for medicinal purposes, due to its antibacterial, antifungal, anti-inflammatory, and analgesic properties. Here we conducted an investigation to evaluate the benefit of consuming oregano oil regarding the adverse and harmful effects of ingested mycotoxins (DON and FB1). The activity of the antioxidative enzymes (catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), glutathione reductase (GR), glutathione peroxidase (GPx), malondialdehyde (MDA), and ferric reducing antioxidant power (FRAP)) was examined in 39 male Wistar rats (n=5), after 5-days treatment. Two groups were administrated with DON and FB1, respectfully; two groups were treated with oregano oil immediately prior to DON and FB1 administration and the control group was not treated. We detected significantly decreased enzymatic activity of SOD, GSH, GPx, GR, and FRAP in rats that were treated with DON and FB1. Contrary, rats from these two groups had significantly increased MDA activity (DON-30%, $p<0.05$; FB1-31.3%, $p<0.05$), while CAT activity was significantly increased (50.9%, $p<0.05$) in the group treated with FB1 compared to control. For most of the estimated parameters, oregano oil-treatment caused significant changes compared to DON and FB1- treated rats. These changes in enzymatic activity gradually started to normalize (GSH, SOD, GR, and FRAP) or completely normalized almost to the control values (CAT, GPx, and MDA) in groups that were ingested with oregano oil. Despite the short length of the experiment, the obtained results confirmed the beneficial effect of preventive consumption of oregano oil towards the production of free radicals during mycotoxicoses. Still, for most of the parameters, the effects of FB1 were more evident than the effects of DON.

Key words: fumonisin B1, deoxynivalenol, oregano oil, antioxidants, rats

Scientific session X
PARASITIC DISEASES IN ANIMALS

Oral presentations

O53

CLINICOPATHOLOGICAL ALTERATIONS IN PET DOGS NATURALLY INFECTED WITH EHRLICHIA CANIS AND LEISHMANIA INFANTUM IN MACEDONIA – CASE STUDY

Elena Atanaskova Petrov[*1], Ksenija Ilievska[2], Todor Novakov[3], Ljubica Rashik[3], Igor Djadjovski[4], Miroslav Kjosevski[5], Irena Celeska[6]

[1]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine – Skopje, Department of internal medicine of small animal and horses, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia
[2]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine – Skopje, Department of Veterinary Surgery, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia
[3]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine – Skopje, University veterinary hospital, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia
[4]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine – Skopje, Department of farm animals, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia
[5]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine – Skopje, Department of animal hygiene, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia
[6]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine – Skopje, Department of pathophysiology, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia

Canine ehrlichiosis and leishmaniosis are vector borne diseases well recognized in Europe, endemic in the Mediterranean region. *E. canis* is transmitted by ticks (mainly *Rhipicephalus sanguineus*), while *L. infantum* is transmitted by phlebotomine sand flies. Both infectious agents are intracellular pathogens that disseminate from the skin to the internal organs (spleen, liver, bone marrow) and activate host immune mechanisms. Coinfections with these diseases are common in the endemic areas, causing severe clinical manifestation and fatal outcomes. The clinical manifestation of both diseases can be unspecific (fever, depression, anorexia, PU/PD etc.) and overlapping (bleeding tendencies like epistaxis, limphadenomegaly and splenomegaly), which makes them diagnostic challenge for veterinarians. The aim of this study was to present the clinicopathological alterations in patients infected with *E. canis* and *L. infantum* and comparation with patients with single *E. canis* infection. Thirty pet dogs admitted in the University veterinary hospital were included in this study, divided in 2 groups: positive for ehrlichiosis and dogs co-infected with ehrlichiosis and leishmaniasis. All data were obtained from the medical records (clinical manifestation, physical examination findings, blood and biochemistry results). *E. canis* infection was diagnosed with PCR, while *L. infantum* by IFAT. The results for clinical signs were analyses using the Fisher exact test, while the hematology and biochemistry results were analyzed using Mann Whitney U test) with significant levels set on $p<0.005$. Polyuria, polydipsia and diarrhea were significantly represented in patients

with coinfections. Laboratory results revealed significant difference in the levels of red blood cells parameters (RBC, Hct and Hb) as well as in the levels of albumin, urea and creatinine. The results from the clinical manifestation and laboratory parameters indicate that patients with coinfection might have more severe renal impairment then patients with single *E. canis* infection. *E. canis* and *L. infantum* are potentially chronic infections and the impact of their coinfection can increase the severity of clinical signs, hematology and biochemistry parameters. It is nearly impossible to determinate the stage of the disease in naturally infected dogs, so the presented results may vary in case-control studies. In future, this study should continue on greater number of patients.

Key words: *Ehrlichia canis*, *Leishmania infantum*, dog, infection, clinicopathological parameters

O54

INVESTIGATION OF GASTROINTESTINAL HELMINTH INFECTIONS OF HORSES IN THRACE REGION, TÜRKİYE

Fatma Nur Dal*[1], Şakir Pehlivan[1], Kerem Öter[2], İbrahim Kurban[3], Aynur Gülanber[2]

[1]Istanbul University-Cerrahpaşa, Institute of postgraduate education, Alkent 2000 Mah. Yiğittürk Cad. A-2 Blok, 34500 Büyükçekmece/İstanbul, Türkiye
[2]Istanbul University-Cerrahpaşa, Veterinary faculty, Department of parasitology, Alkent 2000 Mah. Yiğittürk Cad. A-2 Blok, 34500 Büyükçekmece/İstanbul, Türkiye
[3]Istanbul University-Cerrahpaşa, Vocational school of veterinary medicine, Caddesi No:7, 34320 Avcılar/İstanbul, Türkiye

Gastrointestinal parasites of equids can cause diarrhea, pain and even death, especially in foals and immunosuppressive horses. The use of prophylactic anthelmintic drugs is a very common control strategy. In the equine industry, gastrointestinal (GIS) parasite infections cause significant economic losses. Horse breeding for sports and hobby purposes is an important line of business in the Istanbul and Thrace regions of Türkiye. In the last few decades, intermittent research on GIS helminths of horses in various regions of Türkiye has been reported, but there is no comprehensive survey of the situation in the Thrace region. For the first time, a cross-sectional study was initiated in January 2022 to identify equine GIS helminth species and the prevalence of the infection in the Thrace region. Freshly collected horse feces was examined with mini-FLOTAC technique using NaCl and $ZnSO_4$ flotation solutions. In our study, 37 (42%) stool samples of 88 horses examined were found to be infected with helminths. GIS nematode eggs were detected in 38.6 % (34/88), Fasciola eggs in 12.5% (11/88) and ascarid eggs in 9% (8/88) of the samples. Available data show that helminth infections are common in horses in the Thrace region. The relationship between farm management and prevention strategies and the incidence of infections ($p<0.001$) was found to be statistically significant. The findings about the situation of GIS parasitic infections of horses in the Thrace region, which are revealed for the first time through this study, will be the main source of future studies.

Key words: gastrointestinal helminth, gastrointestinal nematodes, horses, Thrace, Türkiye

O55

PREVALENCE AND GENETIC CHARACTERIZATION OF *ECHINOCOCCUS GRANULOSUS* IN DOMESTIC RUMINANTS IN NORTH MACEDONIA

Ljubica Rashikj*, Aleksandar Cvetkovikj, Ivana Arsovska,
Iskra Cvetkovikj, Jovana Stefanovska

Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine - Skopje, Veterinary institute, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia

Cystic echinococcosis is a zoonotic disease with worldwide distribution caused by the larval form of *Echinococcus granulosus (sensu lato)*, present in rural communities where raising livestock dominates. The aim of this study was to evaluate the current prevalence of echinococcosis and to perform molecular identification of hydatid cysts taken from previously slaughtered cattle and sheep from several regions in the Republic of North Macedonia. Post-mortem inspection of the internal organs of the slaughtered animals was conducted in order to detect hydatid cysts. Fertility and sterility of the cysts was determined with microscopic examination. The DNA was extracted from the protoscoleces and the cyst wall. The genotypes were differentiated by sequencing the cox 1 mitochondrial gene. Sixty percent of the tested cattle and sheep were positive for cystic echinococcosis. The hydatid cysts were present in the lungs and the liver. Twenty cysts taken for molecular analysis (12 from cattle and 8 from sheep) were identified as *Echinococcus granulosus (sensu stricto)* (G1-G3 genotype complex). High fertility rate in sheep confirmed the transmission cycle between them and the dogs and highlighted a high potential risk of zoonotic infection. The results confirmed that North Macedonia is an endemic region with G1-G3 complex of *Echinococcus granulosus* being the predominant circulating genotypes in domestic ruminants in different regions of the country. Active surveillance of livestock should be implemented, as well as raising public awareness about infected organ management and regular treatment of dogs with antiparasitic drugs.

Key words: cystic echinococcosis, domestic ruminants, prevalence, genotypes

O56

INVESTIGATION OF PARASITIC DISEASES IN WILD ANIMALS IN THE FEDERATION OF BOSNIA AND HERZEGOVINA

Jasmin Omeragić[1], Naida Kapo*[1], Darinka Klarić Soldo[1], Vedad Škapur[2], Saša Kunovac[3], Ermin Šaljić[1], Šejla Goletić[1], Adis Softić[1], Sabina Šerić-Haračić[1], Teufik Goletić[1]

[1]University of Sarajevo - Veterinary faculty, Zmaja od Bosne 90, 71000 Sarajevo, Bosnia and Herzegovina
[2]University of Sarajevo - Faculty of agriculture and food sciences, Zmaja od Bosne 8, 71000 Sarajevo, Bosnia and Herzegovina
[3]University of Sarajevo - Faculty of forestry, Zagrebačka 20, Sarajevo 71000, Bosnia and Herzegovina

Even though Bosnia and Herzegovina has a diverse range of fauna, some species, particularly large carnivores, are becoming rare and endangered (bear, wolf and lynx). One of the most significant components in the conservation of wild animal populations is the investigation of wildlife health. Additionally, the role of wildlife in the development of disease in humans and domestic animals has become widely recognized as a factor that cannot be ignored. According to the World Health Organization (WHO), about 75% of new diseases that have affected humans over the past ten years have been caused by pathogens originating from an animal or products of animal origin. Parasitic diseases (trichinosis, echinococcosis, toxoplasmosis, and others), which have a significant economic impact and impact on human and animal health, should be recognized in addition to viral and bacterial infections. Previously, in the territory of the Federation of Bosnia and Herzegovina (FBiH), detailed researches were conducted only on a small number of locations, with no systematic research. The aim of this study was to investigate parasitic diseases of wild animals in the FBiH with an emphasis on preserving and improving biodiversity. This study was undertaken on 13 animal species (n=983) from March 2021 to May 2022. Samples of faeces were collected in the field, as well as samples from dead or shot animals during necropsy, in accordance with applicable legal rules and in cooperation with veterinary and hunting organizations. Standard parasitological and molecular tests were carried out at the University of Sarajevo – Veterinary Faculty's accredited laboratories (BAS EN ISO/IEC 17025: 2018). Of the 983 samples taken, 688 were positive (69.99%). Parasites were found in 15.38% of bears (n=51), 85.07% of foxes (n=201), 67.27% of wolves (n=55), 50.00% of wild cats (n=3), 46.15% martens (n=13), 39.07% wild boars (n=81), 85.67% roe deers (n=307), 77.46% rabbits (n=173), 25.00% badgers (n=3), 100% chamois (n=2), 22.22% pheasants (n=9), 30.00% black grouses (n=10) and 40.00% partridges (n=5). Based on the results of the research, proposals of measures for the control of the identified parasitic species were given. Furthermore, the outcomes of the project had extremely useful elements, through support for scientific research and continuing education.

Key words: Federation of Bosnia and Herzegovina, wildlife, parasites, diagnosis, control

O57

GASTROINTESTINAL HELMINTHS OF GOATS BREEDING IN MOUNTAIN AREAS IN CENTRAL SERBIA

Ivan Pavlović*[1], Oliver Radanović[1], Nemanja Zdravković[1], Božidar Savić[1], Aleksandra Tasić[2], Marija Pavlović [2]

[1]*Scientific veterinary institute of Serbia, Janisa Janulisa 14, 11000 Belgrade, Serbia*
[2]*Scientific veterinary institute of Serbia, Smolućka 11, 11070 New Belgrade, Serbia*

Goats population in Serbia had a drastic decrease after II WW, but in the last decade were stared to a new increase of the its population especially at mountain areas. They are usually kept under extensive conditions and graze or brows on any land that is not being cultivated. Our examination was performed in 2017 and 2018 and each year starts with the beginning of the grazing season in late March and ended with the withdrawal of goats from the pasture in mid-November. During examination 727 fecal samples originating from 62 sheep flocks were examined using standard coprological techniques. A total of 69 animals were analyzed by post-mortem examination after slaughter. Determination of eggs and adult parasites were performed by morphological characteristic. Examinations were performed with Carl Zeiss AxioLab A1 microscope with the Axiocam 105 Color microscope camera and Zen Lite software. During the examination parasites were detected in 65.31% fecal samples and in all post-mortem examined animals. Polyparasitism was established in all infected animals. The postmortem examination of the slaughtered animals revealed the presence of: *Teladorsagia* (*Ostertagia*) *circumcincta* (99.78%), *Ostertagiatrifurcata* (95.23%), *O.ostertagi* (34.33%), *Trichostrongylus axei* (100%), *T.colubriformis* (89.47%), *T. capricola* (40.85%), *T.vitrinus* (80.71%), *Nematodirus filicolis* (41.61%), *N.spathiger* (88.78%), *Hameonchus contortus* (80.95%), *Marshallagia marshalli* (29.57%), *Skrjabinema caprae* (20.08%), *Chabertia ovina* (57.14%), *Oesophagostomum venulosum* (39.79%), *Bunostomim trigonocephalum* (14.28%) and *Cooperia curticei* (9.94%). At the beginning of our research, conducted in March, the real extent of gastrointestinal infections with gastro-intestinal strongylid was 71.62%, reaching a level of 100% at the end of follow-up period. The epidemiology of the helminth parasitic diseases depends on factors such as the infection pressure in the environment and the susceptibility of the host species. Furthermore, the availability of large numbers of susceptible definitive and intermediate hosts will increase the parasites‘ ability to reproduce and result in high parasite abundance. However, since parasitic infections of goats are mostly subclinical, this problem is not given due attention in Serbia. Prophylactic treatment is not carried out in most herds or is carried out only partially when parasites are found on slaughter lines or on the basis of poor production results. In order to control the parasitic infection of goats and prepare measures for its control, we must continue our investigation. This is the only way to obtain higher production and the best quality of goat meat and milk.

Key words: goats, gastrointestinal helminths, mountain area, central Serbia**.**

Acknowledgments: The study was funded by the Serbian Ministry of Education, Science and Technological Development (Contract No 451- 03-68/2022- 14/200030)

O58

IDENTIFICATION OF LICE SPECIES OF WATER BUFFALOES IN THE MARMARA REGION OF TÜRKİYE

Şakir Pehlivan*[1], Fatma Nur Dal[1], Kerem Öter[2], Aynur Gülanber[2]

[1]Istanbul University-Cerrahpaşa, Institute of postgraduate education, Büyükçekmece Yerleşkesi Alkent 2000 Mahallesi Yiğittürk Caddesi No:5/9/1 A2 Blok, Oda A217, 34500 Büyükçekmece/İstanbul, Türkiye
[2]Istanbul University-Cerrahpaşa, Veterinary faculty, Department of parasitology, Büyükçekmece Yerleşkesi Alkent 2000 Mahallesi Yiğittürk Caddesi No:5/9/1 A2 Blok, Oda A217, 34500 Büyükçekmece/İstanbul*, Türkiye*

The aim of this study was to determine the lice species found on water buffaloes in the Marmara Region of Türkiye. For this purpose, 143 water buffaloes from İstanbul, Kırklareli and Sakarya were examined with an electronic comb. Each water buffalo was examined with the electronic comb from neck to tail for approximately 15 minutes. Recovered lice from the examined water buffaloes were taken into tubes containing 70% ethanol and animal information was recorded. Tubes brought to İstanbul University – Cerrahpaşa Veterinary Faculty Department of Parasitology Laboratory. In total 143 water buffaloes were examined and 37 were found infected with lice. From the infected animals, 197 lice were recovered. Specimens were examined morphologically under the light microscope at the species level. All of the examined lice were identified as *Haematopinus tuberculatus*. Out of 197 collected lice, 76 and 121 specimens were determined as male and female, respectively. Lice infestations in animals can cause discomfort, skin lesions, anaemia and loss of production. To our knowledge, no research has been previously done to determine the lice of the water buffaloes in Türkiye. Probably, the determination of the species of lice found on water buffaloes is important for the determination of protection and control measures.

Key words: lice, water buffalo, *Haematopinus tuberculatus*, Türkiye

O59

PATHOGENICITY OF *TRIAENOPHORUS NODULOSUS* (CESTODA: OTHRIOCEPHALIDEA) IN NORTHERN PIKE (*ESOX LUCIUS*) FROM THE MREŽNICA RIVER, CROATIA

Valerija Benko*[1], Krešimir Matanović[1], Snježana Kužir[2], Zrinka Dragun[3], Emil Gjurčević[1]

[1]University of Zagreb, Faculty of veterinary medicine,Department for biology and pathology of fish and bees, Heinzelova 55, 10 000 Zagreb, Croatia
[2]University of Zagreb, Faculty of veterinary medicine, Department of anatomy, histology and embryology, Heinzelova 55, 10 000 Zagreb, Croatia
[3]Ruđer Bošković Institute, Division for marine and environmental research, Bijenička cesta 54, 10 000 Zagreb, Croatia

Triaenophorus nodulosus Pallas, 1781 is a well-known representative of the taxonomically complex genus *Triaenophorus* Rudolphi, 1793 and is widely distributed throughout the Palearctic region. The life cycle of *T. nodulosus* involves copepods as first intermediate hosts, many freshwater fish species of different orders (including members of the Perciformes, and Salmoniformes) as second intermediate hosts, and pikes (*Esox* spp.) as definitive hosts. Northern pike (*Esox lucius*) is an important fish for aquaculture and the fishing industry in Europe. Despite its commercial significance, there is only limited information on the health status of this species in natural waters. Here we report the results of an initial evaluation of the pathogenicity of *T. nodulosus* in naturally infected northern pike collected from two locations (Site 1 and Site 2) along the Mrežnica River, Croatia. In the spring and autumn of 2021, a total of 62 specimens (30/Site 1 and 32/Site 2) were captured by electrofishing and examined as part of a fish health and ecotoxicological study, conducted under the Croatian Science Foundation project, "Metal-binding biomolecules and health disturbances of freshwater organisms exposed to industrial wastes" (IP-2019-04-2636). Immediately after capture, all collected fish were screened for the presence of adult cestodes on site. The precise location of cestodes was noted for each infected fish. The intensity of infection was evaluated, and the prevalence was calculated. The weight and length of collected fish were determined, and the condition factor (CF) was calculated. Differences in CF between infected and uninfected fish were assessed for significance using the Mann-Whitney rank sum test (SigmaPlot, version 11.0). The level of significance was set at $p<0.05$. For histological examination, samples of the intestine of each infected fish were removed and fixed in 10% neutral-buffered formalin, embedded in paraffin, and sliced into 5-µm sections. Sections were stained with haematoxylin and eosin (H&E) and periodic acid-Schiff (PAS). The bothriocephalidean cestode *T. nodulosus* was found in 14 of 62 northern pike examined, and was always located in the anterior part of the intestine. Both the prevalence and infection intensity were higher at Site 1 (prevalence 43.3%, average intensity 1.3) than at Site 2 (3.1%, 1.0). At the time of capture, the affected fish appeared to be 'healthy'. Importantly, CF did not vary significantly between infected and uninfected fish ($p>0.05$). Microscopically, *T. nodulosus* causes only localized histopathological changes at/around the site of attachment.

Key words: *Triaenophorus nodulosus*, northern pike, pathogenicity

POSTER PRESENTATIONS

ORGANIZATION OF THE POSTER-SESSIONS
The meeting will have four poster sessions which will take place on Friday,
23th September and Saturday, 24th September 2022
However, all posters are classifed according to the thematic subject on the general schedule of the meeting and will be available in poster hall during the whole scientifc meeting.

P1

BIOSECURITY IN FRESHWATER AQUACULTURE

Vladimir Radosavljevic*, Dimitrije Glisic, Oliver Radanovic, Jelena Maksimovic-Zoric, Jelena Maletic, Ksenija Nesic, Vesna Milicevic

Scientific Institute of Veterinary Medicine of Serbia,
Janisa Janulisa 14, 11000 Belgrade, Serbia

In this paper, we have presented the most important approaches to biosecurity in freshwater aquaculture. Frequent outbreaks of diseases are becoming one of the major obstacles in ensuring the growth and sustainability of freshwater aquaculture. Unfortunately, aquaculture has taken a long time to adopt many standard veterinary approaches and practices to prevent, control, and eradicate infectious and contagious diseases, many of which are well developed in terrestrial animal agriculture. Therefore, biosecurity programs aiming to prevent, control, and eradicate diseases in aquaculture operations are necessary. In freshwater aquaculture, biosecurity consists of practices that minimize the risk of introducing infective fish disease agents and spreading them to the susceptible fish populations at the aquaculture facility and reducing the risk that diseased animals or infectious agents will leave a facility and spread to other sites. Various approaches to aquaculture biosecurity that might protect aquaculture from infectious diseases are available. Developing emergency preparedness and contingency plans for important species and diseases in aquaculture is of utmost significance. This includes an effective implementation plan and adequate financial resources. Emergency response with strong support from the aquaculture sector should be a core function of government services. Empowering aquaculture producers through their organizations may be a good entry point and mechanism for introducing management practices on biosecurity and aquatic animal health and monitoring their implementation.

Key words: biosecurity, freshwater, aquaculture

P2

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *STAPHYLOCOCCUS AUREUS* ISOLATES FROM BOVINE MASTITIS IN BULGARIA

Nikolina Velizarova Rusenova[*1], Nasko Yovchev Vasilev[2], Anton Georgiev Rusenov[3], Aneliya Milanova Milanova[4], Ivo Nikolaev Sirakov[5]

[1]Trakia University, Faculty of veterinary medicine, Department of veterinary microbiology, infectious and parasitic diseases, 6000 Stara Zagora, Bulgaria
[2]Trakia University, Faculty of veterinary medicine, Department of obstetrics, reproduction and reproductive disorders, 6000 Stara Zagora, Bulgaria
[3]Trakia University, Faculty of veterinary medicine, Department of internal noninfectious diseases, 6000 Stara Zagora, Bulgaria
[4]Trakia University, Faculty of veterinary medicine, Department of pharmacology, animal physiology and physiological chemistry, 6000 Stara Zagora, Bulgaria
[5]Medical University of Sofia, Faculty of medicine, Department of medical microbiology, 1431 Sofia, Bulgaria

Staphylococcus aureus is the leading cause of intramammary infections in cows - the main global health concern in dairy cattle farming. Owing to the widespread use of antibiotics for treatment and control of bovine mastitis, reports of resistant *Staphylococcus aureus* isolates have increased in recent years. The published data in our country on antimicrobial resistance of this agent are scarce. Therefore, the aim of this work was to study the antimicrobial susceptibility pattern of *Staphylococcus aureus* isolates from cows with mastitis by disk diffusion test and determination of minimum inhibitory concentrations and to evaluate the agreement of both methods by Cohen's kappa statistics. To this end, 546 milk samples from cows with subclinical and clinical mastitis originating from 14 farms in 9 districts of Bulgaria were collected. Disk diffusion test was carried out with 14 antimicrobials, while minimum inhibitory concentration tests: with 10 included in the Sensititre Mastitis Plate Format; comparison of results between 9 antimicrobials was performed. A total of 92 *Staphylococcus aureus* isolates were identified by polymerase-chain reaction based on the *nuc* gene. Results from the first method showed resistance to at least one of the tested antimicrobials in 32/92 (34.8%) of the isolates, to penicillin in 29/92 (31.5%), to erythromycin in 9/92 (9.8%), to streptomycin in 8/92 (8.7%), to tetracycline in 6/92 (6.5%), to gentamicin and ceftiofur – in 1/92 (1.1%). Isolates resistant to amoxicillin/clavulanic acid, cefalothin, enrofloxacin, co-trimoxazole, penicillin/novobiocin and pirlimycin were not found. Multidrug resistance to three and more classes of chemotherapeutics was demonstrated in 7/92 (7.6%) of the isolates. Minimum inhibitory concentrations confirmed the results from the disk diffusion method with some exceptions regarding the behavior of penicillin, ampicillin, cefoxitin/oxacillin, ceftiofur and erythromycin of some isolates. A high resistance to sulfadimethoxine (87%) was registered by the minimum inhibitory concentration method. Strong agreement

with a kappa value of 0.836 was established between both methods for all 9 compared antimicrobials and almost perfect agreement for some of them. The results of this study showed preserved susceptibility of isolates to most antimicrobial classes tested, except for penicillinase-sensitive penicillins and sulfadimethoxine, to which moderately high and high resistance, respectively was detected. The established between-method agreement of over 90% ensures the reliability of the tests in monitoring *Staphylococcus aureus* resistance at the phenotypic level.

Key words: *Staphylococcus aureus,* mastitis, cows, antimicrobial susceptibility testing

P3

DETECTION OF *BARTONELLA HENSELAE* IN AN ASYMPTOMATIC CAT

Ivana Arsovska*[1], Aleksandar Cvetkovikj[2], Ljubica Rashikj[2],
Zagorka Popova Hristovska[3], Igor Djadjovski[3], Iskra Cvetkovikj[1]

[1]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine-Skopje, Microbiology and immunology department, Lazar Pop Trajkov 5-7, 1000 Skopje, North Macedonia
[2]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine-Skopje, Department of parasitology and parasitic diseases, Lazar Pop Trajkov 5-7, 1000 Skopje, North Macedonia
[3]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine-Skopje, Department of farm animals, Lazar Pop Trajkov 5-7, 1000 Skopje, North Macedonia

Bartonella spp. are vector-borne bacteria with zoonotic potential. *Bartonella henselae* is the main causal agent of cat-scratch disease (CSD) or "cat scratch fever". Domestic and wild felines are known to be its major reservoirs. Studies indicate that the presence of *B. henselae* infection in Europe is much higher than clinically detected since it is not a reportable disease. Feral and young (<1 year old) cats are more susceptible to *B. henselae* infection than indoor and older cats. The disease may occur after scratches and bites from an infected cat, especially in children and immunocompromised individuals. Here we describe the diagnosis of an infected cat with *B. henselae* after a suspected *Bartonella* infection in a 7-year-old child from a rural area that had direct contact with a cat. The child had a fever and inguinal lymphadenopathy whereas the parents and the cat were asymptomatic. A blood sample from the suspected cat was cultured on a blood agar plate and bacterial DNA was extracted from colonies resembling *B. henselae*. The 16S ribosomal RNA gene was amplified, sequenced and the isolate was confirmed as *B. henselae.* To the best of our knowledge, this is the first detection of *B. henselae* infection in an asymptomatic cat in North Macedonia. This finding reveals that *B. henselae* circulates in our country and it may affect both feline and human health. Year-round flea prevention in cats is recommended to help prevent *Bartonella* infection spread between the cats, and from cats to humans. Cat-scratch disease should be recognized as a present zoonotic threat in our country which needs to be included in the differential diagnosis when symptoms include lymphadenopathy, particularly in immunocompromised and young patients who had previous contact with cats.

Key words: *Bartonella henselae,* vector-borne bacteria, cat-scratch disease, zoonosis, public health

P4

EMERGING DISEASES IN SERBIAN AQUACULTURE

Vladimir Radosavljevic*, Dimitrije Glisic, Jelena Maksimovic-Zoric,
Oliver Radanovic, Nemanja Zdravkovic, Vesna Milicevic

Scientific Institute of veterinary medicine of Serbia,
Janisa Janulisa 14, 11000 Belgrade, Serbia

Frequent outbreaks of diseases are one of the major obstacles in freshwater aquaculture production. The occurrence and spread of carp edema virus disease (CEVD) in common carp and lactococcosis in rainbow trout may significantly affect the health of fish in the Serbian aquaculture. CEVD is becoming one of the biggest threats to carp aquaculture, due to mortality in infected fish, reduced growth and the fact that secondary bacterial and fungal infections are regularly found in diseased fish, causing additional pathologies and mortality. Also, an increase in the incidence and mortality of CEVD at carp farms was noticed. Lactococcosis is a significant fish disease caused by *Lactococcus garvieae*, one of the most important bacterial fish pathogens, causing high losses to the trout aquaculture. The impact of lactococcosis is particularly significant as losses often occur when fish reach market size. In this paper, emerging fish diseases facing aquaculture in Serbia are presented.

Key words: CEVD, common carp, lactococcosis, rainbow trout

Acknowledgments: This work was funded by Serbian Ministry of Education, Science and Technological Development (contract number 451-03-9/2021-14/200030).

P5

GASTROINTESTINAL PARASITES OF CAPTIVE ANIMALS IN THE ZOO IN SKOPJE, NORTH MACEDONIA, 2020-2022

Ljubica Rashikj[1], Jovana Stefanovska[1], Iskra Cvetkovikj[1], Ivana Arsovska[1], Vesna Levajkovikj-Trajkov[2], Aleksandar Cvetkovikj*[1]

[1]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine-Skopje, Veterinary institute, Lazar Pop Trajkov 5-7, 1000 Skopje, North Macedonia
[2]ZOO Skopje, Bul. Ilinden, 1000 Skopje, North Macedonia

The aim of this study was to investigate the prevalence of gastrointestinal parasites in 18 animal species (*Ovis aries, Lama glama, Capra aegagrus hirus, Bison bison, Taurotragus oryx, Giraffa camelopardalis, Equs ferus przewalskii, Hipopotamus amphibious, Sus scrofa domesticus, Vicugna pacos, Dolichotis patagonium, Equus quagga, Camelus dromedaries, Macropus rufogriseus, Canis lupus, Canis lupus arctos, Panthera leo* and *Pan troglodytes*) housed in the ZOO in Skopje, North Macedonia in a 3-year period (2020-2022). To detect the parasites, 105 fecal samples from 15 carnivores, 80 herbivores and 10 primates, were examined with a standard fecal flotation technique using saturated zinc sulfate solution. The overall prevalence of gastrointestinal parasites was 42%. The results showed presence of different parasite groups including protozoa (*Balantidium* sp., *Coccidia* sp., *Giardia* sp.), nematodes (*Nematodirus* sp., *Parascaris* sp., *Trichostrongylus* sp., *Toxascaris leonina*, *Trichuris* sp., *Ancylostoma* sp., *Strongyloides* sp.), and cestodes (*Anoplocephala* sp., *Dipylidium* sp.). No trematodes were detected during the study. Mixed helminth and protozoan infections were identified in 10 out of the 105 fecal samples (9.5%). Protozoan infections were more common in primates, while helminth infections were more common in carnivores and herbivores. The gastrointestinal parasites are common in captive animals which act as their potential reservoir. Regular monitoring as well as good hygiene practice and appropriate deworming protocols should be implemented in order to prevent the infection and minimize the spread of the parasites.

Key words: zoo animals, protozoa, cestoda, nematoda, helminth

P6

A LABORATORY PROCEDURE FOR CHARACTERIZATION OF *EAE* POSITIVE *ESCHERICHIA COLI* AND GENETIC DETERMINATION OF THE INTIMIN TOXIN TYPES

Bilyana Sirakova*[1], Tanya Strateva[2], Nikolina Rusenova[3], Raina Gergova[2], Ralitsa Popova-Ilinkina[4], Ivo Sirakov[2], Ivan Mitov[2]

[1]Medical University of Sofia, Faculty of dental medicine, 1431 Sofia, Bulgaria
[2]Medical University of Sofia, Faculty of medicine, Department of medical microbiology, 1431 Sofia, Bulgaria
[3]Trakia University, Faculty of veterinary medicine, Department of veterinary microbiology, infectious and parasitic diseases, 6000 Stara Zagora, Bulgaria
[4]National diagnostic and research veterinary medical institute, National center of food safety, 1303 Sofia, Bulgaria

E. coli is a facultative anaerobic bacteria, making part of the resident microflora of mammals, birds and reptiles. It appeared 120-160 million years ago, evolving along with mammals to the relationships we know today. Pathogenic *Escherichia coli* (*E. coli*) strains can be divided into: causing extraintestinal disease and causing enteric infections. Enteropathogenic *E. coli* (EPEC) are representatives of the attaching and effacing *E. coli* (AEEC), along with shiga toxin-producing *E. coli* (STEC) due to the nature of the lesions they produce in the intestinal lining. Typical EPEC are strict human pathogens, while atypical EPEC cause diarrhoea in piglets, lambs, calves and pups. Intimin, one of the virulence factors responsible for the A/E lesions, is encoded by *ea*e gene located on the pathogenicity island, named LEE. Many variants of the intimin toxin have been found in nature, with different frequencies and pathogenic potential in animals, children and the elderly. Determination of the toxin type is essential for the food safety, pathogenicity of the strains, from a clinical and epidemiological point of view. Therefore, we propose a procedure involving standard microbiological, molecular and genetic analysis, to detect and identify the type of intimin toxin in *E. coli*. For this purpose, 57 food products and raw materials, were analysed using various liquid and solid culture media. Biochemical identification of the isolates was performed by the catalase and oxidase tests, indole test and the MICRONAUT-E system. PCR based on the detection of the *eae* gene and 16S rDNA was adapted and Mega7 software for sequence and phylogenetic analysis was used. A total of 25 *E. coli* isolates were detected in the analysed samples, 5 (20%) of which were positive for the *eae* gene, with a predominance of "*theta*" type and a specific phylogenetic profile, distinguishing separate groups. The proposed procedure ensures consistency, traceability and precision in the identification of *E. coli* intimin toxin type. The steps in the selection and synthesis of primers will serve as a guidance to any young researcher who starts developing primers for their own investigation, beyond those described in the study.

Key words: intimin toxin, *eae* gene, *E. coli*, sequencing, primers

Acknowledgments: he study was carried out with the support of the Medical University - Sofi a, Bulgaria under Project No. 8456 / 09.12.2016; Contract No. D-64 / 02.05.2017

P7

CO DOMINANT GENETIC MARKER AT GRAY WOLF POPULATION IN OSOGOVO AND SARPLANINIAN MOUNTAIN REGIONS

Igor Esmerov*[1], Nikola Adamov[1], Aleksandra Angelevska [1], Radmila Chrcheva[1], Ljupco Mickov[1], Ljupco Angelovski[1], Nikolaj Markov[2], Branko Atanasov[1]

[1]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine – Skopje, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia
[2]Agricultural Academy - Sofia, Institute of mountain animal husbandry and agriculture, 5600 Troyan, Bulgaria

DNA microsatellite polymorphic data is particularly important in biodiversity characterization in certain populations. The idea of this study was to determine certain specifics of the Gray Wolf living in the area of Osogovo and Sharplaninian mountain regions, by obtaining data from detection of 10 DNA microsatellite loci. For this research, the following DNA microsatellite loci characterized with high polymorphism (FH2361, DGN10, FH3287, FH3924, FH3608, FH3023, FH3489, FH3721, FH4027, FH2141) were screened in 28 samples from both mountains, 14 each. The informativeness of the study was presented through 4 parameters (number of detected alleles by locus, PIC Polymorphism informative content, expected heterozygosity, observed heterozygosity, zero allele frequency). Phenol/chloroform extraction and 70% ethanol precipitation were used for DNA extraction from the collected blood samples. The amplification was performed on a Perkin-Elmer GeneAmp PCR System 2400 according to the following protocol: average denaturation at 94 C°/10 minutes, denaturation at 94 C°/1 minute, cancellation of primers at 56 C°/1 minute, extension at 72 C°/1 minute, end extension at 72 C°/ 8 minutes. The PCR reaction was consists of: H_2O 14.0 µl; 10xRb puffer 2.5 µl; 2.5 mmol $MgCL_2$ 1.5l; 2.5 mmol dNTP 1.0 l; 0.5 U Ampli Taq Gold 0.3l; 2.0 p.mol primer R / F 1.0 l; DNA 4 l final volume of reaction will be 23.3 µl. Allele size detection was performed by single-column capillary electrophoresis Applied Biosystems 7500. The results were processed in the CERVUS software application. The results indicate low genetic variability, which was expected because the Gray Wolf has a wide range of migration and does not linger in its natural habitat. The detection of specific alleles in the population is noticeable, indicating that in the future, more accurate genetic typing of the present Gray Wolf population in the region could be made, using extensive study with an increased numbers from different habitats in the Balkan region.

Key words: gray wolf, DNA microsatellite, genetic variability, polymorphism, microsatellite loci

P8

THE KIDNEY VASCULAR ZONES IN DOMESTIC ANIMALS

Rizah Avdić*[1], Verica Mrvić[2], Faruk Tandir[1], Nedžad Hadžiomerović[1], Pamela Bejdić[1]

[1]University of Sarajevo, Veterinary faculty, Department of basic sciences of veterinary medicine, Zmaja od Bosne 90, 71000 Sarajevo, Bosnia and Herzegovina
[2]University of Belgrade, Faculty of veterinary medicine, Serbia

The kidneys are excretory organs that eliminate waste materials from the body. The normal functioning of the kidneys depends on their vascularization, which is very intensive in comparison with the other organs. Renal ischemia results in pathoanatomical, pathohistological and functional changes manifested by an increase in general arterial blood pressure as a compensatory measure. The vascularization of the kidneys originates from the branches of the *arteria renalis*. The aim of this study was to examine detail anatomical relationship of the vascular systems of domestic animals by injecting vinilyt resin into blood vessels. Also, the additional aim was to allow experimental physiological investigation of the kidneys' normal and pathological function by excluding their specific areas from the circulation. The investigation was performed on the 10 kidneys of the cow, 10 kidneys of the pig, 6 kidneys of the horse and 20 kidneys of the sheep. The kidneys were taken from the slaughterhouse with the adipose capsule and branches of the *a. renalis*. The blood vessels were washed with the saline and then injected with the plastic resin colored with different color. The injection was performed with the automatic infusion pump with controlled pressure. The results showed that relationship between primary and secondary branches of the *a. renalis* are quite independent and their functional independency can have significant practical application in the partial resection of the sheep, pig and cow kidneys. The independent vascularization of the arterial system allows exclusion of some parts of the kidneys in the sheep and pig, but smaller parts in cow kidneys. The horse kidney had centrally oriented branches and did not allow any exclusion of the kidney parts.

Key words: kidney, arteria renalis, horse, cow, sheep, pig

P9

HISTOLOGY OF THE DIGESTIVE TRACT IN THE EUROPEAN HAKE (*MERLUCCIUS MERLUCCIUS*)

Lucija Bastiančić*[1], Krešimir Matanović[2], Emil Gjurčević[2], Nikolina Škvorc[1], Valerija Benko[2], Marin Lovrić[3], Damir Valić[3], Snježana Kužir[1]

[1]University of Zagreb, Faculty of veterinary medicine, Department of anatomy, histology and embryology, Heinzelova street 55, 10 000 Zagreb, Croatia
[2] University of Zagreb, Faculty of veterinary medicine, Department for biology and pathology of fish and bees, Heinzelova street 55, 10 000 Zagreb, Croatia
[3]Ruđer Bošković Institute, Division for marine and environmental research, Bijenička street 54, Zagreb, Croatia

The European hake (*Merluccius merluccius*) is a predatory demersal fish widely distributed in the Mediterranean. The adult European hake feeds mainly on fish and less frequently on crustaceans and mollusks. The aim of this study was to investigate and describe the histology of the digestive tract in the European hake. For this purpose, tissue samples of the esophagus, stomach, intestine and rectum were taken from five European hakes. The tissue samples were fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned with microtome into 6 µm sections. The sections were stained using five different methods: hematoxylin and eosin, Mallory trichrome, Verhöeff-Van Gieson, Alcian blue-PAS kit and the seven-reagent kit for determining reticulin fibers. The histology of the investigated parts was analyzed by an Olympus BX41 microscope. The wall of the digestive tract was composed of mucosa, submucosa, muscularis and adventitia or serosa. According to the anatomy, thickness of the wall, length of mucosal folds and thickness of muscularis, intestine was subdivided into three parts: anterior, middle and posterior. The mucosa of the esophagus was lined by stratified squamous epithelium. The stomach and intestine were lined by simple columnar epithelium. The rectum was lined with epithelium that resembles pseudostratified columnar. Microvilli were present on apical surface of the epithelial cells in all parts of the digestive tract except in the esophagus. Mucous cells were present in all investigated parts except in the stomach. The lamina propria was composed of loose connective tissue, except in the esophagus, where it contained dense connective tissue. Abundant gastric glands were present in the cardiac part of the stomach. The muscular layer of the mucosa was identified only in the stomach. The submucosa was composed of dense connective tissue, except in the esophagus, where it contained loose connective tissue. The muscularis was composed of inner circular and outer longitudinal layers of smooth muscle cells, except in the esophagus, where it was made of striated muscle fibers. Adventitia was found to be the outermost layer of the esophagus, while serosa was present in all other parts. Melanocytes were found in different layers of the esophagus, stomach and in the serosa of the rectum. In further histochemical research the focus will be placed on defining enzyme activities along the digestive tract in the European hake.

Key words: European hake, digestive tract, histology

P10

ENGINEERED EXTRACELLULAR VESICLES FROM MESENCHYMAL STROMAL CELLS AS POTENTIAL NANO-SHUTTLES OF THERAPEUTIC BIO-MOLECULES

Gabriele Scattini[1*], Luca Avellini[1], Olimpia Barbato[1], Antonello Bufalari[1], Stefano Capomaccio[1], Patrizia Casagrande Proietti[1], Maria Rachele Ceccarini[3], Rodolfo Gialletti[1], Samanta Mecocci[1], Laura Musa[1], Giulia Pianigiani[2], Tommaso Beccari[3], Luisa Pascucci[1]

[1]University of Perugia, Department of veterinary medicine, Via San Costanzo, 4 – 06126 Perugia, Italy
[2]University of Perugia, Department of medicine and surgery, Piazzale Gambuli, 1 – 06129 Perugia, Italy
[3]University of Perugia, Department of pharmaceutical sciences, Via San Costanzo – 06126 Perugia, Italy

Extracellular vesicles (EVs) are cell-derived micro and nano-structures involved in intercellular communication in physiology and pathology. They have the potential to serve as natural delivery systems due to their high specificity for the target, low immunogenicity, reduced clearance, great stability in blood circulation, and intrinsic ability to cross biological barriers. For these reasons, strategies for tailoring EV cargo are being explored to enable loading with pharmacological agents. EVs derived from mesenchymal stromal cells (MSC-EVs) are of interest as therapeutic agents *per se* and as natural nanocarriers. MSC-EVs, in fact, could be used to specifically package bio-therapeutics and shuttle them to regenerating tissues. The present work aimed at highlighting the most effective upload mechanism able to drive a protein of interest inside MSC-EVs. The perspective is to develop biological nanocarriers able to specifically deliver promising therapeutic proteins and to sustain their bioactivity in a steady and long-term fashion (controlled delivery). To this aim, MSC were engineered to express a reporter protein (Green Fluorescent Protein- GFP) associated with different tags that were expected to drive it inside EVs. In particular, CD63, Syntenin-1, TSG101, and the Palmitoylation signal from Lck were investigated. Over-expression of tagged-GFP in canine MSC was detected through live confocal microscopy and transmission electron microscopy (TEM) to map its intracellular localization. In addition, western blotting was used to detect GFP associated with EVs. All the tags demonstrated the ability to drive GFP inside EVs. Syntenin-1 displayed a high efficiency but exhibited a diffuse localization pattern in transfected cells. The palmitoylation signal showed a low efficiency and low specificity. TSG101 displayed a localization pattern compatible with a specific localization in endosomal structures, but the low expression did not allow further considerations. Finally, CD63 displayed the highest efficiency and specificity, giving rise to a concentration of GFP inside EVs 5-fold higher than in cell lysates. In conclusion, CD63 resulted the most effective and specific tag for

EVs. Further studies aimed at better dissecting its contribution and clarifying which part of the molecule is involved in trafficking within vesicles, could shed light on EVs biogenesis and bioengineering. The results of this study provide the basis for the use of MSC-EVs as delivery tools of proteins and peptides with therapeutic potential in different diseases of humans and animals.

Key words: mesenchymal stromal cells, extracellular vesicles, EVs engineering

P11

GISMVET: A MODEL FOR VETERINARY REGENERATIVE MEDICINE EDUCATION

Stefano Grolli*[1], Silvia Dotti[2], Maurizio Del Bue[3], Eleonora Iacono[4], Ana Ivanovska[5], Anna Lange Consiglio[6], Luisa Pascucci[7]

[1]University of Parma, Department of veterinary medical sciences, Via del Taglio, 10 43126, Parma Italy
[2]Istituto zooprofilattico sperimentale della Lombardia ed Emilia-Romagna, Via Bianchi 9, 25124 Brescia, Italy
[3]Freelance veterinary medical doctor, 43121, Parma, Italy
[4]University of Bologna, Department of veterinary medical sciences, Alma Mater Studiorum, via Tolara di Sopra 50,40064 Ozzano Emilia (Bologna), Italy
[5]National University of Ireland Galway, Regenerative medicine institute (REMEDI), Newcastle Road, Dangan, Galway City, Ireland
[6]University of Milan, Department of veterinary medicine and animal sciences, Via dell'Università 6, 26900 Lodi, Italy
[7] University of Perugia, Department of veterinary medicine, Via S. Costanzo, 4, 06126 Perugia, Italy

GISMVet (Italian Mesenchymal Stem Cell Group – Veterinary Section) is a section of GISM (Italian Mesenchymal Stem Cell Group) and represents a team effort between scientists and clinicians with multi-year experience in veterinary regenerative medicine working in academic and public healthcare institutions in Italy and Ireland. Motivated by the current need to accelerate the progress of cell-based therapies on a scientific and practical clinical level, we designed an online model of veterinary regenerative medicine post-graduate education in format of webinars offered to veterinary surgeons, scientists and students in Italy in collaboration with the online veterinary platform "Cogito Ergo Vet". Veterinary regenerative medicine is a dynamic fast-growing medicinal area stimulating great interest, as it can offer innovative solutions where current medical and surgical treatments fail to deliver a favourable outcome. In terms of cells, the current front-runners widely used in clinical practices are mesenchymal stromal cells (MSCs). MSCs are the central argument of our education program and multiple aspects related to their isolation, characterisation, biology, manufacturing, regulatory frameworks and clinical application are covered. We started the cycle with introduction webinars on cell biology, tissue sources, and mechanisms of action. Each webinar was delivered by a group of clinicians, scientists and young researchers addressing a clinical condition, followed by a scientific overview of a mechanism of action and finalised by a relevant journal club presentation. The next cycle covered another level of depth of knowledge addressing the clinical evidence of various diseases treated with MSCs in veterinary patients as well as best practices for optimal clinical application. All webinars had a total duration of 1 hour including questions and

discussion. The webinars were offered to members of GISM in Italian language, each participant in the live event was awarded credits for continuous professional development and a recording of the video was made available as well. We have learned that although there is great interest in cell products, there are still multiple scientific questions that need to be answered to pave the way toward a correct clinical application. These types of interactive platforms are of utmost importance as they represent a valid forum for discussion and the exchange of ideas between scientists and clinicians. Additionally in absence of consolidated veterinary regenerative medicine courses in veterinary schools, they represent a direct opportunity for veterinary surgeons to get input based on current scientific knowledge and acquire a needed set of skills for the correct treatment of their patients.

Key words: mesenchymal stromal cells, veterinary regenerative medicine, education platform, clinical application, multidisciplinary approach

P12

LOCAL AND SYSTEMIC APPLICATION OF AUTOLOGOUS MESENCHYMAL STROMAL CELLS IN CATS SUFFERING FROM CHRONIC GINGIVOSTOMATITIS: A PILOT STUDY

Priscilla Berni*[1], Tommaso Magni[2], Maurizio Del Bue[3], Virna Conti[1], Valentina Andreoli[1], Rosanna Di Lecce[1], Anna Maria Cantoni[1], Roberto Ramoni[1], Stefano Grolli[1]

[1]University of Parma, Department of veterinary medical science, Strada del Taglio 10, 43121, Parma, Italy
[2]Clinica Veterinaria Pet Care, Via Marzabotto 1, 40043, Bologna, Italy
[3]Freelance veterinary medical doctor, 43121, Parma, Italy

Feline Chronic Gingivostomatitis (FCGS) is a severe inflammatory oral disease characterized by painful mucosal lesions, oral discomfort, inappetence, reduced grooming, weight loss, and hypersalivation, seriously affecting the patient quality of life. Since there is no cure, the current standard of care consists of invasive full or near-full mouth tooth extraction and long-term antibiotic/immunosuppressive/analgesic treatments, with a high rate of relapse. Since FCGS is probably immune-mediated, Mesenchymal Stromal Cells (MSCs) represent a promising tool for this disorder because of their immunomodulatory properties. Different studies have reported the efficacy of systemic administration of autologous/allogeneic adipose-derived MSCs (Ad-MSCs) in cats with non-responsive FCGS, while a pilot study reported a lack of efficacy when the treatment is performed prior to full-mouth tooth extraction. This study aims to determine the efficacy of local and systemic administration of Ad-MSCs in cats with FCGS, with or without full-mouth tooth extractions. Eleven client-owned cats with FCGS for at least one year and with long-term anti-inflammatory or immunosuppressive clinical history, with or without teeth, were treated with a double application of autologous Ad-MSCs at 30-day intervals. The cats were enrolled in two groups: one group was treated with local injections of 5x106 autologous Ad-MSCs and the other group was treated with local injections associated with systemic infusions of 2x106/Kg autologous Ad-MSCs. An oral examination, with photographs and oral biopsies, was performed at the enrolment and 30-days after each treatment. An SDAI (Stomatitis index) scoring was calculated at the same intervals, with a brief owner questionnaire about appetite, activity level, grooming behaviour and perceived oral comfort, in addition to a veterinarian scoring of the weight and the severity of the oral inflammatory lesion. Furthermore, a complete blood count, blood immune cell phenotyping and biochemical profile were planned on day-0 and 3 months after the first treatment. At the time of writing, eight cats have been treated with double MSCs application. Seven cats have completely suspended any pharmacological treatment after the first application. The clinical assessment at day-60 showed a marked clinical improvement reported by the owners, except for one patient that showed the maximum SDAI score at the enrolling,

which improved only in the body weight parameter. A statistically significant difference was observed in the SDAI between day-0 and day-60 for the other seven cats, two with a complete resolution of the oral inflammation ($p<0.05$). Immunohistochemical analysis and blood immune cell phenotyping are needed to confirm the observed clinical improvement.

Key words: mesenchymal stromal cells, regenerative medicine, immunomodulation, gingivostomatitis

P13

MESENCHYMAL STROMAL CELLS AND CRANIAL CRUCIATE LIGAMENT INJURY: FRIENDS OR FOES?

Luisa Pascucci*[1], Gabriele Scattini[1], Alessandro Fruganti[2], Fabrizio Dini[2], Aurora Barbetta[3], Piero Boni[4]

[1]University of Perugia, Department of veterinary medicine, Via San Costanzo, 4 – 06126 Perugia- Italy
[2]University of Camerino, School of biosciences and veterinary medicine, Via Raffaele Fidanza, 15, 62024 Matelica, Italy
[3]Veterinary practitioner, Cannara (PG), Italy
[4]Veterinary practitioner, Via Martiri di Modena, 2 06033 Cannara (PG), Italy

In recent years, mesenchymal stromal cells (MSC) have received a strong boost in veterinary medicine due to their interesting pro-regenerative properties. MSCs are virtually present in all organs possessing a vascular stroma. The aim of this study was to evaluate the potential therapeutic use of MSCs from adipose tissue in dogs with complete cranial cruciate ligament (CCL) rupture that has not undergone surgical treatment. CCL rupture is the most common cause of lameness in dogs. The condition can be treated conservatively, but surgical treatment is the treatment of choice to restore the stability and functionality of the joint. However, surgery does not prevent the development of osteoarthritis, which over time leads to relapse of lameness and pain. It is worth mentioning that an increasing number of public and private veterinary hospitals treat partial CCL rupture with MSC as a second-line or even first-line therapy. There is no evidence of MSC use in complete ruptures. This study refers to Tyson, an American Staffordshire Terrier, male, 4 years old who received a diagnosis of complete CCL rupture followed by 4 intra-articular infiltrations of autologous MSC. Post-infiltration monitoring was performed by orthopedic, echographic, radiographic examination, and nuclear magnetic resonance (NMR). A 16 months follow-up was performed. MSCs did not trigger adverse effects in the short to medium term, nor do they cause regeneration of the CCL. However, they displayed a strong anti-inflammatory activity responsible for symptom remission. The therapeutic effectiveness of MSCs also seems to be attributable to their chondroprotective effect which slows down the development and progression of osteoarthritis. The results described in this study suggest that the use of MSC for the treatment of complete rupture of the CCL could represent a valid option: (i) As an alternative to surgery when the patient or owners are unable to deal with it; (ii) As an adjuvant to surgical therapy both in the peri-operative and in the post-operative in order to obtain a stabilization and reduce the development of osteoarthritis; (iii) as a substitute of non-steroidal anti-inflammatory drugs with the advantage of reducing their side effects.

Key words: mesenchymal stromal cells, cranial cruciate ligament rupture, regenerative medicine

P14

METASTATIC PANCREATIC ADENOCARCINOMA

Trpe Ristoski[1], Mehmet Fatih Bozkurt[2], Ivica Gjurovski[1],
Spiro Bozinoski[1], Aleksandar Janevski*[1]

[1]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine, Department of pathology, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia
[2]Afyon Kocatepe University, Faculty of veterinary medicine, Department of pathology, 03030 *Afyonkarahisat, Turkey*

The case of 9 years old sterilized stray bitch has a symptoms of chronic weight loss, lethargy, difficult mobility, incontinence and fever of 40.4 ºC. Clinical examination was performed, as well as biochemical and complete blood count, X-ray of the pelvis and coxofemoral joints. The results showed increased ALKP, hyperphosphatemia and mild thrombocytopenia ($135*10^9$/L) and anemia (erythropenia – $3.59*10^{22}$/L; HCT – 25.6 %; HGB – 8.8 g/dL). The X-ray showed bilateral osteolysis of the auriculas of the ilium bone with sclerotic and proliferative processes. Considering the clinical findings and worse general condition of the bitch, human euthanasia was recommended and performed. The postmortem X-ray showed neoplastic changes of proximal epiphysis and entire diaphysis of left humerus,completely right humerus, with proximal diaphysis of tibia. Also, bilateral, auricles of the ilium, alongside sacrum and four ribs on the right side were affected. Post mortem necropsy was performed with proliferative neoplastic changes of the pancreas, other abdominal organs and osteolysis of the bones explained in x-ray finding. Samples of macroscopic changed organs and bones were fixed in 10% neutral buffered formalin (for bones after a 24 hour soaking in osteomol). Samples were processed for routinely staining with Hematoxylin and Eosin (HE) for histopathological evaluation. Immunohistochemistry was perform using the Ae1Ae3 Cytokeratin, S-100, Ki67, MNF, Vimentin and En Vision Kit. Prepared histopathological samples revealed malignant neoplastic cells in the pancreas and metastasis on almost all internal organs and bones. Immunohistochemistry was positive for Ae1Ae3 Cytokeratin meaning that the malignant neoplasia was form epithelial origin. Besides anamnestic data, clinical examination, routine laboratory methods and x-ray examination, metastatic pancreatic adenocarcinoma, was confirmed with immunohistochemistry procedure.

Key words: pancreas, adenocarcinoma, x-ray, pathohistology, immunohistochemistry

P15

A COMPARATIVE STUDY OF CLASSICAL SMEAR EXAMINATION AND CELL BLOCK METHOD IN THE DIAGNOSIS OF EFFUSIONS IN THE BODY CAVITIES OF DOGS AND CATS – PRELIMINARY RESULTS

Valerija Benko[1], Lucija Bastiančić[2], Marin Torti[3], Siniša Faraguna*[4]

[1]University of Zagreb, Faculty of veterinary medicine,Department for biology and pathology of fish and bees, Heinzelova 55, 10 000 Zagreb, Croatia
[2]University of Zagreb, Faculty of veterinary medicine, Department of anatomy, histology and embryology, Heinzelova 55, 10 000 Zagreb, Croatia
[3]University of Zagreb, Faculty of veterinary medicine, Clinic for internal diseases, Heinzelova 55, 10 000 Zagreb, Croatia
[4]University of Zagreb, Faculty of veterinary medicine, Department of pathophysiology, Heinzelova 55, 10 000 Zagreb, Croatia

The cell block preparation method is widely used in human cytopathology. In veterinary medicine, cytological examination of different effusions from the body cavities of dogs and cats is one of the most useful methods, especially when malignant process is suspected. Diagnostic challenges arise in everyday clinical practice, as it is difficult to differentiate reactive atypical mesothelial cells and malignant cells by the routine classical cytological examination. Instead of applying only conventional cytology, effusions can be further processed by using the cell block method, which may augment the diagnostic utility. Several techniques have been proposed to convert fluid specimens of different effusions into solid material. The aim of this study was to compare the diagnostic utility of classical cytological examination, cell blocks and their combination, regardless of the etiology of effusions. A total of 4 effusions in body cavities (from 2 dogs and 2 cats) were subjected to routine laboratory analysis. For classical examination, a minimum of two thin smears were prepared from the sediment, air dried and stained with the May-Grünwald-Giemsa stain. Cell blocks were prepared after fixation in 10% neutral buffered formalin, embedded in paraffin and sectioned on 4 and 5 μm using a microtome. Sections were stained with hematoxylin and eosin (H&E). The histomorphological features were examined using an Olympus BX41 microscope. The results of both methods were compared to the final diagnosis. When compared to the classical smear examination, the results obtained from the paraffin-embedded cell blocks yielded more cellularity and better architercural patterns, which led to more reliable diagnosis. As these are only preliminary results, a larger study is needed for higher efficiency of cell block method value. As far as it can be observed, cell block method is a diagnostic tool which is not yet used in the veterinary medicine in Croatia. Paraffin-embedded cell blocks can be used for additional staining and testing, such as histochemistry, immunohistochemistry and molecular diagnostics.Therefore, the cell block method could be considered a useful diagnostic tool in evaluating fluid specimens for a final cytodiagnosis, along with the classical smear examination of effusion.

Key words: cytology; effusions; smear; cell block method

P16

CHEMOTHERAPY TREATMENT PROTOCOLS USED IN MULTICENTRIC LYMPHOMA IN GOLDEN RETRIEVER: A CASE REPORT

Elena Mitrevska*, Irena Celeska[1], Sara Gjorgjievska[2], Elena Atanaskova Petrov[3]

[*]Student, Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine-Skopje, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia
[1]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine-Skopje, Department of pathophysiology, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia
[2]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine-Skopje, University veterinary hospital, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia
[3]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine-Skopje, Department of internal medicine of small animal and horses, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia

Multicentric lymphoma is one of the most often diagnosed malignant diseases in dogs. The disease comprises a number of clinically and morphologically distinct forms of lymphoid cell neoplasia. There are many different treatment protocols available to date, but multi-agent therapy seems to be most effective. This case report presents an 11-year-old golden retriever with multicentric lymphoma. The patient had previous history of hypothyroidism and dilatative cardiomyopathy. Clinical examination revealed enlarged peripheral lymph nodes; blood and cytology FNA samples were taken. The cytology results revealed large numbers of lymphoblast with characteristics of anisocytosis that were individual or in clusters. Abdominal ultrasonography was in accordance with the diagnosis of multicentric lymphoma (splenomegaly with focal hyper-echogenic areas). The hematology analysis showed a decrease of red blood cells parameters, platelets and lymphocyte count. On the serum biochemistry, liver enzymes and cholesterol levels were mildly elevated. COP protocol by University of Wisconsin was initiated after the diagnosis. On the blood analysis progressive elevation of the PLT count and lymphocyte count but lowering of the RBC was present, while the biochemistry results showed a progressive elevation on the liver enzymes. At the fourth week of treatment, the patient presented moderate reduction of the lymph nodes. Additionally, the PLT and lymphocyte count at week 6 were normalized, while the RBC was still mildly decreased. Relapse of the disease was reported 2 weeks after finishing the protocol, accompanied by hepatomegaly, splenomegaly and enlarged lymph nodes alongside with decreased thrombocytopenia and anemia. CHOP Protocol by University of Wisconsin was administered for 15 weeks with modification of epirubicin instead of doxorubicin due to the previously diagnosed cardiomyopathy and cardiotoxicity of the doxorubicin. Normalization of the patient vital signs was established, and the lymph nodes were normalized by the third week. Unfortunately, in the middle of the second protocol, lymphoma relapsed. Due to deterioration of the clinical signs and hematology

analysis, humane euthanasia was recommended. Multicentric drug resistant lymphoma is therapeutically challenging. This is a result partly from the onset of drug resistance, but in part also from the lack of new chemotherapeutic agents. A potentially major step forward in the treatment of multicentric lymphoma would be the possibility to prevent or delay drug resistance.

Key words: multicentric lymphoma, dog, chemotherapy protocol

P17

DISPLACEMENT OF THE MEDIAL FABELLA IN WEST HIGHLAND WHITE TERRIER – CASE REPORT

Aleksandar Janevski*[1], Boris Dimitrievski[1], Marko Mitrović[2], Dine Mitrov[1]

[1]Ss. Cyril and Methodius University, Faculty of veterinary medicine, Department of visual diagnostics, Lazar Pop Trajkov 5-7, 1000 Skopje, North Macedonia
[2]University of Belgrade, Faculty of veterinary medicine, Department of radiology and radiation hygiene, Bulevar Oslobođenja 18, 11000 Belgrade, Serbia

The displacement of the fabella is an asymptomatic condition in dogs where its prevalence regarding the medial and lateral fabella is 1.7% and 0.3% respectively. It is usually bilateral, which may indicate that it is a congenital anomaly. The lateral fabella (LF) and medial fabellae (MF) along with the patella and the popliteal bone are the four sesamoid bones that are typically part of the knee joint in dogs. MF and LF in dogs are located at the caudal aspect of the femoral condyles and each fabella is in the tendon of origin of the corresponding head of the gastrocnemius muscle. MF is smaller and angular, and often slightly more distal than the lateral fabella; the lateral fabella is globular, except for its truncated end. Normal ossification of the fabella occurs at 3 months of age in dogs. The case is about a male, unsterilized West Highland White Terrier (WHWT) at the age of 12 months. The dog fell from the bed while playing with its owner and hit the floor with the rear part of the body, which resulted in the dog limping on its right hind limb. After the initial physical examination by the family veterinarian, the patient was admitted to the Department for Visual Diagnostics for a radiological examination of the pelvis, hips, and right hind limb. The native X-ray images showed no radiologically visible changes regarding the continuity and structure of the pelvis, hips, and right hind limb. Medio-distal displacement of the MF on both limbs was observed, while LF was compact and in the correct anatomic position. After the negative radiological findings, an additional orthopedic examination of the knee was performed during which no soft tissue injuries were found. A control examination was performed seven days after the recommended rest time and application of analgesic therapy during which the dog showed no visible clinical signs. Our case is consistent with the only epidemiological study that indicates that MF displacement is more common in WHWT that in other breeds and occurs exclusively in small dog breeds (<10 kg). It is a condition that has no particular clinical significance but should be distinguished from the pathological displacement of the fabellae which may occur due to avulsion injury of the tendon of origin of the corresponding head of the gastrocnemius muscle or from the luxation of the patella which may be the primary cause of displacement of the MF.

Key words: dog, displacement, medial fabella, WHWT

P18

SCHIRMER TEAR TEST VALUES IN DOGS WITH ATOPIC DERMATITIS

Tajna Kovač*[1], Nejra Subašić[2], Lidija Medven Zagradišnik[3], Boris Pirkić[4], Nikša Lemo[2], Valentina Plichta[4], Marija Mamić[4], Petra Dmitrović[4]

[1]PhD student at Doctoral studies in veterinary sciences, University of Zagreb, Faculty of veterinary medicine, Radićeva 20, 10000 Zagreb, Croatia, tkovac@vef.hr
[2]University of Zagreb, Faculty of veterinary medicine, Internal diseases clinic, Heinzelova 55, 10000 Zagreb, Croatia
[3]University of Zagreb, Faculty of veterinary medicine, Department of veterinary pathology, Heinzelova 55, 10000 Zagreb, Croatia
[4]University of Zagreb, Faculty of veterinary medicine, Clinic for surgery, orthopedics and ophthalmology, Heinzelova 55, 10000 Zagreb, Croatia

Atopic dermatitis is a genetically predisposed, chronic, pruritic, inflammatory skin disease. It occurs as a consequence of immune system hypersensitivity to environmental allergens. Atopic dermatitis is a frequent dog disease that presumably affects approximately 10% of the world's dog population. Itching and secondary skin lesions, such as erythema and lichenification, result from an inflammatory process. Those typical skin lesions, as well as pruritus, occur on the face (periocular and mouth), concave aspect of the ear pinnae, ventrally on the neck and stomach, axillary, inguinal, interdigital, perianal region and around the joints on extremities. The pathogenesis of canine atopic dermatitis is complex and the diagnosis is still clinical. As opposed to human medicine, in veterinary medicine, there exist no clear criteria for diagnosing allergic conjunctivitis. Although atopic dermatitis is a common disease, little is known about its ophthalmological aspect of it. Clinical symptoms are periocular edema and pruritus, conjunctival congestion, chemosis and lacrimation along with serous or mucous discharge. Dry eye disease or keratoconjunctivitis sicca is an eye surface inflammatory disease caused by a pathological decrease of a lacrymal component of the tear film. Immune-mediated etiology is most frequent in dogs, and as opposed to human medicine, where it is well known that it's an enviromentaly caused allergic disease, in veterinary medicine this fact is seldomly investigated. Schirmer tear test, as a method of measuring tear production in animals as well as in humans, physiological counts between 15 and 25 mm/min in dogs. The subject of this research are dogs, patients of the University of Zagreb Veterinary hospital, diagnosed with atopic dermatitis. The goal is to perform an ophthalmological examination on these dogs and investigate if any deviation from physiological measures exists. Therefore a presumable connection between atopic dermatitis and allergic conjunctivitis will be revealed. In 24 dogs, from aged 1 to 15, that underwent no systemic or topical therapy, an ophthalmological examination was performed. Tear film production was measured using Shirmer tear test strips (STT strips, Eickermeyer) conventionally. 25% (6/24) of dogs had low measurements (mean and median 11,5). None had increased STT values, so the rest (75%, 18/24) dogs had normal STT values. T-test showed very high statistical significance ($p<0.0001$) between decreased and normal STT groups in atopic dogs. Therefore, we can conclude that decreased STT (and KCS) is frequent in atopic dogs.

Key words: atopic dermatitis, canine, keratoconjunctivitis sicca

P19

STRATEGY FOR DIAGNOSIS, TREATMENT AND LONG TERM MANAGEMENT OF CANINE ATOPIC OTITIS EXTERNA

Ivelina Vacheva[1], Tandzhu Mehmedov*[2], Krasimira Genova[2]

[1]Multidisciplinary veterinary clinic Bulgaria, Lilya 8 str., Sofia 1614, Bulgaria
[2]University of Forestry Saint Kliment Ohridski, 10 Blvd, Sofia 1756, Bulgaria

Atopic otitis externa is an inflammation of the external ear canal caused by environmental allergen. It is often characterized by a correlation between otitis flare episodes and seasonal allergy periods. In many cases, the otitis is the first sign of an underlying allergy condition. Successful management involves identification and analysis of all involved factors, being primary, secondary, predisposing and/or perpetuating. Our study was comprised of a total of 29 dogs (n=29) of different breeds, the youngest being 11 months old and the oldest 10 years old. Diagnosis of atopic otitis was based on history, clinical signs, general examination, dermatological and otoscopic examination, cytology of the ear canal, culture and sensitivity testing, MRI and food diet trial with hydrolysed protein in order to differentiate atopic vs food induced otitis. Treatment was based on a reactive therapy followed by a proactive therapy aiming the long-term well-being of the patients. The general principles of the treatment include reducing inflammation, ear cleaning, video-otoscopy, deep ear flushing, elimination of microbial infections, use of glucocorticoids and addressing the primary causes of the otitis. Long-term management is an important part of a well build strategy. It involved long-term treatment of the primary cause, monthly check-up at the clinic, weekly proactive therapy with 1% dexamethasone eardrops, at-home cleaning of the ear canal with personalized frequency and materials and detailed instructions for proper care and symptom evaluation to the owners. Long-term management therapy involves ear cleaner with N-Acetyl Cysteine + EDTA or a Cerumenolytic ear cleaner; Allergen specific immunotherapy (ASIT), Oclacitinib, Cyclosporine and only in persistent cases use of systemic glucocorticoids. Owner feedback is extremely valuable as early detection of symptom recurrence is crucial for the ultimate success. In this study, all but one of the patients exhibited long term dissipation of symptoms without relapsing for a period of 6 to 8 months.

Key words: atopic otitis externa, long-term management, video-otoscopy, deep ear cleansing, ear cytology

P20

SURGICAL LIGATION OF PATENT DUCTUS ARTERIOSUS IN A DOG

Nihan Dikbaş*[1], Oktay Düzgün[2]

[1]Istanbul University- Cerrahpaşa Institute of graduate studies, Avcılar, 34320, Istanbul, Turkey
[2]Istanbul University- Cerrahpaşa Faculty of veterinary medicine, Department of surgery, Avcılar 34320, Istanbul, Turkey

The ductus arteriosus provides a connection between the truncus pulmonalis and the aorta descendens during the fetal period. If this vessel does not close after birth and continues for more than a few days, it is called ductus arteriosus persistens (PDA). It is the most common congenital heart defect in dogs. It is more common in purebred female dogs. Some breeds such as Maltese Terrier, Toy Poodle, Yorkshire Terrier and Pomeranian are predisposed. Most young animals are asymptomatic, but mild exercise intolerance may occur. It typically causes left-to-right shunt and left ventricular dilatation, resulting in volume overload in the left ventricle. It usually causes left congestive heart failure and pulmonary edema up to 1 year of age. Depending on this, developmental delay, exercise intolerance, cough, and anorexia can be seen in the later stages. Rarely, the flow in the shunt changes from right to left and pulmonary hypertension develops. It mostly develops as a complication of PDA that is not closed, and it can rarely be seen after birth due to persistent pulmonary hypertension and its closure is contraindicated. The only treatment option in left-to-right shunt is duct closure. The most appropriate period is 8-16 weeks of age, and in this period, the morphological changes return to normal within 2 months after the operation. If complications do not develop, the patient completely recovers from the heart defect. Extravascular surgical ligation and intravascular transcatheter occlusion options are available. In this presentation, the clinical examination findings, diagnosis, surgical ligation method and results of PDA in a dog are presented. The case was a 4-month-old cross-breed female dog brought to Istanbul University- Cerrahpaşa Veterinary Faculty with a history of cough and exercise intolerance. In the clinical examination, a continuous (machinery) murmur was detected on auscultation, and a diagnosis of PDA from left to right was made with the echocardiogram taken after the radiological examination. After the hemogram and biochemical blood analyzes were evaluated, the patient was anesthetized. The operation area was prepared with routine asepsis and antisepsis rules. After the approach with the left fourth intercostal space thoracotomy, the ductus was ligated. Echocardiographic findings of the patient were evaluated every 3 months. At the 1st year follow-up, it was observed that the morphological changes regressed considerably and the clinical symptoms disappeared completely. As a result, in this case, the prognosis was positive with early diagnosis, surgical ligation method and appropriate postoperative treatment.

Key words: dog, patent ductus arteriosus, surgical ligation

P21

IS fPl2 A RELIABLE BIOCHEMICAL MARKER FOR DIAGNOSING FELINE PANCREATITIS?

Larisa Shakjiri, Elena Atanaskova Petrov, Irena Celeska,
Goce Atanasovski, Sara Gjorgjievska

Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine – Skopje, Lazar Pop-Trajkov 5-7, 1000, Skopje, North Macedonia

By definition, pancreatitis is an inflammatory disease with a vague nature of manifested clinical signs. The nonspecific clinical and laboratory findings make the disease difficult to diagnose and for a definite diagnosis, invasive methods are needed such as histopathology of a biopsied specimen of the pancreas. Results of the routine biochemistry and complete blood count are highly variable and most of the time not contributory to the diagnosis. Abnormalities commonly seen are leukocytosis or leukopenia, elevated liver enzymes, elevated amylase concertation, hypoalbuminemia, hyperglycemia. Useful asset is the fact that the pancreas is source of some substances, which are solely synthetized in this organ and might be detected in the blood stream if a disease is present. This includes the feline trypsinogen, trypsin-activation peptide and *pancreas specific lipase.* However, normal values do not exclude the presence of pancreatitis. The aim of this report is to describe a case of feline pancreatitis with clinical manifestation of the disease and normal fPl2 levels. Other proximate causes of the clinical sings were excluded. Our patient, a 13 years old, male, mix breed cat, was admitted to the University Veterinary Hospital with the following symptoms: lack of appetite, vomiting, diarrhea, lethargy. During physical examination, the cat showed severe abdominal pain, dehydration 8%, low body weight index and a normal body temperature and vital signs. The ultrasound exam and the x-rays showed no apparent specific changes. Biochemical and hematological results showed a mild leukopenia, elevated liver enzymes and amylase levels. The initial fPl2 was normal, while the following one, which was made two days later was increased but within normal reference ranges. The use of the specific pancreatic lipase as a diagnostic and prognostic tool might be useful but it is not always dependable in all the cases of feline pancreatitis. Our case was one of those where this parameter was not a marker of the inflammatory disease even though all the symptoms were present and differential diagnosis was made which ruled out all other possible causes of the clinical signs. Our therapeutic approach was directed towards eliminating the symptoms, nutritional support and pancreatic enzymes supplementation, after which the patient got better. This case showed that the fPl2 levels should not be taken straightforward and should be interpreted in conjunction with the clinicopathological findings.

Key words: cat, pancreatitis, fPl2

P22

HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF BLOOD IN SPORTS AND WORK HORSES AT REST AND AFTER PHYSICAL ACTIVITY

Ermin Šaljić*[1], Maja Varatanović[2], Ratko Ralević[3]

[1]University of Sarajevo, Veterinary faculty, Clinic for internal diseases, oncology and emergency medicine, Zmaja od Bosne 90, 71000 Sarajevo, Bosnia and Herzegovina
[2]University of Sarajevo, Veterinary faculty, Department of animal production and biotechnology, Zmaja od Bosne 90, 71000 Sarajevo, Bosnia and Herzegovina
[3]Private veterinary institution "Dr Ralević," Berane, Svetog Save bb, 84300 Berane, Montenegro

The development of the equestrian sports in BiH and the use of horses in heavy work in the forest indicates the need for relevant monitoring of the health status of these animals. The aim of the study is assessment of blood counts in sports and working horses during rest and after physical activity, since it is most often associated with horse training and is used to assess sport performance. Examination of certain biochemical parameters, such as total proteins and serum albumin, also provides useful data on the health status of the horse. This study was conducted on 37 horses of different breed, divided into two groups: sport horses and work horses. The group of sports included Arabian thoroughbreds (n=17), and the group of workers included crossbreeds (n=20) aged 6 to 11, which were used or are still used in long-distance riding competitions and for work in the forest. Blood sampling was done before the training and 5-10 minutes after completion. The results showed statistically significant differences for erythrocyte values after physical activity in both groups of examined horses in both sampling periods. Hemoglobin and hematocrit values differed statistically significantly in the post-exercise period between sport and work horses. The statistically significant difference was found in the MCH and MCHC between sport and work horses after the physical activity. Mean leukocyte counts showed a statistically significant difference between sport and work horses at rest. The obtained mean values for urea, glucose, creatinine showed statistically significant differences within both groups between sampling periods. A statistically significant difference of albumin levels was found for working horses between the examined periods. Mean values for ALT showed a statistically significant difference between sport and work horses only at rest. The activities of enzymes amylase and alkaline phosphatase had statistically significant differences between sport and work horses in both study periods. The physical activity in sport horses had a more significant impact on hematological and biochemical parameters compared with the working horses.

Key words: Arabian thoroughbred, working horse, hematological and biochemical parameters

P23

INFLUENCE OF SEX, AGE AND SEASON ON LYMPHOCYTE SUBPOPULATIONS IN PHEASANTS

Tandzhu Mehmedov*[1], Krasimira Genova[1], Ivelina Vacheva[2]

[1]University of Forestry Saint Kliment Ohridski, Faculty of veterinary medicine, 10 Blvd, Sofia 1756, Bulgaria
[2]Multidisciplinary veterinary clinic Bulgaria, Lilya 8 str., Sofia 1614, Bulgaria

Immunocompetent cells respond to various external and internal factors by changes in the expression of surface or intracellular functional molecules. Thus, the cell adapts and perform better its inherent regulatory or effector functions. Today there is an intensification of hunting on a global scale, but pheasant farming and technology have created the conditions for the emergence of a number of diseases. The lymphocytes, together with the cells of innate immune system, provides increased protection against different pathogens. In this regard, the absolute amount of lymphocytes, phenotyping of peripheral blood lymphocyte subpopulation and their functional activity are of extremely great importance. Our objectives were to estimate subpopulations of lymphocytes in peripheral blood from phasans and to estimate role of the sex, age and season. The research is based on 104 pheasants (n=104) of different sexes and ages. According to the age birds were divided in 4 groups - newly hatched (n=20), on 28 day (n=20), on 40 day (n=20) and adults (n=44). The adult pheasants were further divided into two subgroups (n=22) - male and female. From them the blood were taken for immunological studies during the different seasons. For the phenotyping of lymphocytes we used spontaneous rosette tests and indirect rosette tests. Immunocytoadherent technic revealed that there is no difference in the absolute number of T- (E-ROK) and B- (EAC-rosettes) cells according to the sex. We found that the number of B-lymphocytes and helper cells increases with age, and we showed the highest percentage of lymphocytes in autumn, which correlated with a higher percentage of Th1 and Th2 helpers and an increase in the content of cytotoxic T cells. The obtained data show not only the trends of the changes, but they are also the basis for analysis in various pathological conditions.

Key words: pheasants, immune phenotyping, immunocytoadherent technic, E-ROK and EAC-rosettes

P24

DETERMINATION OF PESTICIDE RESIDUES IN STERILIZED MILK USING MODIFIED QUECHERS SAMPLE PREPARATION FOLLOWED BY GC/MS

Aleksandra M. Tasić*[1], Ivan Pavlović[1], Tanja Šolovic Knudsen[2]

[1]Scientific institute of veterinary medicine of Serbia,
Janisa Janulisa 14, 11107 Belgrade, Serbia
[2]University of Belgrade, Institute of chemistry, technology and metallurgy,
Department of chemistry, Njegoševa 12, 11000 Belgrade, Serbia

In recent years, pesticide residues in processed food have become the focus of consumer attention. Processed food contamination with pesticide residues is a source of serious concern. Free of contaminants, sterilized milk is an essential augmentative and healthy food for infants and the elderly population. Animal digestion and absorption of pesticides from the environment and animal feed are the main way of possibly finding residues in products such as sterilized milk. The occurrence of pesticide residues in sterilized milk is not so rare. For this reason, a large number of modified QuEChERS methods have been developed for the determination of different classes of pesticides in milk. The QuEChERS method is primarily used for non-fatty food samples, including natural agricultural products. The aim of this research was to modify the QuEChERS method for pesticide multiresidue analysis in sterilized milk samples using gas chromatography coupled to mass spectrometry (GC/MS). The following pesticide were determined: Fenthion, Alpha Endosulfan, Beta Endosulfan, Endosulfan sulphate, Fensulfothion, Iprodione, Tetramethrin and Spirodiclofen. Pesticide residues were extracted using the modified QuEChERS technique with acetonitrile and then clean-up using the dispersive solid phase extraction (d-SPE). The conditions for extraction solvent and freezing time were optimized. Compared to the currently established EN 1528 method for determination of organochlorine pesticides, this validated method uses much less solvent, it is quick and easy to prepare a sample for analysis. The matrix effects were compensated with matrix match calibration. Matrix-matched solutions were prepared by suitably diluting the intermediate solution with blank milk sample extracts containing none of the tested analytes to perform matrix-matched calibration. The linearity of the analytical response across the studied range of concentrations (0.01-0.10 mg kg^{-1}) was excellent, obtaining correlation coefficients higher than 0.99. The average recoveries of the pesticide ranged from 75.6 to 109.2 %, for fortification levels of 0.01; 0.02 and 0.1 mg kg^{-1}. The precision values associated with the analytical method, expressed as RSD values, were less than 20% for the pesticide in the milk matrixes. The limit of quantification of the tested pesticides was reached at the level of 0.006 mg kg^{-1}. The developed method complies with all validation parameters according to SANTE/12682/2019 for the studied sterilized milk matrix and achieves a sensitivity suitable for the purpose of all considered analytes.

Key words: pesticides, sterilized milk, GC/MS, extraction

Acknowledgments: The study was funded by the Serbian Ministry of Education, Science and Technological Development (Contract No 451- 03-68/2022- 14/200030)

P25

NITRITES IN SMOKED MEAT PRODUCTS

Tanja Č Bijelić*[1], Aleksandra M Tasić[1], Djordje D Radojičić[1]

[1]Scientific institute of veterinary medicine of Serbia,
11107, Janisa Janulisa, 14, Belgrade, Serbia

Nitrite is used for preparation in meat products as an additive because they have preservative properties and have the ability to inhibit the growth of some pathogens. Salting was one of the first methods used for meat preservation, while the use of nitrite occurred first through the accidental presence of nitrate in the salt, and then the use was retained. The importance of these salts is due to the fact that they give dried meat a characteristic taste and color, and in addition the presence of nitrite enables prolonged storage of meat products. Nitrites as additives prevent the rancidity of lipids in meat, prevent the growth of microorganisms and contribute to the taste of smoked meat products. In the form of their salts, they are used for drying meat, and they are also found in the form of natural compounds in many food products and thus represent a part of the human diet. As nitrites are common additives added to cured meat products, due to their potentially dangerous effect, research on the safety of cured meat and smoked meat products was started in the late 1960s. Nitrites react with proteins and form the carcinogenic compound nitrosamine, which increases the risk of cancer in people who like meat foods. This research was done with the aim of determining the amount of nitrite in smoked meat products in the period from 2020 to 2022 in Serbia. There are many methods for the determination of nitrite in food, which can be divided into two categories: spectroscopic methods and chromatographic methods. In addition to them there are also electrochemical, as well as the method of rapid detection. For this research, a spectroscopic method was used, ISO 2918:1999, at a wavelength of 538 nm. An analysis of 39 samples of smoked meat product was performed, of which 23 samples of smoked pork neck and 16 samples of smoked pork ribs. The test results gave mean levels of nitrite content expressed as sodium nitrite ($NaNO_2$) for smoked pork neck of 30.99 mg/kg (maximal concentration 150 mg/kg), while for smoked pork ribs a value of 26.54 mg/kg (maximal concentration 150 mg/kg) was obtained. Based on this research, it can be concluded that the concentration of nitrite in the investigated smoked meat products, expressed as $NaNO_2$, was below the established maximum allowed values according to National and European regulations.

Key words: nitrite, pork meat, smoked ribs, smoked neck

Acknowledgments: The study was funded by the Serbian Ministry of Education, Science and Technological Development (Contract No 451- 03-68/2022- 14/200030)

P26

EFFECT OF MUSCLE TYPE ON FATTY ACID PROFILE OF ROE DEER MEAT

Marija Pavlović*, Aleksandra Tasić, Ksenija Nešić, Mihajlo Vićentijević, Vladimir Radosavljević, Ivan Pavlović

Scientific veterinary institute of Serbia, Janisa Janulisa14, 11000 Belgrade, Serbia

Roe deer meat is considered as highly valuable red meat, which quality parameters varies according to species, nutrition, hunting season, age, gender, carcass weight, type of muscle etc. The aim of this study was to examine and compare selected meat quality parameters of roe deer (Capreolus capreolus L.) obtained from two different muscles (musculus gluteus superficialis and musculus longissimus thoracis). During the hunting season in 2021, twenty samples of roe deer meat were collected: 10 samples of musculus gluteus superficialis (GS) and 10 samples of musculus longissimus thoracis (LT). In examined samples was determined chemical composition through the following parameters: moisture (ISO 1442:1998), fat (ISO 1443:1992), ash (ISO 936:1999), protein (ISO 937:1992), as well as fatty acid profile. The AOAC 996.06:2001 method was applied for the lipid extraction from the tissue. After the lipid hydrolysis, the fatty acids were esterified to methyl esters, that were analyzed by an internal standard method using a gas chromatograph and comparing peak areas and retention times with a standard mix of FAMEs. Conditions of analyses: detector temperature - 250°C, injector temperature - 225°C, column temperature - 200°C, carrier gas - helium, carrier gas flow rate - 50 mL/min. Obtained data for fatty acids composition were expressed in percentage by weight of the identified total fatty acids. The differences between means were compared by t test at the level of significance of 95% and 99%. Proximal chemical composition of roe deer meat (moisture 71.40% and 71.80%; fat 1.65% and 2.21%, protein 22.14% and 22.11%, ash 1.19% and 1.01% for LT and GS, respectively) showed no significant differences ($P>0.05$) for all the examined parameters except for total fat content ($P<0.05$). Furthermore, there were differences among all determined saturated fatty acids ($P<0.05$), except for Arachidic acid, and for the unsaturated the siginificant differences were among the content of Myristoleic, Oleic, Eicosenoic and Linoleic acid. The ratio of polyunsaturated to saturated fatty acids (0.476 and 0.546 for LT and GS, respectively) and monounsaturated to saturated fatty acids (0.385 and 0.436 for LT and GS, respectively) differed significantly ($P<0.05$). Indicators of the nutritional value and health benefits of fat determined based on analysis of the fatty acid profile of roe deer meat shows that GS had a more favorable characteristics compared to LT. In general, obtained results suggest that type of muscle has a significant influence on the fatty acid profile in roe deer meat.

Key words: roe deer, meat, muscle, fatty acids

P27

EVALUATION OF HEPATO-RENAL TOXIC HAZARDS OF GLUTAMATE AND SULFITE SODIUM IN BROILER CHICKENS

Eman E. Elsharkawy*[1], Doaa S. Abd El-Maguid[2]

[1]Assiut University, Faculty of veterinary medicine,
Department of forensic medicine and toxicology, Assiut, Egypt
[2]Assiut University, Faculty of veterinary medicine,
Department of forensic medicine and toxicology, Branch of New Valley, Egypt

Monosodium glutamate (MSG) and sulfite are frequently used as flavor enhancer and most applied as food additives in modern nutrition globally. This study planned to investigate the potential toxic effect of MSG on liver and kidney when administered to broiler chickens during the growth period. Forty, day-old, unsexed Ross broiler chicks, they assigned into 4 groups: 10 chicks each, fed on standard diet mixed with 0.75g of MSG/kg (group A), sodium metabisulfite 3.5/kg (group B), 0.75g MSG + 3.5g sulfite /kg (group C) and control group (group D). Oxidative stress indicators assay such as malondialdehyde (MDA), and superoxide dismutase (SOD), activities were determined. Liver and kidney function testes foe alp enzyme and creatinine metabolite were examined. Histo- architecture of liver and kidney tissues were conducted in all exposed groups. The results indicated that the increase in the determination levels of serum ALP, creatinine, MDA and SOD in all exposed groups in comparison with control. Disturbance in hepatic architecture with hydropic changes in hepatic cells with congestion of the interstitial blood vessels and necrobiosis of renal tubular epithelium.

Key words: monosodium glutamate**,** metabisulfite, oxidative stress SOD, liver, kidney, broiler

P28

THE IMPACT OF THE UKRAINE WAR ON FOOD PRICES IN MACEDONIA

Blagica Sekovska*, Radmila Crceva Nikolovska,Aleksandra Angeleska,
Katerina Blagoevska, Viktor Denkovski, Vasilka Poposka Trenevska

Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine, Food institute, Lazar Pop – Trajkov 5-7, 1000 Skopje, North Macedonia

The impact of the war in Ukraine on food prices in Europe, and especially in the Western Balkans, speaks to the fact that Ukraine is one of the largest producers of cereals and other crops in Europe. Conflict causes price shocks and supply issues, especially with respect to cereals and cooking oil, and thus contributes to an increase in commodity market prices, and consequently the increase in trading prices and finally, increase in retail prices.Through the analysis of the available data, a correlation will be made between the consumption, stocks and prices of cereals and other crops that are of exceptional importance for Macedonia and the wider region. The use of the statistical method in the form of statistical time series correlated with the current situation will show the impact of the war in Ukraine on market movements in the region, and especially in Macedonia. All the processed data indicate that conflict in the Ukraine contribute significantly to the price of food not only due to the fact that much of it is produced in the Ukraine, but also due to the interruption of supply chains and rising transaction costs. As far as Macedonia is concerned, the analysis shows that we are an import-dependent country. Therefore an appropriate strategy for the development of agriculture is necessary, to a large extent this refers to measures for the possibility of larger plantations for cereals, especially wheat. In addition to this, policies and strategies are needed that will use analyzes from the past, which will refer to sufficient replenishment of commodity reserves so that the possibility of a significant shortage of basic food products such as bread or cooking oil, and also other critical food products, is mitigated.

Key words: food, shortage, Ukraine

P29

VALIDATION OF THE RAPID ELISA METHOD FOR DETERMINATION OF METHYLTESTOSTERONE IN URINE AND MUSCLE BASED ON THE NEW REGULATION (EU) 2021/808

Risto Uzunov*, Zehra Hajrulai-Musliu, Stefan Jovanov, Elizabeta Dimitrieska-Stojkovikj, Aleksandra Angeleska, Biljana Stojanovska-Dimzoska, Velimir Stojkovski

Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine-Skopje, Food institute, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia

Methyltestosterone is a synthetic anabolic steroid that can be used in meat production animals to improve the weight gain and the meat/fat-ratio, but the residues of this anabolic steroid in meat present a potential risk for public health. In the European Union and most countries worldwide the use of methyltestosterone and other anabolic steroids as growth promoter in livestock production is completely banned. In this study an enzyme-linked immunosorbent assay (ELISA) screening method for detection of methyltestosterone in urine and meat has been validated according to the Regulation (EU) 2021/808. Regulation (EU) 2021/808 is a new regulation that replaces the Commission Decision 2002/657. The sample preparation procedure for urine involved hydrolysis and clean-up with solid phase extraction (SPE) using C18 cartridges, while the sample preparation for muscle included extraction with methanol, defatting with hexane and clean-up with SPE using C18 cartridges. The concentrations of the residues were measured with ELISA reader at 450 nm. In the validation procedure were included linearity, selectivity/specificity, limit of detection (LOD), decision limit (CCβ), trueness (expressed through recovery) and precision (expressed through repeatability and reproducibility). The method demonstrated excellent linearity with coefficient of variation (R^2) 0.9966 for urine and 0.9997 for muscle, in the calibration range from 0.125 to 4.5 µg/L. The results for selectivity/specificity indicated that there are not matrix interferences. LOD was 0.25 µg/l for urine and 0.25 µg/kg for muscle, while CCβ was 0.45 µg/l for urine and 0.60 µg/kg for muscle. These values for CCβ were lower than new Minimum Method Performance Requirements (MMPR) values for methyltestosterone. The new MMPR values for methyltestosterone, which were adopted in 2020, for urine is 0.5 µg/l, while for muscle is 1.0 µg/kg. The method yielded acceptable recovery rate between 70-120% in the fortified samples from urine and muscle. The coefficient of variation (CV) for repeatability was less than 15.0 %, while the CV for reproducibility was less than 20.0%. The method fulfils the criteria prescribed in Regulation (EU) 2021/808 and can be used as rapid screening method for the routine detection of methyltestosterone in urine and muscle.

Key words: methyltestosterone, urine, muscle, validation, Regulation (EU) 2021/808

P30

VALIDATION OF UHPLC-MS/MS METHOD AFTER MODIFIED QUECHERS SAMPLE PREPARATION FOR MONITORING OF PESTICIDE RESIDUES IN BOVINE MILK ACCORDING TO EC/2018/555

Elizabeta Dimitrieska-Stojkovikj*[1], Aleksandra Angeleska[1], Dushica Koceva[1], Biljana Stojanovska-Dimzoska[1], Goran Stojković[2], Gordana Ilievska[1], Risto Uzunov[1], Zehra Hajrulai-Musliu[1]

[1]Ss Cyril and Methodius University from Skopje, Faculty of veterinary medicine-Skopje, Institute for food, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia
[2]Ss Cyril and Methodius University from Skopje, Faculty of natural sciences and mathematics, Institute for chemistry, Arhimedova br. 5, 1000 Skopje, North Macedonia

The EU Regulation EC/555/2018 has foreseen implementation of monitoring program for selected pesticide residues in bovine milk, aimed for further dietary exposure assessment. Therefore, since 2019 the required pesticide substances were introduced into the National Residue Control Program for animal origin food in North Macedonia. For this purpose, UHPLC-MS/MS method was optimized and validated after modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) sample preparation. The MS/MS method was optimized for 19 pesticide substances and metabolites: carbaryl, carbofuran, fenvalerate, indoxacarb (carbamate pesticides), cypermethrin, deltamethrin, bifenthrin permethrin (pyrethroids), malathion, parathion, diazinone, dichlorvos, chlorpyriphos, chlorpyriphos-methyl, pyrimiphos-methyl (organophosphates), famoxadone, fipronil, including metabolites fipronil sulfone, fipronil sulfide. For each compound the two most sensitive MRM transitions were selected for further determination. Analysis was performed on Waters UHPLC-MS/MS system consisted of H-class UHPLC and Xevo TQ-S micro triple quadrupole detector, in ESI+ and ESI- mode. Chromatographic separation was performed on C18 column with 100 mm length and 1.7 μm particle diameter, using gradient elution with water and methanol, both modified with formic acid and ammonium formate (0.1 % v/v and 5 mmol/L, respectively), at flow rate of 0.45 mL/min. The optimized method was validated according to DG SANTE Document 2017/11813. Validation parameters were linearity, limit of quantification (LOQ), precision, accuracy, and matrix effect. The determined LOQs from the concentration level at which S/N ratio was higher than 1:10, were in the range 1.0 – 8.3 μg/L, and were equal or below the MRL values for the pesticides of interest. Linearity was determined in the range 1-100 μg/L, obtaining five-level calibration curves with correlation coefficients ≥0.99 for all tested analytes. Method precision was tested at two concentration levels - 10 and 50 μg/kg), except for carbofuran, for which the target levels were 1 and 10 μg/kg. The calculated relative standard deviation (RSD) ranged from 6.3 to 15.5 %. The method accuracy, estimated from the recovery at two concentration levels, was in the range 73.8 – 107.3 %. Matrix effect did not have significant influence on the analyte signal suppression or enhancement, thus solvent

standards could be used for calibration. The method performance characteristics were satisfactory according to the SANTE document requirements (LOQ ≤ MRL, precision ≤20 %, and recovery 70-120 %). With this, it was proven that the proposed method was suitable for analysis of the defined scope of substances, even for pyrethroids, for which gas chromatography was considered to be a more appropriate analysis technique.

Key words: pesticide residues, monitoring, bovine milk, UHPLC-MS/MS, validation

P31

VALIDATION PROTOCOL FOR DETERMINATION OF FUMONISINS IN CORN

Biljana Stojanovska Dimzoska*, Elizabeta Dimitrieska Stojkovic, Zehra Hajrulai-Musliu, Risto Uzunov, Katerina Blagoevska, Aleksandra Angeleska

Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine, Food institute, Lazar Pop Trajkov 5-7, 1000 Skopje, North Macedonia

Fumonisins are a group of at least 15 closely related mycotoxins produced principally by *Fusarium verticillioides* and *Fusarium proliferatum*. Fumonisin B_1 is the most frequently found in food products (especially corn) representing 70–80% of the total of fumonisin content and, together with FB_2 and FB_3, seem to be the major fumonisin due to its toxic properties. It is most important in veterinary medicine as a cause of leukoencephalomalacia in horses, liver cancer in rats, and porcine pulmonary edema. In areas of high maize consumption fumonisins may be responsible for esophageal cancer in humans. FB_1 is classified as possibly carcinogenic to humans (IARC group 2B). As an animal and human health threat, fumonisins are regulated by legislation worldwide. Maximum limits for the total content of fumonisins have been established in the EU in maize and maize based products (EC No 1126/2007). Among various analytical methods for the determination of mycotoxins, ELISA method is still the method of choice for screening purposes. The aim of this paper was to test and validate a commercial ELISA kit for determination of fumonisins. The validation procedure was performed in compliance with Commission Decision 2002/657/EC. For linearity test, six standard solutions were used in a concentration range of 0 – 2 mg/kg and a satisfactory coefficient of correlation was found. The LOD was accomplished from the measurement of the background response from 20 blank corn samples and it was found to be 50 µg/kg. The determination of trueness was performed by means of an analysis of six replicates of the CRM and the obtained value was 83.5%. Recovery, the other accuracy parameter, was achieved by fortifying blank samples at level of one-half of MRL and the obtained value was 112.6%. The both values (trueness and recovery) were in accordance to the performance criteria. CCβ was established by analyzing at least 20 blank samples fortified at level one-half of MRL and the achieved value was 0.63 ± 0.31 mg/kg. Repeatability was estimated using the data from the recovery and the RSD_r was 17.33%. The within-laboratory reproducibility (RSD_R) of the method was 27.8% which is in accordance to the EU validation criteria. The realised validation protocol shows that this rapid ELISA method is simple, because it employs staff with lesser technical training; easier, it saves time and costs, saves investments in complex instruments and it is accurate. It can be implemented for routine analysis of fumonisins.

Key words: validation, ELISA, fumonisins, corn

P32

SMALL ANIMAL MODEL IN THE DEVELOPMENT OF RADIOPHARMACEUTICALS - THE STEP FORWARD TO CLINICAL STUDIES

Emilija Janevik-Ivanovska[1]*, Icko Gjorgoski[2], Boris Aleksovski[2], Toni Tripunovski[3], Marija Darkovska Serafimovska[1], Katarina Smilkov[1], Darinka Gjorgieva Ackova[1], Marija Arev[1], Paulina Apostolova[1], Lajos Balogh[4]

[1]Goce Delcev University-Stip, Faculty of medical sciences, North Macedonia
[2] Ss. Cyril and Methodius University in Skopje, Faculty of natural sciences and mathematics, Institute of biology, North Macedonia
[3] Ss. Cyril and Methodius University in Skopje, Faculty of medicine, Institute of pathophysiology and nuclear medicine, North Macedonia
[4]Gamma-VET Ltd, Deak Ferenc utca 20, Budapest, Hungary

Experimental design is a critical component for the success of research activities involving development and evaluation of new radiopharmaceuticals. Experimental animal models have substantially contributed to a better understanding of mechanisms of disease and show the novel approaches in imaging and image analysis were equally important to meet the challenges of analyzing the complex mechanisms underlying pathophysiological processes in vivo. Proper animal models are key factors for successful pharmaceutical and medicinal experiments. To reduce animal number for ethical and financial reasons, cost-efficient methods where high quantities of data are achieved fast are optimal. Biodistribution and pharmacokinetics studies diagnostic or therapeutic radiopharmaceuticals by SPECT or PET imaging followed by *post mortem* analysis in diseases model gives a good start point for further steps toward clinical applications. In this presentation, targeting properties, biodistribution and pharmacokinetics of different molecules, as potential radiopharmaceuticals have been studied in small animal models using suitable imaging modalities and *post mortem* analysis. The following experimentally designed animal models have been introduced in our work so far as an essential part in the development of new radiopharmaceutical products and quality control of existing radiopharmaceutical products. Rat models were used to establish: stasis-induced thrombus in the femoral vein after injection of thromboplastin to demonstrate Deep Venous Thrombosis; induced amyloidosis by multiple application of beta2-microglobulin for determination of the existence of the depositing osteoarticular tissues, condition associated with hemodialysis in patients with chronic kidney diseases; collagen-induced arthritis as a model of inflammatory arthritis; bacterial abscesses by the injection of *Staphylococcus aureus*. Mouse models were used for: in vivo evaluation of the radiolabelled conjugated antibodies in normal Balb/c mice and nude mice xenografts; *per os* administration of iodine labeled BSA loaded microspheres to show the strong adjuvant effect by inducing IgA secretion at the genito-urinary mucosa; athymic nude mice tumor bearning to demonstrate

specifity of pre-targeting technique referred to the Affinity Enhancement System (AES) uses bispecific antibodies and radiolabeled bivalent haptens. The use of experimental animal models in the design of new drugs including radiopharmaceuticals is a key part of preclinical trials. Usually this approach can not fully replicate human disease or the varied and complex physical and psychological manifestations of human conditions. For these reasons the process of experimental design should be carried out routinely to ensure the generation of valid, reproducible and published data.

Key words: experimental design, rat models, Affi nity Enhancement System (AES), radiopharmaceutical products

P33

ESTABLISHMENT OF SAFER ANIMAL RESCUE CAPACITY: ERASMUS+ PROJECT

Cezmi Türkmen[1], Lazo Pendovski[2], Jože Starič[3], Halil Selcuk Biricik*[4], Zehra H. Musliu[2], Yücel Türk[1], Osman Karabulut[5], Sedat Bilgiç[1], Miroslav Kjosevski[2]

[1]Disaster and Emergency Management Directorate (AFAD) - Aksaray, Turkey
[2]Ss. Cyril and Methodius University in Skopje,
Faculty of veterinary medicine, North Macedonia
[3]Ljubljana University, Veterinary faculty, Ljubljana, Slovenia
[4]Afyon Kocatepe University, Şuhut vocational school, Afyonkarahisar, Turkey
[5]Aksaray University, Faculty of veterinary medicine-Aksaray, Turkey

The project was carried out under "Erasmus+ Adult Education Programme**",** Key Action 2: Cooperation for Innovation and Exchange of Good Practices program, and it was conducted during 2015/2017. The coordination was by Aksaray AFAD (Aksaray Disaster and Emergency Management Directorate), partnered by University of Ljubljana, Veterinary faculty - Slovenia, Ss. Cyril and Methodius University in Skopje, Faculty of Veterinary Medicine - Macedonia, and Aksaray University, Faculty of Veterinary Medicine to contribute the further development of animal rescue in Turkey and partner countries. The scope of the project was training of search and rescue teams for animals. Safer animal rescue workshop was carried out in Ljubljana, Slovenia with participation of future trainers of animal search and rescue teams. Trainees were acquainted with basic anatomy, physiology, nutrition, biosecurity, welfare, safe handling of animals and clinical examination techniques. Role of veterinarians, fire fighters and military personel in technical rescuing was introduced. International Animal Rescue Conference as a part of the project was held on 8 July 2017 in Aksaray. Brochures regarding the technical rescuing were compiled for the future trainees to be prepared them for safer animal rescuing in case of disasters. Different cases of technical rescuing were studied and introduced in the book. Within the scope of the project, animal rescue event, TV commercials, Safer animal rescue brochures and technical books, flyers and posters for gaining public awareness about needs of animals and safer rescuing during disasters were released. All the goals of the project were successfully achieved, including increase of animal rescue capacity of governmental organisations through the trainings, decrease of unwanted complications during rescuing, reduction of economic losses and increase of public awareness on animal rescue.

Key words: Erasmus+, adult education, animal rescue

P34

LET ANIMALS BREATHE EASIER IN FIRE

Halil Selçuk Biricik*, İbrahim Durmuş

Afyon Kocatepe University, Şuhut vocational school, Department of laborant and veterinary health, Afyon Street, 69, 03100 Afyonkarahisar, Turkey

Sivil Düşün is an European Union programme supporting active citizens and civil society organisations throughout Turkey. This study is supported by Sivil Düşün programme. Fire brigade teams make the first response in forest and house fires. Like other disasters, firefighters give priority to human rescue activities in case of fires, and animal rescue activities are overlooked. The aims of this study were to determine current emergency animal rescue capacities of fire departments through questionnaires and to raise awareness on the use of oxygen masks in fire. The target group of this study is the firefighters working in the cities of Aegean region in Turkey. The main activity is to encourage firefighters to use Oxygen masks for animals, unconscious due to smoke or have breathing difficulties in fires. In recent years, animal owners have suffered greatly in fires and other disasters in our country. Last year, especially because of the forest fires, many large and small animal barns were burned and animals such as cows and sheeps perished. In recent years, animal owners have therefore suffered greatly in fires and other disasters in our country. With the widespread use of Oxygen masks in fire, it will make a positive contribution to animal welfare at the regional level. Within the scope of the study, awareness activities as seminars and events have been achieved. Current study will contribute to animal rescue strategies in case of fire and other emergencies in Turkey.

Key words: Sivil Düşün, EU Programme, animal, fire

P35

REVIVING OF FARRIERY DISAPPEARING PROFESSION: ERASMUS+ PROJECT

Halil Selçuk Biricik*[1], Lazo Pendovskı[2], Kaspars Kovalenko[3], Ksenija Ilıevska[2], Aija Malnıece[3], İbrahim Durmuş[1]

[1]Afyon Kocatepe University, Şuhut vocational school, Afyon Street 69, 03100 Afyonkarahisar, Turkey
[2]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine-Skopje, Lazar Pop-Trajkov 5-7, 1000 Skopje, North Macedonia
[3]Latvia university of life sciences and technologies, Faculty of veterinary medicine, 8 K. Helmaņa Street, Jelgava LV-3004, Latvia

"Reviving of Farriery; Disappearing Profession" was carried out under "Erasmus+ Vocational Education**",** Key Action 2: Cooperation for Innovation and Exchange of Good Practices program between 2018 and 2021. The partner institutions were Afyon Kocatepe University-Turkey. Ss. Cyril and Methodius University in Skopje and Latvia University of Life Sciences and Technologies. The objectives were: to contribute improvement of working equines and cows hoof health and animal welfare, to start long-term cooperation and partnership among project partners, to develop farriery field capacities of partner associations. International Conference on Farriery and Allied Veterinary Sciences as a part of the project was held to share experiences among participants (>200) from Turkey, Pakistan, North Macedonia, Slovenia, Serbia and other parts of the world. International Farriery Workshop has been held by participation of experienced farriers, veterinary students, academicians. First Short-term joint staff training event consisting of basic farriery practices under spervision of experienced farriers took place in Jelgava, Latvia with the participation of representatives from the partner institutions. Additionally, blended mobility of higher education students to increase awareness level of veterinary and Veterinary technician students on hoof health welfare of horses and cows was organized. A field survey with face-to-face interviews among experienced farriers to form farriery satisfaction analysis was conducted in Afyonkarahisar and surrounding provinces. Photo exhibitions about the history of farriery in Turkey have been organized in partner countries. A scientific article about horseshoeing principles was published in Turkish Journal of Agriculture. Project social media accounts (twitter, facebook, linkedin, instagram, youtube channel) have been used actively exceeding 400 000 visitors. To make project results more visible, we participated in 4rd and 5th edition of the ErasmusDays which took place on 2020 and 2021. Farriery families documentary was published at project youtube channel (https://www.youtube.com/channel/UCH6u7_QVVoYJ7qYBpFNEPEQ). A hand written farrier's memories, with 87 years old were prepared. As part of this project, the Erasmus+ Inter-institutional agreement 2021-2027 was signed between the Universities of Afyon Kocatepe, Ss. Cyril –Methodius and Novi Sad. Afyonkarahisar Agriculture, Livestock and

Farriery Museum, first farriery museum in Turkey, has been established with collaboration of Afyonkarahisar Governorship, Municipality, Afyon Kocatepe University. The museum includes formerly used (dating back 300 years) antique agricultural and farriery tools. We would like to thank our citizens who donated the materials free of charge. Currently it is open to local and foreign visitors under administration of Afyonkarahisar Municipality.

Key words: Erasmus+, vocational education, Turkish national agency, farriery

P36

GENETIC DATA FROM 19 MICROSATELLITE MARKERS FOR INDIVIDUAL IDENTIFICATION AND PARENTAGE ANALYSES OF CANINES RAISED IN REPUBLIC OF NORTH MACEDONIA

Nikola Adamov[1]*, Zagorka Popova[2], Igor Esmerov[3], Branko Atanasov[4], Ljupco Mickov[4], Monika Dovenska[4], Martin Nikolovski[5], Toni Dovenski[4]

[1]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine – Skopje, Department of animal science, Lazar Pop Trajkov 5-7, 1000 Skopje, North Macedonia
[2]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine – Skopje, Laboratory for serology and molecular diagnostics, Lazar Pop Trajkov 5-7, 1000 Skopje, North Macedonia
[3]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine – Skopje, Department of biochemistry and cell biology, Lazar Pop Trajkov 5-7, 1000 Skopje, North Macedonia
[4]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine – Skopje, Department of reproduction, Lazar Pop Trajkov 5-7, 1000 Skopje, North Macedonia
[5]Ss. Cyril and Methodius University in Skopje, Faculty of veterinary medicine – Skopje, Department of functional morphology, Lazar Pop Trajkov 5-7, 1000 Skopje, North Macedonia

Microsatellite or STR DNA markers have become internationally recognized as a genetic tool for individual identification and parentage control in dogs. Eighteen STR loci and one sex determining locus (amelogenin) recommended by the ISAG which are included in the Thermo Scientific Canine Genotypes Panel 1.1 Multiplex Reagent Kit were screened in the Macedonian dog population. Microsatellite DNA polymorphism was examined using automated DNA sizing technology, providing number of alleles per locus, allele frequencies and population genetic parameters necessary for determining the application usefulness of these STR panel to individual identification and parentage analyses. A total of 30 dogs were genotyped and the obtained data were analyzed with the CERVUS software. In the analyzed material, a total of 86 alleles were detected at the 18 microsatellite DNA loci (excluding amelogenin locus). The number of alleles per locus varied from 3 (INU030) to 7 (REN162 and AHT137). Except the INU030 locus which showed the lowest genetic variation (PIC=0.416, H=0.445), the calculated PIC values exceeded 0.6 and H values ranged from 0.655 (INU055) to as much as 0.85 (REN162). Based on the combined exclusion probability (PE_C) it was found that incorrect pedigree in this canine population can be excluded with 99.9994% accuracy using DNA analysis.

Key words: canine, microsatellite DNA markers, parentage control

P37

MODIFIED SAMPLING METHODS FOR *MYCOPLASMA BOVIS* DETECTION IN DAIRY CATTLE

Lelde Titmane, Aija Malniece, Margarita Terentjeva, Kaspars Kovalenko*

Faculty of Veterinary Medicine, Latvia University of Life Sciences and Technologies, Kristapa Helmana street 8, Jelgava, LV-3004, Latvia

Several species of *Mycoplasma* spp. cause significant economic losses in dairy farms due to caused diseases in cows, including mastitis, arthritis, pneumonias, otitis media and reproductive disorders. Traditionally, the isolation and identification of *Mycoplasma* spp. is carried out using bacteriological cultivation and real-time polymerase chain reaction (qPCR). Limitations for application of existing methodology include high numbers of asymptomatic animal cases, problems associated with *Mycoplasma* spp. culturing from clinical cases and low sensitivity of serological testing. Therefore, a modified sampling was developed in order to isolate or detect respiratory mycoplasmas such as *M. bovis* in dairy cattle. The sampling included deep nasal swabbing by using commercially produced swabs and different size steel probangs (pharyngeal probe) according to the size of an animal as well as bulk milk samples. Sampling was performed in 10 dairy cattle farms of various sizes in different regions of Latvia during the year 2021. The nasal samples were placed immediately in a coal-free transport medium. The technique for obtaining of pharyngeal samples with probing was performed as for sampling in case of Foot and mouth disease. The contents of the probe cup with a sterile micropipette was transferred into a separate tube with 2 ml of mycoplasma selective broth. All samples were transported to the laboratory at 4±2°C within 4-6 hours for further isolation, extraction of DNA and qPCR reaction. Isolation of *Mycoplasm*a from samples was performed on solid mycoplasma selective medium in microaerophilic conditions (Kovalenko et al., 2021). DNA was extracted with a DNA extraction kit after what qPCR was performed (Chauhan et al., 2021). In result, 3% (6/200) and 6.5% (13/200) of nasal and pharyngeal samples contained *Mycoplasma* spp. With PCR, 10.5% (21/200) and 17% (34/200) of nasal and pharyngeal samples were positive. In total, 27% (54/200) of sampled animals were positive with one or more methods performed while all bulk milk samples were negative (0/10). The pharyngeal samples were significantly ($p \leq 0.05$) associated with the presence of *Mycoplasma* spp. either detected by qPCR or bacteriology. In conclusion, the most effective and accurate sampling method in order to detect *Mycoplasma* spp. a combination of deep nasal swabbing investigated with both qPCR and bacteriology.

Key words: pharyngeal probe, bulk milk, qPCR, Mycoplasma

P38

MORPHOMETRIC PARAMETERS OF THE MANDIBLE OF DELTARI ILIR DOG IN KOSOVO

Tefik Jashari[1], Oya Kahvecioğlu[2], Sokol Duro[3]*, Ozan Gündemir[2]

[1]Institute of Graduate Studies, Istanbul University-Cerrahpasa, Istanbul, Turkey
[2]Department of Anatomy, Faculty of Veterinary Medicine, Istanbul University-Cerrahpasa, Istanbul, Turkey
[3]Faculty of Veterinary Medicine, Agricultural University of Tirana, Tirana, Albania

The Deltari Ilir is a dog breed of the livestock guardian type which is living in the area between Kosovo, Albania, and North Macedonia. The Deltari Ilir dog is very strong and quite protective of the sheep and goats in the alpine pastures where the presence of the wild beast is very frequent. Mandible or the low jaw is a pairs bone and is part of the facies skeleton. The mandible forms the base of the oral cavity, which supports many structures such as, the tongue, salivary glands, tooth, mandibular and mental nerves, etc. Usually, the animals have different problems that need to be solved by veterinarians with the help of regional anesthesia. The aim of this study was to identify all morphometric parameters of the mandible of Deltari Ilir dog and especially the parameters with clinical importance. The study was conducted in Kosovo from February 2021 to January 2022 and we assessed mandibles from 24 (12 females and 12 male) adult Deltari Ilir dogs (more than 2 years old). Measurements of 22 parameters were made by metric rules and, electronic calibre. All results were evaluated and presented in millimeters as average and standard deviations. The total mandible length was 175.29 ±12.04 mm, respectively 178.87 ±13.2 mm and, 171.72 ±10.05 mm in males and females. The mandible height was 89.87 ±9.67 mm; 94.42 ±8.78 mm and, 85.32 ±8.55 mm in males and females respectively. The distance of mandibular foramen to the angular process was 31.78 ±3.17 mm; 32.23 ±3.19 mm and, 31.32 ±3.22 mm in males and females respectively. In general, all morphometric parameters were significantly higher in male mandibles than in females except; Distance from the condyloid process to the caudal angle of the coronoid process, Distance from the condyloid process to the angular process, and Distance from mandibular foramen to pogonion, where parameters in females were higher compare to male samples. This study provided us with valuable data on morphologic and morphometric parameters of the mandible of the Delatri Ilir dog. These data will serve us not only as a base for comparison studies with other dog breeds but also in the practical veterinary clinic.

Key words: dog, low jow bone, morphometric parameters, mandibular foramen

INDEX OF AUTHORS

In partnership with:

ВКМ
ВЕТЕРИНАРНА КОМОРА на Република Северна Македонија | VETERINARY CHAMBER of Republic of North Macedonia

GENERAL PARTNER

VITA-VET
SKOPJE
++389 2 / 2614-681 ; 2636-588
Str. Tajmiska No.32, 1000 Skopje, R. Macedonia

SUPPORTING PARTNERS

УНИВИГО
ДОО Експорт - Импорт Скопје

VETERINARSKI ZAVOD Subotica

PARTNERS

BI EL

eXpecta medical

SIEMENS Healthineers

ВИН СКОПЈЕ

ВИН Скопје доо
Франце Прешерн 61,
1000 Скопје, Македонија
телефон: +389 2 2032397
факс: +389 2 2032138
e-mail: vin@on.net.mk

ФАРМАХЕМ ФАРМАЧЕМ

Elta 90
more than technology

Virbac ANIMAL HEALTH
Allflex
KRUUSE
Anigen Animal Genetics Inc.

VEZËSHARRI
LECKER
elektro SHARRI

ВЕТЕРИНАРСТВО МАКЕДОНИЈА

NOTES

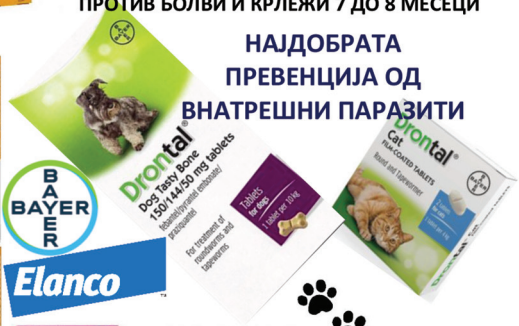
MSD

NOBIVAC ВАКЦИНИ ЗА КУЧИЊА И МАЧКИ

КОНТИНУИРАНА ЗАШТИТА ЗА КУЧИЊА И МАЧКИ

ПРОТИВ БОЛВИ И КРЛЕЖИ 7 ДО 8 МЕСЕЦИ

НАЈДОБРАТА ПРЕВЕНЦИЈА ОД ВНАТРЕШНИ ПАРАЗИТИ



СЕОПФАТНА ЗАШТИТА ЗА МАЧКИ И КУЧИЊА ОД НАДВОРЕШНИ И ВНАТРЕШНИ ПАРАЗИТИ

BAYER

Elanco

ВИТА-ВЕТ СКОПЈЕ

Увозник и дистрибутер
за МК:
ВИТА ВЕТ ДОО
vita-vet@vita-vet.mk

Тајмишка бр. 32 1000 Скопје
02 2614 681 02 2638 588
+070/270-963
www.vita-vet.mk

/vita-vet

NOTES

NOTES

BI EL

SINCE 1992

SKOPJE CANNABIS EXPO 2023

BUCHI · Promega · memmert · DWK LIFE SCIENCES DURAN WHEATON KIMBLE

FL MEDICAL · Biosigma · BRAND · KartellLABWARE

Hettich · IKA · huber · Condalab

interscience for microbiology · Metrohm · RADWAG · FRITSCH

www.biel.mk | +389 71 239 767 | info@biel.mk

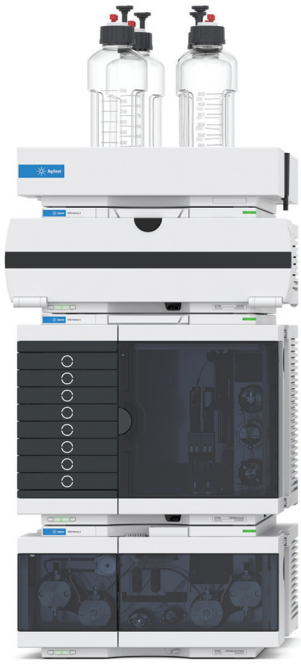
NOTES

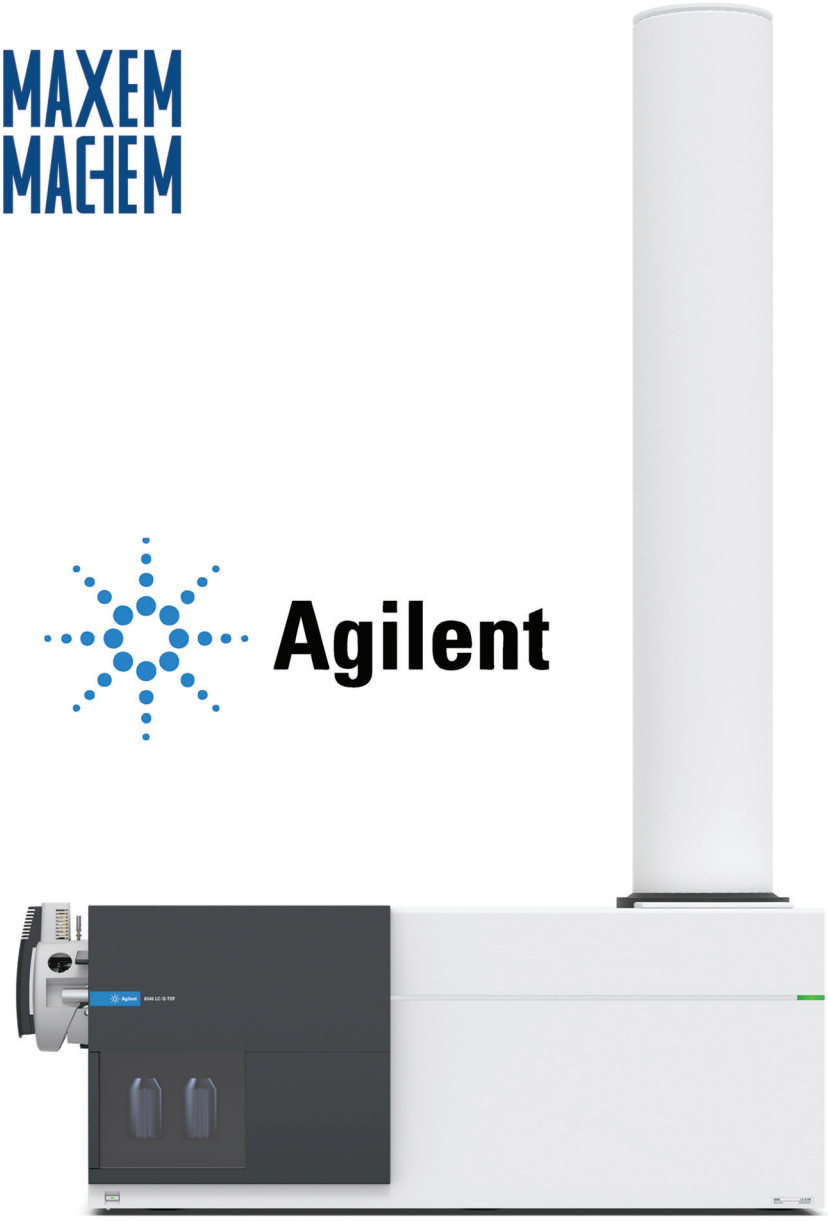

eXpecta
medical
SIEMENS
Healthineers
SOMATOM go.Now
SIEMENS
Healthineers

NOTES

ФАРМАХЕМ
PHARMACHEM

Agilent





NOTES

BRUKER

Elta 90
more than technology

Elta 90 Molecular Medicine DOO, Skopje
Address: Kosta Shahov, Number 12
Phone: +389 02 5117 017
Email: *office@elta90mm.mk*

Changing Microbiology

MALDI Biotyper® sirius RUO System

The *sirius* solution for extended research capabilities for microbiology

The original - often imitated, never duplicated

- Microorganism identification within minutes from culture, covering ~4700 species of bacteria, yeasts and molds
- Analysis of up to 600 samples/hour
- Direct identification from positive blood cultures within 15-20 minutes
- Fast phenotypic resistance detection of carbapenemase/cephalosporinase activity
- Positive/negative ion mode, with the negative ion mode enabling new applications such as fast colistin resistance detection

www.bruker.com/microbiology

RUO

For Research Use Only. Not for use in clinical diagnostic procedures.
Please contact your local representative for availability in your country.

NOTES

ВИН

ВАШИОТ ДОВЕРЛИВ ПАРТНЕР ЗА ВЕТЕРИНАРНА ОПРЕМА

ВЕТЕРИНАРНА ЛАБОРАТОРИСКА ОПРЕМА
ХИРУРШКИ ИНСТРУМЕНТИ
ТЕСТОВИ ЗА БРЗА ДИЈАГНОСТИКА
ПОТРОШЕН МАТЕРИЈАЛ

KRUUSE BIONOTE VET Eickemeyer® Medicinteknik for Dyrlæger

30 ГОДИНИ СО ВАС

vinskopje@gmail.com
www.vin.mk
02/2032-138

NOTES

NOTES

VEZË SHARRI®

LECKER

elektro SHARRI

Design and printing
STV Prizma - Skopje
September 2022
185 copies

ISBN 978-9989-774-38-6