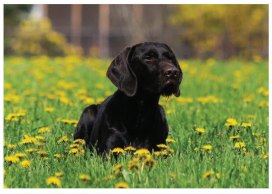
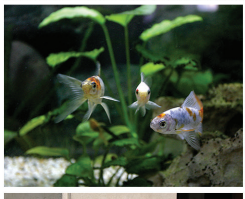
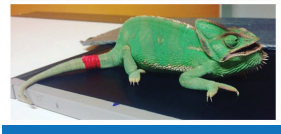
OCTOBER 5TH - 7TH 2017 • ZAGREB • CROATIA









7TH INTERNATIONAL CONGRESS "VETERINARY SCIENCE AND PROFESSION"

BOOK OF ABSTRACTS


UNIVERSITAS STUDIORUM ZAGRABIENSIS
MDCLXIX


VETERINARSKI FAKULTET SVEUČILIŠTA U ZAGREBU
1919

7TH INTERNATIONAL CONGRESS

"VETERINARY SCIENCE AND PROFESSION"

//// OCTOBER 5TH - 7TH 2017 ////

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Faculty of Veterinary Medicine

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Dear Colleagues,

It is with great pleasure that I welcome you to the 7th International Congress "Veterinary Science and Profession" on behalf of the Organizing Committee.

This year we are offering a three day program divided into 6 sessions - Veterinary Public Health, Farm Animals, Horses, Exotic and Wild Animals, Small Animals and Free Communications.

We are pleased to present a panel of distinguished invited speakers from multiple countries, as well as a robust number of oral and poster presentations in a wide variety of topics. There are also 10 workshops run by experts dedicated to a "hands on" learning environment in order to advance the clinical, laboratory, and analytic skills of participants.

For the first time, we have coordinated a "PhD Day" of the Faculty of Veterinary Medicine. This is a valuable opportunity for young scientists to present their research and contribute to the advancement of veterinary medical science.

The concept of "One Health" is a vital part in the future of human welfare, and the final day of the conference will explore the role of veterinarians in the global One Health initiative.

I hope that you enjoy your time in Zagreb and discover new ideas and techniques during the congress. I am grateful to all of the invited speakers, participants, sponsors, and workshop leaders who have made this congress possible.
Thank you all for supporting this event.

Assist. Prof. **Zoran Vrbanac**, DVM, PhD, DACVSMR
President of the Organizing Committee

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/ / / / / • THURSDAY OCTOBER 5TH 2017 - MAIN LECTURE HALL / / / / /

07.30 - 09.00 **REGISTRATION**

09.00 - 09.30 **OPENING CEREMONY**

09.30 - 10.10 **KEYNOTE LECTURE**

Assoc. Prof. **Janet Daly**
University of Nottingham, School of Veterinary Medicine and Research, UK:

- ***Factors in interspecies transmission of influenza A viruses***

10.10 - 10.30 ***Coffee break***

VETERINARY PUBLIC HEALTH - MAIN LECTURE HALL

Chairpersons: Zrinka Štritof, Nevijo Zdolec

• INVITED LECTURES
10.30 - 11.10
Assist. Prof. **Dean Jankuloski**
Head of Food Institute, Faculty of Veterinary Medicine - Skopje, Macedonia:

- ***Molecular characterization of foodborne pathogens***

11.10 - 11.50
Doc. dr. sc. **Josipa Habuš**
Zavod za mikrobiologiju i zarazne bolesti s klinikom, Veterinarski fakultet Sveučilišta u Zagrebu:

- ***Rodent borne zoonoses in Republic of Croatia***

• ORAL PRESENTATIONS
11.50 - 12.05
Tena Oreški, Miša Korva, Tatjana Vilibić-Čavlek, Vladimir Stevanović, Pavle Jeličić, Božana Miklaušić, Ljubo Barbić, Irena Tabain,Tatjana Avšič-Županc

- ***Prevalence of lymphocytic choriomeningitis antibodies in persons with rodent exposure***

12.05 - 12.20
Pavle Jeličić, Lorena Jemeršić, Vlatka Brumen, Nataša Janev-Holcer, Alef Prohić, Ljubo Barbić, Irena Tabain, Vladimir Stevanović, Tatjana Vilibić-Čavlek

• *Seroprevalence of hepatitis E in professionally exposed groups in Croatia: preliminary results*

12.20 - 12.35
Selma Pintarić, Luka Špelić, Klara Zubak Novak, Lana Hadžić, Branka Šeol Martinec

• *Extended spectrum beta-lactamase (ESBL) - producing Klebsiella pneumoniae isolated from dogs*

12.35 - 12.50
Ines Škoko, Lidija Kozačinski, Ivana Lojkić, Merica Carev, Eddy Listeš

• *Comparative phylogenetic analysis of norovirus from shellfish and patients with gastroenteritis in Croatia*

12.50 - 13.05
Ana Marquiza M. Quilicot, Danijela Horvatek Tomić, Željko Gottstein, Maja Lukač, Estella Prukner-Radovčić

• *Comparison of sampling procedures for the detection of Chlamydiaceae in free-range chicken flocks*

13.05 - 14.30 *Lunch break and poster presentation (Student's Hall)*

14.30 - 14.45
Suzana Hađina, Vladimir Stevanović, Snježana Kovač, Tatjana Vilibić Čavlek, Matko Perharić, Vilim Starešina, Ljupka Maltar, Tomislav Kiš, Josip Madić, Ljubo Barbić

• *Serological evidence of tick borne encephalitis virus infections in dogs in Croatia – importance for the veterinary medicine and public health*

14.45 - 15.00
Lejla Velić, Toni Eterović, Sadeta Hamzić, Dženo Hadžović

• *Brucellosis in cattle and humans in Bosnia and Herzegovina*

15.00 - 15.15
Vitomir Ćupić, Biljana Antonijević, Saša Ivanović

• *Use of antimicrobial agents in laying hens*

16.30 - 18.30 **Zagreb ZOO guided tour**

18.30 - 20.00 **WELCOME PARTY**

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FARM ANIMALS - CLINICS DEPARTMENT LECTURE HALL

Chairpersons: Goran Bačić, Nino Maćešić

• INVITED LECTURES
10.30 - 11.10
Assist. Prof. **Neelesh Sharma**
Faculty of Veterinary Science & Animal Husbandry Sher-e-Kashmir, India:

• *Current scenario of mastitis in India and possible stem cell based approaches in its management*

11.10 - 11.50
Prof. dr. sc. **Anamarija Ekert Kabalin**
Zavod za stočarstvo, Veterinarski fakultet Sveučilišta u Zagrebu:

• *The dark side of selection: negative-side effects of selection for high productivity*

• ORAL PRESENTATIONS
11.50 - 12.05
Ivan Vlahek, Kristina Starčević, Anamaria Ekert Kabalin, Boro Mioč, Marija Špehar, Sven Menčik, Maja Maurić, Sofija Džakula, Željko Mikulec, Velimir Sušić

• *Polymorphism of the mtnr1a gene in seasonal and nonseasonal estrous sheep breed*

12.05 - 12.20
Goran Bačić, Nino Maćešić, Tugomir Karadjole, Martina Lojkić, Nikica Prvanović Babić, Iva Bačić, Jelena Šuran, Neelesh Sharma

• *Importance of dry period for the next lactation – review*

12.20 - 12.35
Iva Bačić, Nino Maćešić, Neelesh Sharma, Goran Bačić

• *Fresh cows protocol*

12.35 - 12.50
Tihana Josipović, Nino Maćešić, Goran Bačić

• *Prototheca caused mastitis on dairy farms in Slavonija*

12.50 - 13.05
Jelena Šuran, Karla Milošević, Mihael Galović, Tomislav Mašek, Diana Brozić, Krešimir Matanović, Lada Radin, Jasna Aladrović, Branka Šeol Martinec, Božo Radić, Saša Radić, Miroslav Benić, Antun Kostelić, Frane Božić, Goran Bačić, Nino Maćešić

- ***Intramammary propolis solution (APIMAST) for subclinical mastitis treatment in dairy cows***

13.05 - 14.30 *Lunch break and poster presentation (Student's Hall)*

14.30 - 14.45
Marina Prišlin, Blanka Beer Ljubić, Nadica Maltar Strmečki, Renata Laškaj, Lana Vranković, Martina Lojkić, Vedran Nervo, Jasna Aladrović

- ***Efect of age and folicle size on lipid profile and antioxidant potential of bovine follicular fluid***

14.45 - 15.00
Jasna Aladrović, Martina Lojkić, Blanka Beer Ljubić, Renata Laškaj, Lana Vranković, Ivančica Delaš, Zvonko Stojević, Vedran Nervo

- ***Lipid composition of bovine follicular fluid during folliculogenesis***

15.00 - 15.15
Silvijo Vince, Ivona Žura Žaja, Kristina Rakić, Velimir Berta, Branimira Špoljarić, Ivan Butković, Anamaria Sluganović, Igor Nazansky, Hrvoje Valpotić, Nina Poljičak-Milas, Suzana Milinković-Tur

- ***Spermatozoa subpopulations in buck based on head and tail morphometric parameters***

15.15 - 15.30
Ivona Žura Žaja, Silvijo Vince, Kristina Rakić, Velimir Berta, Marko Samardžija, Nina Poljičak-Milas, Matko Kardum, Sara Strelec, Marinko Vilić, Vladimir Nazansky, Suzana Milinković-Tur

- ***Association of spermatozoa morphometric characteristics with age of bucks and proportion of motile spermatozoa in ejaculate***

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EXOTIC AND WILD ANIMALS - PHYSICS DEPARTMENT LECTURE HALL

Chairpersons: Danijela Horvatek Tomić, Magda Sindičić

• INVITED LECTURE
10.30 - 11.10
Dr. sc. **Luka Jurinović**, dipl. ing. biol.
Hrvatski veterinarski institut, Centar za peradarstvo:

• ***Seagulls, Laridae, as a model for research of wild birds´ diseases***

• ORAL PRESENTATIONS
11.10 - 11.25
Tomislav Gomerčić

• ***On-line information system for monitoring of protected species - an example of marine mammals and Eurasian lynx***

11.25 - 11.40
Ana Tominac, Danijela Horvatek Tomić, Maja Lukač, Estella Prukner-Radovčić, Željko Gottstein

• ***Prevalence of Macrorhabdus ornitogaster in pet bird breeders and pet shops from 2010 to 2015 in Croatia***

11.40 - 11.55
Ira Topličanec, Mateja Stipić, Slaven Reljić, Đuro Huber, Magda Sindičić

• ***Analysis of efficiency of brown bear hunting from hides at feeding sites***

11.55 - 12.10
Dean Konjević, Miljenko Bujanić, Ljubo Barbić, Damir Ugarković, Maja Lukač, Branka Artuković

• ***Fibropapillomatosis in a male mouflon (Ovis musimon) – a case report***

12.10 - 12.25
Miljenko Bujanić, Željko Gottstein, Maja Lukač, Ivica Križ, Martina Lojkić, Danijela Horvatek Tomić, Dean Konjević

- *Artificial insemination of capercaillie (Tetrao urogallus l.)*

12.25 - 12.40
Daria Octenjak, Lana Vranković, Ivančica Delaš, Josip Kusak, Slaven Reljić, Đuro Huber, Zvonko Stojević, Jasna Aladrović

- *Fatty acid composition of different tissues of free-ranging gray wolf (Canis lupus) in Croatia*

12.40 - 12.55
Magda Sindičić, Franjo Martinković, Miljenko Bujanić, Nikolina Tuškan, Marina Špehar, Dean Konjević

- *Morphological and molecular identification of golden jackal intestinal parasite*

12.55 - 14:30 *Lunch break and poster presentation (Student's Hall)*

14.30 - 14.45
Andrea Martinović, Andrija Musulin, Petar Kostešić, Mirta Vučković, Jadranko Boras, Dražen Matičić

- *Anaesthetic management of unilateral adrenalectomy in a eastern quoll (Dasyurius viverrinus) - case report*

14.45 - 15.00
Slavko Žužul, Željka Mesić, Tomislav Mikuš, Kristina Matković, Željko Pavičić, Mario Ostović

- *Attitudes among Croatian veterinary students toward welfare of game animals*

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FREE COMMUNICATIONS - PHYSICS DEPARTMENT LECTURE HALL

Chairpersons: Jasna Aladrović, Mario Ostović

• INVITED LECTURE
15.00 - 15.40
Prof. **David Peter Eckersall**
VetMedZg ERA Chair Laboratory, Faculty of Veterinary Medicine, University of Zagreb

• *Applications of proteomics in veterinary research*

• ORAL PRESENTATIONS
15.40 - 15.55
Mirjana Milovanović, Đorđe S Marjanović, Saša M Trailović

• *Analgesic activity and mechanism of action of the monoterpene p-cymene in the rat model of inflammatory pain*

15.55 - 16.10
Ana Frangeš, Aleksandra Maria Đurić, Boris Pirkić, Zoran Vrbanac, Dino Stanin, Dražen Vnuk, Hrvoje Capak

• *Spinal trauma in dogs in emergency rescue Program „Noina arka"*

16.10 - 16.25
Ana Zupčić, Marta Šurbek, Anamaria Ekert Kabalin, Mario Ostović

• *Impact of missing cat features on their returning to owners*

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SMALL ANIMAL PRACTICE - MAIN LECTURE HALL

Chairpersons: Ivana Kiš, Boris Pirkić

• INVITED LECTURES
09.00 - 09.40
Dr. **René Dörfelt**, DECVECC, DECVAA
Medizinische Kleintierklinik, München, Germany:

• Congestive heart failure patient - criticalist's view

09.40 - 10.20
Doc. dr. sc. **Marin Torti**
Klinika za unutarnje bolesti, Veterinarski fakultet Sveučilišta u Zagrebu:

• Congestive heart failure patient - cardiologist's view

10.20 - 10.50 *Coffee break*

• ORAL PRESENTATION
10.50 - 11.05
Ines Jović, Iva Šmit, Filip Kajin, Jelena Gotić, Vesna Matijatko, Ivana Kiš, Marin Torti

• Prevalence and clinical characteristics of congenital heart diseases in dogs examined at the clinic for internal diseases, Faculty of Veterinary Medicine, University of Zagreb in the period from 2013-2017

• INVITED LECTURE
11.05 - 11.45
Prof. dr. sc. **Dražen Vnuk**
Klinika za kirurgiju, ortopediju i oftalmologiju, Veterinarski fakultet Sveučilišta u Zagrebu:

• Portosystemic shunt – drugs or surgery?

• ORAL PRESENTATIONS
11.45 - 12.00
Petra Dmitrović, Andrija Musulin, Vladimira Erjavec, Dražen Vnuk

• Central venous pressure measurement for intraoperative detection of portosystemic shunt – a case report

12.00 - 12.15
Valentina Percan, Nikolina Škvorc, Monika Živković, Andrea Gudan Kurilj, Marko Hohšteter, Branka Artuković, Lidija Medven Zagradišnik, Željko Grabarević, Ivan-Conrado Šoštarić-Zuckermann

• Anal sac apocrine adenocarcinoma of dogs – a histological, immunohistochemical and clinical characterization

12.15 - 12.30
Lina Nakevska, Igor Ulchar, Irena Celeska

- *Skin cytology – useful diagnostic tool for cutaneous lesions and lumps*

12.30 - 12.45
Ana Manojlović, Dražen Vnuk, Nika Brkljača Bottegaro, Hrvoje Capak

- *Comparison of dogs and cats gunshot injuries in urban areas*

12.45 - 13.00
Doris Švob, Lucija Vidović, Snježana Kazazić, Josipa Habuš, Suzana Hađina, Krešimir Martinković, Matko Perharić, Nenad Turk, Zrinka Štritof

- *Isolation and species identification of Campylobacter spp. from healthy and diarrheic dogs*

13.00 - 14.30 *Lunch break and poster presentation (Student's Hall)*

14.30 - 14.45
Relja Beck, Daria Jurković, Stjepan Krčmar, Tomislav Šarić, Renata Brezak, Sanja Bosnić, Marija Stublić

- *Diversity of piroplasms detected in ticks: individual sequencing approach*

14.45 - 15.00
Ana Beck, Boris Pirkić, Filip Kajin, Ines Jović, Doroteja Huber, Jelena Mraović, Daria Jurković, Relja Beck

- *Canine ocular dirofilariasis-a case report*

15.00 - 15.15
Daria Jurković, Ana Beck, Doroteja Huber, Renata Brezak, Sanja Bosnić, Marija Stublić, Relja Beck

- *Bacteria and protozoa in fleas and ticks from dogs and cats died due to hemolytic anemia*

15.15 - 15.30
Daria Jurković, Ana Beck, Doroteja Huber, Renata Brezak, Sanja Bosnić, Franjo Martinković, Damir Lukačević, Miroslav Pilat, Marija Stublić, Kristina Skrbin, Relja Beck

- *Seroprevalence of vector - borne pathogens in dogs from Croatia*

15.30 - 15.45
Doroteja Huber, Ana Beck, Daria Jurković, Relja Beck

- *Vector-borne infections in Croatian cats: pathologic and molecular study*

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HORSES - PHYSICS DEPARTMENT LECTURE HALL

Chairpersons: Nikica Prvanović Babić, Nika Brkljača Bottegaro

• INVITED LECTURES
09.00 - 09.40
Ao. Univ.- Prof. Dr. **Christine Aurich**
Dipl. ECAR, University of Veterinary Medicine, Vienna, Austria:

• ***Management of early pregnancy and early pregnancy loss in the mare***

09.40 - 10.20
Dr. **Petra Kramarič**, PhD
University of Ljubljana, Veterinary Faculty, Ljubljana, Slovenia:

• ***Colic horse as a diagnostic and therapeutic challenge***

10.20 - 10.50 ***Coffee break***

10.50 - 11.30
Dr. sc. **Jelena Gotić**
Klinika za unutarnje bolesti, Veterinarski fakultet Sveučilišta u Zagrebu:

• ***Tick borne diseases in horses***

• ORAL PRESENTATIONS
11.30 - 11.45
Karla Klobučar, Agata Kučko, Zoran Vrbanac, Jelena Gotić, Krunoslav Bojanić, Jelena Šuran, Diana Brozić, Nika Brkljača Bottegaro

• ***Metabolic and oxidative stress in endurance horses during racing***

11.45 - 12.00
Ana Manojlović, Darko Grden, Jelena Gotić, Zrinka Štritof, Nika Brkljača Bottegaro

• ***Billateral guttural pouch mycosis without epistaxis-a case report***

12.00 - 12.15
Petra Dmitrović, Nika Brkljača Bottegaro, Darko Grden, Marija Mamić, Boris Pirkić

• ***Enucleation with optic nerve ligation in a horse – a case report***

12.15 - 12.30
Darko Grden, Mirta Vučković, Jelena Gotić, Nika Brkljača Bottegaro

• ***Nonsurgical removal of uroliths in horse***

12.30 - 14.30 ***Lunch break and poster presentation (Student's Hall)***

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PHD DAY OF THE FACULTY OF VETERINARY MEDICINE - CLINICS DEPARTMENT LECTURE HALL

PhD program: Veterinary Sciences

09.00 - 09.15 Introduction to a PhD day: Assist. Prof. **Dean Konjević**, Dipl. ECZM

• INVITED LECTURES
09.15 – 09.45
Prof. **Dražen Matičić**

• *Centers of Research Excellence*

09.45 - 10.15
Prof. **Vladimir Mrljak**

• *Increasing the research competences through ERAChair project*

• ORAL PRESENTATIONS
10.15 - 10.30
Katarina Špiranec

• *The effects of natriuretic peptides on the bradykinin signaling in primary culture of neurons and astrocytes*

10.30 - 10.45
Diana Brozić

• *Effect of dietary eicosapentaenoic and docosahexaenoic fatty acid supplementation during last month of gestation on lipid metabolism and FASN and ACACA gene expression in Charolais cows and calves*

10.45 - 11.00
Maja Maurić

• *Influence of DGAT1, FASN, PRL, BRCA1 and TLR1 gene polymorphism on milk production and udder health in cows*

11.00 - 11.15
Tomislav Sukalić

• *Virulence factors of Escherichia coli and their impact on the pathomorphological and histopathological lesions in piglets died from colibacillosis*

11.15 - 11.30
Franjo Martinković

• *Morphological and proteomic analysis of flagellates from Trypanosoma genus in Croatia*

11.30 - 11.45
Marko Pećin

• *Effects of autologous interleukin 1 receptor antagonist proteine on achilles tendon healing in rabbits*

11.45 - 12.00
Dunja Grabarević

• *Correlation between pathological changes, virus and regulatory T lymphocytes tropism in organs of cows and heifers infected with bovine leukemia virus*

12.00 - 12.15
Nina Krešić

• *Molecular epizootiology of bovine respiratory syncytial virus infection*

12.15 - 12.25
Closing remarks: Prof. **Frane Božić**

12.30 *Coffee, lunch break and poster presentation*

POSTER LIST

Miljenko Bujanić:
Three different types of hosts for Fascioloides magna in wild animals

Doroteja Huber:
Morphology of organ dysfunction in dogs with lethal Babesia canis infection

Petar Kostešić:
A sheep model for autologous osteochondral bioreactor engineered graft transplant

Tomislav Mikuš:
Utjecaj dobi mliječnih krava i spola teladi na stupanj stresa pri ranom odbiću

Mirela Pavić:
Gender influence on the expression and distribution of NA/K-ATPase in porcine small intestine

Ana Marquiza M. Quilicot:
Molecular detection of Chlamydiaceae in free-range chicken flocks

Dora Stojević:
Biochemical and molecular analysis of Escherichia coli isolated from food of different animal origin and carcass swabs

/ / / / / • SATURDAY OCTOBER 7TH 2017 / / / / /

LABOKLIN PRESENTING - MAIN LECTURE HALL

Chairpersons: Mirna Brkljačić, Vesna Matijatko

• INVITED LECTURE
Prof. dr. sc. **Urs Giger**, Dipl. ACVIM, ECVIM & ECVCP
University of Pennsylvania, USA, Veterinär - Medizinische Fakultät, University of Zürich, Switzerland:

09.00 - 09.45

- *Advances in Hereditary Diseases in Dogs - Diagnosis and Management*

09.45 - 10.30

- *Approach to the Bleeding Dog - Diagnosis & Management*

10.30 - 11.00 *Coffee break*

11.00 - 11.45

- *Immune-mediated Hemolytic Anemias in Dogs*

11.45 - 12.30 CLOSING CEREMONY

/ / / / / • SATURDAY OCTOBER 7TH 2017 / / / / /

"ONE HEALTH" - MAIN LECTURE HALL

Voditelji sekcije / Chairpersons: **Jelena Šuran i Lada Radin**

Koncept **"One Health"** (Jedno zdravlje) i uloga veterinara u suvremenom društvu
Sekcija je otvorena za građanstvo

12.30 - 13.00
Doc. dr. sc. **Vladimir Stevanović**
Zavod za mikrobiologiju i zarazne bolesti s klinikom, Veterinarski fakultet Sveučilišta u Zagrebu:

One Health pristup u nadzoru i suzbijanju zaraznih bolesti ljudi i životinja
One Health approach in human and animal infectious diseases control

13.00 - 13.30
Prof. dr. sc. **Tatjana Živičnjak**
Zavod za parazitologiju i invazijske bolesti s klinikom, Veterinarski fakultet Sveučilišta u Zagrebu:

One Health koncept: Postoji li zid ili most između ljudi i parazita životinja?
One Health concept: is there a wall or a bridge between humans and animal parasites?

13.30 - 14.00 ***Coffee break***

14.00 - 14.30
Prof. dr. sc. **Frane Božić**
Zavod za farmakologiju i toksikologiju, Veterinarski fakultet Sveučilišta u Zagrebu:

Koncept „One Health": važnost pametne primjene antimikrobnih lijekova
One Health concept: the importance of smart antimicrobial drugs use

14.30 - 15.00
Dr. sc. **Iva Šmit**
Klinika za unutarnje bolesti, Veterinarski fakultet Sveučilišta u Zagrebu:

Pretilost pasa i mačaka - bolest današnjice
Canine and feline obesity - a modern life disease

15.00 - 15.15 ***Discussion***

POSTER PRESENTATIONS - STUDENT'S HALL

Chairpersons: Lana Vranković, Ivona Žura Žaja

1. Boris Pisinov, Nikola Roković, Ksenija Nešić, Snežana Ivanović, Milan Radović, Jasna Kureljušić, Svetlana Vuković: Mercury determination and sensory analysis of fish from Serbian market

2. Nevijo Zdolec, Vesna Dobranić, Snježana Kazazić, Ivan Šimunić, Zvonimir Dumbović: Isolation and identification of *Yersinia enterocolitica* strains from food production chain

3. Liča Lozica, Danijela Horvatek Tomić, Maja Lukač, Estella Prukner-Radovčić, Željko Gottstein: Antimicrobial resistance of *Escherichia coli* isolated from poultry and pet birds

4. Branimira Špoljarić, Silvijo Vince, Ivan Butković, José Fernando Vázquez Armijo, Juraj Grizelj: Ovsynch efficiency in anoestrous dairy goats

5. Slavomír Marcinčák, Martin Bartkovský, Dana Marcinčáková, Marek Hudák, Peter Popelka, Tatiana Klempová, Milan Čertík, Ján Mačanga: Impact of feeding 10 % fermented bioproduct enriched with biologically active compounds on fatty acid profile and quality of chicken breast meat

6. Dana Marcinčáková, Martin Bartkovský, Ján Mačanga, Iveta Jaďuttová, Slavomír Marcinčák: The use of humic substances in broiler chickens during the fattening period

7. Bartkovský Martin, Marcinčáková Dana, Mačanga Ján, Čertík Milan, Molnár Ladislav, Sesztáková Edina, Marcinčák Slavomír: Effect of feeding 10 % fermented feed enriched with gamma-linolenic acid and beta-carotene on blood biochemical parameters of broiler chickens

8. Morana Orban, Lana Vranković, Ivančica Delaš, Biserka Zidar, Ivanka Majić Balić, Zvonko Stojević, Jasna Aladrović: Fatty acid composition in semen of boars

9. Bejo Bizhga, Eglantina Xhemollari, Dardan Shehdula: Fasciolosis in Albania

10. Gordana Gregurić Gračner, Slavko Žužul, Nataša Lončarić, Juraj Grizelj, Alenka Dovč, Željko Pavičić, Damjan Gračner: Ruminants' environmental enrichment

11. Maja Ivana Smodiš Škerl, Ivana Tlak Gajger: Quality of honey bee (*Apis mellifera carnica*) attendants in transport queen cages and Nosema spp. prevalence

12. Eglantina Xhemollari, Luljeta Dhaskali, Bejo Bizhga, Elenica Dimço, Jetmira Abeshi: Analysis of serum enzymes in *Dicrocelium dendriticum* infected sheep

13. Marko Pećin, Elena Valković, Teodor Banković: Ventral abdominal hernias in birds; diagnostic and surgical repair – review paper

14. Ana Grgoić, Ira Topličanec, Danijela Horvatek Tomić, Željko Gottstein, Gordana Nedeljković, Maja Belić, Ivan Cizelj, Estella Prukner-Radovčić, Maja Lukač: Bacterial and fungal flora of western caspian turtles (*Mauremys rivulata*)

15. Nika Dvojković, Tena Galešić, Maja Belić, Maja Lukač, Mirna Robić, Romana Turk: Diverse morphology of chelonian blood cells

16. Tena Galešić, Nika Dvojković, Maja Belić, Maja Lukač, Mirna Robić, Romana Turk: Evaluation of blood parameters in western caspian turtle (*Mauremys rivulata*)

17. Branka Artuković, Lidija Medven Zagradišnik, Andrea Gudan Kurilj, Marko Hohšteter, Ivan-Conrado Šoštarić-Zuckermann, Ingeborg Bata, Jadranko Boras, Željko Graberević: Metastasing scapular osteosarcoma in a brown bear (*Ursus arctos*)

18. Petar Kostešić, Marija Mamić, Jadranko Boras, Ingeborg Bata, Maja Lukač, Mirta Vučković, Dražen Vnuk, Zoran Vrbanac, Dražen Matičić: Orchiectomy of the lowland tapir (*Tapirus terrestris*) in field conditions

19. Ema Velčić, Lana Vranković, Dean Konjević, Miljenko Bujanić, Ivančica Delaš, Zvonko Stojević, Jasna Aladrović: Fatty acid composition of different tissues of indian small mongoose (*Herpestes auropunctatus*) in Croatia

20. Sara Došen, Tatjana Trbojević Vukičević, Martina Đuras: Arterial vasculature of the bottlenose dolphin heart

21. Lana Vranković, Blanka Beer-Ljubić, Slaven Reljić, Đuro Huber, Zvonko Stojević, Jasna Aladrović: Haematological and biochemical values in Croatian free-ranging brown bears or how to sample a bear

22. Željka Sabol, Magda Sindičić, Miljenko Bujanić, Franjo Martinković, Dean Konjević: First identification of the nematodes Setaria tundra in roe deer in Croatia by molecular method

23. Sara Ančić, Nicole Marie Auguštin, Lana Vranković, Ivančica Delaš, Josip Kusak, Dean Konjević, Miljenko Bujanić, Slaven Reljić, Đuro Huber, Zvonko Stojević, Jasna Aladrović: Comparison of fatty acid composition of subcutaneous adipose tissue of gray wolf (*Canis lupus*) and small indian mongoose (*Herpestes auropunctatus*) in Croatia

24. Ira Topličanec, Danijela Horvatek Tomić, Željko Gottstein, Gordana Nedeljković, Petar Kostešić, Mirta Vučković, Jadranko Boras, Ingeborg Bata, Maja Lukač: Monitoring health status in a group of captive nutrias (*Myocastor coypus*)

25. Mirta Vučković, Petar Kostešić, Jadranko Boras, Hrvoje Capak, Iva Šmit, Ozren Smolec, Andrea Martinović, Dražen Matičić: Total oral/dental treatment in a serval (*Leptailurus serval*) - case report

26. Maša Efendić, Marko Samardžija, Nikica Prvanović Babić, Goran Bačić, Tugomir Karadjole, Martina Lojkić, Hrvoje Capak, Zoran Vrbanac, Nino Maćešić: Postovulatory egg retention (dystocia) in bearded dragon (*Pogona vitticeps*) - a case report

27. Emilian Shabani, Avni Robaj, Eglantina Xhemollari, Kastriot Belegu: Radiological findings of mucopolysaccharidosis in dogs and cats

28. Asier Galán, Anita Horvatić, Josipa Kuleš, Petra Nižić, Nicolas Guillemin, Renata Barić Rafaj, Vladimir Mrljak: LC-MS/MS analysis of dog serum phosphoproteome reveals novel phosphorylation sites and differential phosphoprotein patterns in babesiosis caused by B. canis

29. Marija Lipar, Marko Pećin, Jadranka Bubić Špoljar, Gordana Gregurić Gračner, Berislav Radišić: Masticatory muscle myositis in maltese

30. Luka Pajurin, Dina Jelenčić, Boris Pirkić, Tomislav Babić, Damir Mihelić, Ivana Stolić, Danijel Špoljarić, Perta Dmitrović, Mirna Abaffy Kirin, Mirela Pavić, Hrvoje Lucić, Maja Popović: Founding the animal eye bank at the Faculty of veterinary medicine of the University of Zagreb

31. Zdravka Vidić, Damir Lukačević, Sanda Katić, Ines Škoko, Eddy Listeš: Dermathophytosis in clinically suspected dogs during 2014- 2015 in Split

32. Josipa Kuleš, Petra Nižić, Blanka Beer Ljubić, Renata Barić Rafaj, Vladimir Mrljak: Analytical validation of canine kidney injury molecule -1 (KIM-1) immunoassay in urine samples

33. Petra Nižić, Josipa Kuleš, Blanka Beer Ljubić, Nicolas Guillemin, Vladimir Mrljak: Urinary immunoglobulin G and retinol binding protein as biomarkers of renal dysfunction in canine babesiosis

34. Marko Pećin, Marina Sinković, Anja Samardžić: Evaluation of recovery after cranial cruciate ligament rupture surgery in 60 dogs – questionnaire and clinical examination

35. Anita Horvatić, Petra Nižić, Josipa Kuleš, Nicolas Guillemin, Vladimir Mrljak: Identification of potential serum biomarkers for kidney dysfunction in canine babesiosis by label-based quantitative proteomic approach

36. Lidija Medven Zagradišnik, Hrvoje Capak, Andrea Gudan Kurilj, Ivan - Conrado Šoštarić - Zuckermann, Željko Grabarević, Branka Artuković, Nikolina Tuškan, Marko Hohšteter: Radiographic and pathologic pattern of canine primary lung tumors

37. Nicolas Guillemin, Anita Horvatić, Josipa Kuleš, Asier Galan, Petra Nižić, Blanka Beer Ljubić, Albert Marinculić, Vladimir Mrljak, David Eckersall: Establishment of a DNA-based test for quick diagnostics of canine babesiosis

38. Lina Nakevska, Miroslav Radevski ,Vlatko Ilieski: Aggressive dogs:assessment and treatment considerations

39. Klara Marić, Petra Šoštarić, Hrvoje Valpotić, Jelena Šuran, Marija Lipar, Frane Božić, Andreja Prevendar Crnić, Diana Brozić: Activity of salivary enzymes and level of salivary urea in gingivitis of dogs

40. Matej Krištić, Iva Šmit, Valentina Plichta, Jelena Šuran, Diana Brozić, Lada Radin, Nika Brkljača Bottegaro, Zoran Vrbanac: Lactate concentration changes during treadmill exercise in cats

41. Biserka Zidar, Jasna Aladrović, Lana Vranković, Ljiljana Bedrica: ECG monitoring during elective surgical procedure in cat

42. Mirta Vučković, Ana Petak, Nikša Lemo, Ines Jović, Petar Kostešić, Dražen Matičić, Andrea Gudan Kurilj: Chronic ulcerative stomatitis in maltese dog-a case report

43. Jelena Mraović, Doroteja Huber, Ana Beck, Filip Kajin, Nikola Šanta, Daria Jurković, Relja Beck: Preliminary evaluation of IDEXX COMBO SNAP® test for usage on postmortal sample

44. Renata Brezak, Sanja Bosnić, Daria Jurković, Kristina Skrbin, Marija Stublić, Relja Beck: Prevalence of gastrointestinal parasites in dogs from Zagreb area

45. Valentina Brgles, Branimir Škrlin, Martina Crnogaj, Iva Šmit, Darko Capak, Hrvoje Capak: Chewed bones in canine stomach – treatment options and outcome

46. Tomislav Bureš, Iva Šmit, Diana Brozić, Jelena Šuran, Nika Brkljača Bottegaro, Božo Radić, Lada Radin, Zoran Vrbanac: Hematological and serum biochemical parameters in search and rescue dogs before and after a whole day fieldwork

47. Dunja Rukavina, Ivona Božić, Ćazim Crnkić, Atifa Ajanović, Dajna Preldžić, Agnesa Ćoralić, Denis Čamo, Dženita Hadžijunuzović-Alagić, Amir Zahirović: Serum biochemical parameters in clinically healthy adult Bosnian and Herzegovinian mountain horses

48. Katarina Miljak, Jelena Gotić, Damir Žubčić, Nika Brkljača Bottegaro: Equine metabolic syndrome in a pony – a case report

49. Ana Kajmić, Jelena Gotić, Agata Kučko, Zrinka Štritof, Nikica Prvanović Babić, Nika Brkljača Bottegaro: Skin nodules prevalence in grey and white horses

50. Agata Kučko, Karla Klobučar, Jelena Gotić, Krunoslav Bojanić, Nika Brkljača Bottegaro, Zoran Vrbanac: What should be considered when interpretating biochemistry blood results in healthy endurance horses?

51. Nika Konstantinović, Ivan-Conrado Šoštarić-Zuckermann, Lidija Medven Zagradišnik, Andrea Gudan Kurilj, Branka Artuković, Željko Graberević, Darko Grden, Berislav Radišić, Nikica Prvanović Babić, Jelena Gotić, Nika Brkljača Bottegaro, Marko Hohšteter: Bowel dislocation as cause of death in horses at the Faculty of veterinary medicine Zagreb

52. Roberta Čordaš, Tea Ćorić, Jelena Gotić, Nikica Prvanović Babić: Microphthalmia in "Croatian coldblooded" foal – case review

53. Sanja Bosnić, Renata Brezak, Daria Jurković, Relja Beck: Entomological survey of insects of the genus *Culicoides Latreille*, 1809 (Diptera: ceratopogonidae) in 2016 in Croatia

54. Ivan Milosević, Jelena Danilović Luković, Tijana Luzajić, Danica Marković, Anita Radovanović, Sophie Sourice-Petit, Jerome Guicheux , Milica Kovacević Filipović: Subclinical hypothyroidism in gravid rats cause delayed osteogenic differentiation in pups

55. Tijana Lužajić, Ivan Milošević, Jelena Danilović Luković, Danica Marković, Sandra Milošević, Milica Kovačević Filipović, Anita Radovanović: Thyroid hormones affect the proliferation and / or mobilization of bulge stem cell population

56. Jelena Danilovic Lukovic, Aleksandra Korac, Ivan Milosevic, Tijana Luzajic, Milica Kovacevic Filipovic, Anita Radovanovic: Ovarian surface epithelium in newborn rats: germ cells warehouse

57. Andreja Pađen, Ivona Matić, Kristina Starčević, Vedran Micek, Krešimir Bošnjak, Marina Vranić, Luka Krstulović, Ivana Stolić, Snježana Kužir, Maja Maurić, Andrea Gudan Kurilj, Marcela Šperanda, Mislav Đidara, Tomislav Mašek: Influence of dietary lipids on the hepatic expression of genes for the β-oxidation of fatty acids in the rats with streptozotocin induced diabetes

/ / / / / WORKSHOPS

/ / / / / • THURSDAY OCTOBER 5TH 2017 / / / / /

14.30 - 16.30 **Elastografija** - nova ultrazvučna metoda u oslikavanju mekih tkiva životinja u veterinarskoj praksi (prof. dr. sc. **Damir Stanin**, Zavod za rendgenologiju, ultrazvučnu dijagnostiku i fizikalnu terapiju, Veterinarski fakultet, Sveučilište u Zagrebu; mr. sc. **Tomislav Brajko**, dr. med. vet., MIDES ZAGREB d.o.o.)

14.30 -18.30 **Laboratorijska hematologija pasa i mačaka**
(Doc. dr. sc. **Maja Belić**; Izv. prof. dr. sc. **Romana Turk**; prof. dr. sc. **Mirna Robić**; prof. dr. sc. **Nina Poljičak Milas**, Zavod za patološku fiziologiju Veterinarski fakultet, Sveučilište u Zagrebu)

14.30 - 18.30 **Genetska selekcija i zdravstveni parametri u reprodukciji mliječnih krava**
(mr. sc. **Josip Daud**, dr. med. vet., „BOSGEN" d.o.o. i doc. dr. sc. **Maja Maurić**, Zavod za stočarstvo, Veterinarski fakultet, Sveučilište u Zagrebu)

/ / / / / • FRIDAY OCTOBER 6TH 2017 / / / / /

09.00 - 14.00 **Plinska kromatografija u analizi materijala biološkog podrijetla**
(prof. dr. sc. **Ivančica Delaš** Medicinski fakultet , Sveučilište u Zagrebu; izv. prof. dr. sc. **Jasna Aladrović**, izv. prof. dr. sc. **Tomislav Mašek**, dr. sc. **Lana Vranković**, Veterinarski fakultet, Sveučilište u Zagrebu)

09.00 - 13.00 **Službene kontrole u proizvodnji hrane**
(doc. dr. sc. **Nevijo Zdolec**, Zavod za higijenu, tehnologiju i sigurnost hrane, Veterinarski fakultet, Sveučilište u Zagrebu; **Frane Rupčić**, dr. med. vet., Uprava za veterinarstvo i sigurnost hrane; **Mirela Juras**, dr. med. vet., Veterinarska stanica Vrbovec)

11.30 - 14.00 **Praktičan pristup konju s kolikom / Practical approach to a colic horse**
(Dr. sc. **Petra Kramarič**, dr. med. vet., University of Ljubljana, Veterinary Faculty)

14.30 - 18.00 **Reprodukcija rasplodnih kobila / Broodmare reproduction**
(Ao. Univ.-Prof. Dr. **Christine Aurich**, Dipl. ECAR, University of Veterinary Medicine, Vienna)

14.30 - 18.00 **Praktikum hitne veterinarske medicine / Veterinary Emergency Medicine Workshop** (dr. **René Dörfelt**, DECVECC, DECVAA, Medizinische Kleintierklinik, München; izv. prof. dr. sc. **Ivana Kiš**, doc. dr. sc. **Marin Torti**, Klinika za unutarnje bolesti, Veterinarski fakultet, Sveučilište u Zagrebu)

/ / / / / • SATURDAY OCTOBER 7TH 2017 / / / / /

09.00 - 14.00 **Klinička i laboratorijska dijagnostika gmazova**
(dr. sc. **Maja Lukač**; doc. dr. sc. **Maja Belić**; dr. sc. **Hrvoje Capak**, Veterinarski fakultet, Sveučilište u Zagrebu)

12.00 - 16.00 **Osnove ehokardiografije**
(doc. dr. sc. **Marin Torti**, **Ines Jović**, dr. med. vet., Klinika za unutarnje bolesti, Veterinarski fakultet, Sveučilište u Zagrebu)

//// KEYNOTE LECTURE /////

FACTORS IN INTERSPECIES TRANSMISSION OF INFLUENZA A VIRUSES

Janet M. Daly

School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, LE12 5RD, UK

Influenza A viruses (IAVs) occur as different subtypes (e.g. H3N8) named according to the proteins that project from their surface (haemagglutinin, H, and neuraminidase, N). The traditional view of the ecology of influenza A viruses was that ducks and shorebirds are the reservoir hosts; all known subtypes were found in these species and infection usually results in no or mild clinical signs. Occasionally, certain subtypes have 'spilled over' and, more rarely, become established in other hosts, including terrestrial poultry, pigs, horses and people. What allows certain subtypes to jump to a new host is not fully understood.

Early studies established that specific recognition of α2,3-linked host cell receptors by avian IAVs plays a role in restricting transmission to humans; the cells lining the human upper respiratory tract primarily express α 2,6 linkages. In contrast, α2,3 receptors are found in equine trachea, permitting direct transmission from ducks to horses, and a mixture of α2,3 and α2,6 receptors on porcine trachea. It was postulated that pigs may act as a 'mixing vessel' as cells could be infected simultaneously with avian and human IAVs allowing exchange of gene segments (reassortment). The emergence of the 2009 pandemic H1N1 virus, a triple reassortant of avian, human and porcine IAVs, from pigs lent credence to this theory. However, the critical role of receptor specificity has recently been challenged. Viruses that are more pathogenic may overcome the barrier posed by lack of relevant receptors. For example, this appears to be the case for highly pathogenic avian influenza (HPAI) viruses that infect people. However, despite circulating for 20 years, HPAI viruses of the H5N1 subtype have not yet acquired the ability to transmit person-to-person.

In 2005, the first evidence for transmission of equine influenza virus to dogs (greyhounds in the USA) was reported. The virus subsequently became endemic in the USA. It was demonstrated retrospectively that equine influenza virus had also jumped from horses to foxhounds in the UK in 2002, but did not become established. Experimental infection of ponies with an equine influenza virus strain isolated in 2003 confirmed the more severe clinical signs reported from the field than with earlier isolates. This was associated with greater induction of pro-inflammatory cytokines. Genome sequencing of the 2003 equine influenza virus strain revealed a truncation in the viral NS1 protein, which modulates the host cytokine response. This suggests that the species jump occurred as a result of emergence of a more pathogenic equine influenza virus strain. Interestingly, the NS1 truncation was lost on adaptation to dogs.

There is also mounting evidence that differences between species in cell signalling pathways that determine how cells respond to infection play a key role in interspecies transmission. We have been focussing on involvement of the PI3K pathway, which regulates cell proliferation and survival. Activation of the PI3K pathway by IAV infection differs depending on the host species from which cells are derived and the virus strain. The NS1 protein interacts with the PI3K pathway by binding to a regulatory subunit. Understanding how different interactions between host PI3K pathways and IAV NS1 proteins are responsible for interspecies transmission may enable better prediction of whether emerging viruses have the potential to cause future pandemics and to breed resistant livestock.

//// INVITED LECTURES /////

MOLECULAR CHARACTERIZATION OF FOODBORNE PATHOGENS

Dean Jankuloski, Sandra Mojsova, Katerina Blagoevska, Lazo Pendovski

Faculty of Veterinary Medicine-Skopje, "Ss. Cyril and Methodius" in Skopje, Republic of Macedonia

The ability to accurately distinguish strains of foodborne pathogens is crucial for efficient epidemiological and surveillance analysis, studying microbial population structure and developing improved public health control strategies. To accomplish such goals, several molecular typing methods have been proposed that can identify disease outbreak isolates. Monitoring of PFGE profiles makes it possible to assess the molecular diversity and circulation of food pathogens within the food chain. Finding the PFGE profile of a strain isolated from food that matches a human strain profile does not necessary imply that this food is the source of the contamination. Pulsed-field gel electrophoresis (PFGE) is considered as the "gold standard" method for Listeria monocytogenes and other epidemiological investigations. There other ultimate molecular methods, such as multi locus sequence typing (MLST) and whole genome sequencing (WGS), with great promise for typing in the future, but they are currently not widely available and accepted. For the food industry, tracking of major foodborne pathogens can give scientific evidence about where food poisoning bacteria are entering a process, where cross contamination may be occurring, whether a particular strain is endemic and/or persistent in a factory environment, and, most importantly, where controls should be directed. All epidemiological information gathered in the food chain, such as timeline, product description, food processing and sampling stage, should be crossed with PFGE, MLST or WGS typing data. This is necessary to conclude the relationship between different contaminations events. The aim is therefore to collect as much scientific evidence as possible to help the authorities to decide whether to withdraw a product from the market or to locate the source of a foodborne outbreak. This demonstrates that establishing and sharing molecular typing data is the best way to link cases from one source to another, from one country to another, thereby enabling the identification of a potentially common source of a national or multinational outbreak. Nevertheless, detecting a human strain profile in food should improve the rapidity and precision of outbreak detection. Therefore, the European Food Safety Authority (EFSA) collects information on food and animals, as well as food-borne outbreaks from the Member States (MSs) and publishes an annual report. That is why the EFSA has created a Task Force on Zoonoses Data Collection, with participants from all the MSs, as well as the DG SANCO and the European Centre for Disease Prevention and Control. The ECDC coordinates a network of national public health laboratories (NPHLs) in charge of typing food pathogens strains isolated from national clinical cases. The ECDC has developed a European molecular surveillance database with the objective of sharing, in real time, molecular epidemiological information and PFGE and WGS data on strains of *L. monocytogenes, E.coli, Salmonella* isolated from humans. All these tools and networking will enable the location of the source of the pathogen microorganisms in the food chain and their successful eradication.

RODENT BORNE ZOONOSES IN REPUBLIC OF CROATIA

Josipa Habuš

Department of Microbiology and Infectious Diseases with Clinic, Faculty of Veterinary Medicine, University of Zagreb, Croatia

Rodents are the most abundant and diversified mammals that account for almost half the total number of mammalian species. Their role in maintenance and transmission of different pathogens has been well known since the Middle Ages when the Black Death (plaque) took more than 20 million lives across Europe. Rodent-borne diseases still pose a threat to human and animal health worldwide. Moreover, due to global warming, urbanization and intensified trade and travel, population density and the distribution of rodent/arthropod species, as well as changed human settlement patterns, certain diseases are now reemerging.

Rodent-borne diseases can be transmitted from rodents to other animals and humans via two different pathways. The first pathway is direct or indirect contact with rodents or an environment contaminated with rodent urine, faeces or saliva. The second pathway is transmission through various species of arthropods (ticks, flees, etc.) that often serve as biological vectors of the disease. A great deal of research has been undertaken across the globe to identify rodents that serve as reservoirs for certain pathogens, and to clarify the role of different biotic and abiotic factors that are important for rodent/vector population dynamics and the transmission patterns of various diseases. In Croatia, a multidisciplinary team, consisting of infectious disease specialists, veterinarians and foresters, is engaged in on-going research and monitoring of rodent-borne zoonoses in forest habitats. To date numerous studies have been undertaken to determine the presence of pathogens such as: Hantaviruses, *Leptospira* spp., *Francisella tularensis*, *Borrelia* spp., *Bartonella* spp., *Ehrlichia* spp., *Anaplasma* spp., *Rickettsia* spp., *Coxiella burnetii*, *Babesia microti*, *Toxoplasma gondii*, *Orthopoxvirus,* lymphocytic choriomeningitis virus and tick-borne encephalitis virus, in different rodent species.

The longest and best studied pathogens are hantaviruses and *Leptospira* spp. Outbreaks of both diseases (hemorrhagic fever with renal syndrome and leptospirosis) in Croatia correlate with an increase in rodent population density, and the appearance of so-called "mouse years". Certain biotic and abiotic factors have been identified which influence rodent population dynamics and consequently outbreaks of both diseases. In addition, high infection rates of *Leptospira* spp. and hantaviruses with numerous multiple co-infections have been documented in some studies. Further identification of potential temporal risk zones and better understanding of rodent reservoir ecology are essential for developing a prediction model that will quantify the risk of a certain disease and formulate an adequate public health response.

CURRENT SCENARIO OF MASTITIS IN INDIA AND POSSIBLE STEM CELL BASED APPROACHES IN ITS MANAGEMENT

Neelesh Sharma

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India is the largest milk producer, however, it still faces the challenge of improving the quality of milk as per international standards, even after extensive efforts by the Food Safety and Standards Authority of India (FSSAI). The dairy sector in India is continuously growing and undergoing major changes, to meet the higher demand for milk and milk products, with the pressure of quality control. Our target is to achieve the goal of about 180 million tons in milk production per annum by the year 2020-2021. The most important aspect is to control production diseases in dairy animals, including mastitis, which causes heavy economic losses to the dairy industry worldwide. "Bovine mastitis" is the inflammation of the mammary parenchyma, with or without visible changes in the milk and udders, and has a direct impact on the quality of milk and milk products, and milk production.

In India, there has been a significant (about 135 fold) increase in economic losses in last few decades. The clinical form of mastitis is a matter of potential concern from an animal welfare perspective, as it is an acutely painful condition. The prevalence of bovine mastitis in India ranges from 29.34% to 78.54% in cows, and 27.36% to 45.00% in buffaloes. There are many factors involved, such as the lack of knowledge of farmers about mastitis, poor hygienic and milking practices, delay in diagnosis and treatment, which play an important role in this high prevalence of mastitis. Staphylococci, streptococci and *E. coli* are the major etiological agents in dairy cows. Early and rapid diagnosis of bovine mastitis has the potential to improve the efficacy of treatment and bacteriological cure rate, which is still challenging to vets, in terms of timely, specific and effective treatment.

The high prevalence and the low cure rate of this highly economically harmful and sometimes deadly disease is alarming for the dairy sector, vets and policy makers. The increased occurrence of pathogens that are resistant to conventional antibacterial treatments is another serious health concern and increases the need to find potential therapeutic options for mastitis. Unfortunately, presently it is very difficult to find a therapeutic strategy to improve or revert the more than 50% post-mastitis structural damage to mammary glands. One technology, which may be of use in improving the structural defects associated with mastitis, is the use of adult stem/progenitor cells. Stem cells have an important property in that they serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the animal is alive. We have established bovine mammary epithelial stem cells (bMESCs) using various markers such as Nanog, Oct4, Sox9, CK14, CK18, CSN2, CSN3 etc. We targeted a mastitis causing organism using antibacterial genes (e.g. Lipocalin-2) into bMESCs by successful transfection using mammary specific vectors. Our findings have opened up new hope in the management of bovine mastitis.

THE DARK SIDE OF SELECTION: NEGATIVE - SIDE EFFECTS OF SELECTION FOR HIGH PRODUCTIVITY

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Long-term systematic selection has considerably increased livestock productivity. However, the selection for economically interesting production traits has influence on growth, metabolism and reproduction parameters. In most livestock species, beside the breeding goal to achieve high economic production efficiency (which usually means high production combined with low feed intake and the regular repetition of reproductive cycles), some negative side effects of selection have become apparent. This is based on the fact that production traits are the result of different synchronous metabolic processes that are to some extent genetically determined. Therefore, the biochemical reactions and gene control mechanisms influence several traits, some of which are desirable, and some are considered "negative" for animal welfare and longevity.

Milk, meat, egg production, food conversion, carcass composition and similar traits are directly related to the economic efficiency of the livestock production systems. However, high production selection criteria used in breeding programs may lead to indirect selection for other, unfavourable traits, and the disruption of animal homeostasis. As different authors have pointed out, the main physiological, immunological and behavioural problems that may arise while enhancing livestock productivity through breeding and selection are: metabolic and/or physical exhaustion after prolonged high production, the occurrence of hunger or metabolic diseases as a consequence of imbalance between nutrient demand and supply, increased susceptibility to metabolic or infectious diseases associated with the decreased immunity of animals, chronic pain due to body shape distortion and condition loss, etc.

Some examples of the negative side effects of selection for high productivity in livestock are summarized below. They are presented from information based on field data or the experimental findings of numerous authors.

Selective breeding for increased milk yield is the root cause of declining longevity and increasing incidence of some production-related health problems in dairy livestock. In the case of dairy cattle, selective breeding has resulted in more than doubled milk production per cow over the past 40 years. A negative energy balance in early lactation and the mobilisation of body reserves for milk production may be associated with a higher incidence of metabolic disorders and health problems, such as impaired fertility (lower pregnancy rate at first service, abnormal oestrus cycles and an increase in early foetal loss), lameness or mastitis. The average production lifetime in many high genetic merit dairy herds in developed countries is up to three lactations. From the point of view of sustainability, a cow becomes profitable only when she reaches her fourth lactation. Furthermore, optimal efficiency for highly productive dairy cows is reached after five or six lactations. So, in the light of sustainable production, selection criteria must include not only high milk yield, but also longevity, lower occurrence of metabolic disorders and other health problems, as well as smaller loss of body condition score during lactation and impaired fertility.

Selection in pig production is focused primarily on an increase in growth rate, a decrease in feed conversion and back fat thickness of carcasses and, recently, litter size in sows. These production traits are mostly of moderate to high heritabilities, meaning that the increase in productivity is, to a high extent, of genetic origin. Unfavourable side effects that accompany long-term selection for meat production traits may be noticeable as responses in some reproductive traits (the delayed onset of puberty in gilts, less intense symptoms or shorter

duration of proestrus and oestrus, a prolonged interval from weaning to farrowing, a greater number of piglets per litter, but a higher percentage of stillborn, mummified or lightweight piglets), as well as different metabolic or health disorders (the more frequent occurrence of stress susceptibility and, consequently, the appearance of pale, soft and exudative meat, more frequent problems with legs, as well as behavioural problems in highly productive herds).

Similarly, the primary aim in poultry meat production is to reach rapid growth accompanied by superior feed efficiency and high processing carcass yield. As many authors have stated, the increase in broiler growth performance mostly resulted from genetic selection, and it has been accompanied by an increasing incidence of health problems. Some negative responses in metabolic traits (decrease in metabolic rate, a high rate of food passage and digestion, higher enzymatic activities in the small intestine) and altered growth patterns (broilers/ turkeys reach slaughter weights at a younger age; faster increase in body fat), negative responses in reproduction traits (reduced fertility and the inability of natural mating caused by excess body weight, a higher percentage of defective eggs, lower semen concentration, volume and motility, and a higher percentage of dead and abnormal sperm cells), undesirable responses in health traits (negative immune performance due to lower antibody responses, higher mortality mainly caused by infectious diseases and heart and circulation problems, ascites, incidence of leg problems), as well as stress susceptibility (and consequential glycolytic changes in muscles), are more frequent in flocks selected for high production efficiency.

Selection for rapid growth also causes some health problems in beef cattle. The impact of selective breeding is illustrated by the selection of Belgian Blue or Piedmontese cattle for double muscling, which leads to larger, heavier muscles, particularly in the hind quarters. This mutation (in the myostatin protein) increases muscle mass, decreases body fat and improves the feed efficiency of animals. As a result, cows often carry calves which are too large for natural birth, which leads to the frequent use of caesarean sections. Furthermore, myostatin mutations are pleiotropic in their effects, and negatively affect a number of different body systems (as muscle mass is significantly increased, fat and bone mass and the weight of other internal organs are decreased). But, myostatin gene mutation is not the only one associated with more muscular and leaner animals. A similar, known mutation in sheep is the callipyge mutation (known as "beautiful buttocks" mutation), which causes an increase in muscle mass primarily located in the back and hindquarters. Unlike the myostatin mutations in cattle, this mutation is the result of hypertrophy (an increase in the size of the muscle fibres), which begins after birth, but causes a negative side effect - a lack of tenderness of lamb meat.

Today's commercial laying hens have been selectively bred to produce more than 250 or even 300 eggs per year. This unnaturally high level of productivity often causes hens to suffer from so-called production (including osteoporosis and accompanying bone fractures, which may lead to reproductive disorders) or behavioural (such as canibalism) diseases. This is expected, knowing that the amount of calcium that a hen deposits in her egg shells over a laying year can be up to 20 times the amount retained in her body.

From all the above, we may conclude that the selection of farm animals for high productivity may result in correlated, undesirable responses in some other traits important for their welfare and longevity in production. Also, new molecular technologies implemented in many breeding schemes have increased the efficiency of selection in farm animals by allowing breeders to make selections, at an earlier phase in the animal's life, on the basis of genotype rather than phenotype. However, overselection for production traits may cause animal welfare problems. Having that in mind, selection programs should include genetic correlations between favourable production traits and accompanying metabolic, reproductive and health traits in farm animals. In such a way, the breeding goal will be turned toward sustainable production, i.e. the breeding of animals with a long (re)productive life at an economical production level, without disturbed welfare.

SEAGULLS, *LARIDAE*, AS A MODEL FOR RESEARCH OF WILD BIRDS' DISEASES

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Gulls are one of the most common birds in human surroundings. They are present in most aquatic habitats (from sea coasts and ports to lakes and rivers). In the last few decades, whenever there is no separation of the organic component of household garbage, they have found an endless source of food on open rubbish tips. They are especially numerous during the colder part of the year (Nov – Feb), when tens of thousands of gulls can be found feeding on rubbish tips.

We have been studying gulls on Zagreb's city rubbish tip since 1999. After the first ecological research we obtained new data about their movements and dynamics on the tip, with most attention paid to reading rings to determine their origin. There were 9 species of gulls recorded on the tip; Black-headed Gull, *Larus ridibundus,* Yellow-legged Gull, *L. michahellis,* Common Gull, *L. canus,* Caspian Gull, *L. cachinnans,* Lesser Black-backed Gull, *L. fuscus,* Greater Black-backed Gull, *L. marinus,* Ring-billed Gull, *L. delawarensis,* Mediteranean Gull, *L. melanocephalus,* and Pallas's Gull, *L. ichthyaetus.* In the winter of 2006/2007 we started catching and ringing gulls on the tip. Our results show that gulls feeding on Zagreb city rubbish tip originate from all over Europe: from central Russia in the east, the UK in the west, Finland in the north, while southernmost recovery was from Tunisia. As gulls are capable of crossing great distances in short periods, and tend to form huge flocks of individuals from different populations and age groups on adequate feeding sites, they can easily transmit and spread viruses and bacteria across a huge area, and therefore can potentially pose a serious threat to both humans and farm animals. Also, environmental conditions during these months are the most suitable for transmission of bacterial and viral pathogens, due to both low temperatures and huge aggregating flocks while feeding or roosting.

In the winter of 2006 we started taking biological samples from captured gulls, mostly swabs, but also blood samples. We tested swabs for the presence of 2 viral (avian influenza and avian paramyxovirus) and 2 bacterial (*Salmonella* and *Campylobacter*) pathogens. Blood samples and smears were tested for the presence of avian haemosporidian parasites of the genera *Haemoproteus*, *Plasmodium* and *Leucocytozoon,* using a combination of molecular and microscopic approaches.

During the past 11 years we have isolated the first H13 and H16 subtypes of avian influenza virus in Croatia. The vast majority of avian paramyxoviruses isolated from gulls belong to serotype 1. During our research we detected more than 10 different serovars of *Salmonella.* Also we found the first *Campylobacter lari* in Croatia. Most of the *Campylobacter* isolated from the gulls showed multiple resistance to antimicrobial drugs which contributes to the thesis of gulls as an important reservoir of these bacteria. In total, we screened 173 individual Black-headed Gulls (*Chroicocephalus ridibundus*) and 97 blood samples of large gulls (*Larus* sp.) for haemosporidian parasites over two consecutive years (2015 and 2016). The preliminary results showed higher parasite prevalence in the large gulls (37.2% and 22.2% for the two years, respectively) compared to 2.7% in 2015 and 3.3% in 2016 in the Black-headed gulls. The predominant infections were by *Haemoproteus* spp. while haemosporidians of the genus *Leucocytozoon* were not detected. *Plasmodium* parasites, the causative agents of malaria, were recorded in one sample of *Larus cachinnans*, one sample of *Larus michahellis* and three samples of Black-headed gulls. The latter two species represent new host records for *Plasmodium* spp. among gulls.

APPLICATIONS OF PROTEOMICS IN VETERINARY RESEARCH

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In the last few decades veterinary research, as with all of biomedical science, has benefited enormously from genome sequencing and gene function analysis. However there has also been an expansion in the use of post-genomics tools with the development of additional omic sciences which have allowed an unparalleled approach to an overall view on how organisms and in particular domestic animal species, work and how they react to particular conditions and disease. Omics now includes several sub-disciplines. These include transcriptomics, proteomics, lipidomics and metabolomics. Each of these sub-disciplines is dedicated to a particular aspect of research. For instance, proteomics may be defined as the science that studies the proteome, or the proteins present in a given organelle, cell, tissue, fluid, organ, organism or population. By analogy other omics are dedicated to particular classes of compounds: transcriptomics for the transcriptome or all RNAs in a given organelle, cell, tissue, fluid, organ, organism or population, lipidomics for the lipids and metabolomics for the metabolites. Veterinary research is being, and will in the future be greatly enhanced by multi-omics research, which is integral to a Systems Biology Approach.

Among the omic sciences, applications of proteomics has been recently developing rapidly in veterinary research. The primary purpose of livestock farming and aquaculture is production of protein for human nutrition and other uses. Animal products such as dairy produce, meat, fish, eggs and wool have an enormous potential to be analysed using proteomics in order to provide greater insight and understanding of their highly complex roles in vivo and as they are processed post-harvest. In companion animal sciences the applications of proteomics mirrors the major advances that this technology has provided in human clinical research in areas such as disease biomarker discovery, vaccine candidate identification, drug target analysis and an increased understanding at a molecular level of physiological and pathophysiological processes.

Biomarker discovery studies have made significant progress in different species of interest in veterinary research. In dairy cow research, proteomic investigations have an increasing ability to demonstrate the change in the milk proteome during mastitis, caused by intra mammary infection. A quantitative proteomic investigation of milk from a model of *Streptococcus uberis* mastitis revealed over 300 proteins that changed significantly following infection. A number of proteins were identified that increased over a thousand fold in response to the pathogen, including haptoglobin, cathelicidin and mammary associated serum amyloid A. These proteins are candidates for development as biomarkers of mastitis. In aquaculture of Atlantic salmon, proteomics has been able to identify serum enolase as a viable biomarker of pancreas disease caused by salmonid a-virus infection, reflecting the effect that this virus has on the skeletal muscle of the fish. Proteomic investigation of the change in serum proteins in dogs infected with *Babesia canis canis* have identified potential biomarkers such as clusterin and leucine-rich-a2-glycoprotein which could be incorporated into multiplexed diagnostic profiling as a means to diagnose and monitor therapy of this parasitic infection.

The potential for applications of advanced proteomics in veterinary research is great and the use of the technology for veterinary applications is in reality in its infancy. However in order to fully exploit proteomics in veterinary research a multi-disciplinary approach is needed covering protein biochemistry, mass spectrometry and bioinformatics to generate and interpret the big data sets that result from a single experiment. A collaborative approach between experts in these omic disciplines with researchers from all areas of veterinary science is essential and will yield immeasurable benefits to advance veterinary research into the future.

CONGESTIVE HEART FAILURE PATIENT– CRITICALIST'S VIEW

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Most dogs and cats presented to an emergency service due to decompensated congestive heart failure (CHF) are suffering from backward failure. Depending on the affected valve, this leads to pulmonary congestion or ascite formation. Most often, the mitral valve is affected, leading to pulmonary edema and dyspnea. Therefore, respiratory distress is the most common emergency presentation and should be addressed primarily.

The emergency treatment of patients in respiratory distress consists of stress reduction and oxygen support.

Avoiding stressful situations, such as strong fixation, rectal thermometry, placement of a venous catheter and taking radiographs is mandatory. Pharmacological stress reduction using sedatives may reduce muscle activity and therefore breathing. This may lead to hypoxemia and death. Therefore, sedation is only recommended after careful evaluation of the patient and assessing the risk benefit ratio.

As patients suffer from hypoxemia secondary to pulmonary edema, oxygen support is mandatory. This can be performed via flow by oxygen, oxygen mask, oxygen nasal tubes or oxygen cages. Depending on the method, an inspiratory oxygen concentration of 30–60% can be reached. Higher concentrations can be reached with some closed oxygen cages, by intubation, or hyperbaric chambers.

The next step in the emergency evaluation of the patient in respiratory distress is to find the localization and if possible the cause of respiratory distress. If the lung sounds are increased on auscultation, a pulmonary parenchymal disease may be suspected. If these are present on both sides of the chest, and perhaps a heart murmur is present, a cardiogenic pulmonary edema is very likely.

In cats the auscultation findings may be variable, as they commonly additionally develop pleural effusion. This leads to decreased ventral lung and heart sounds on auscultation. Percussion is dull, especially at the ventral thorax. Looking at the jugular vein, this is often distended in animals with decompensated right-sided heart failure.

The classical way of diagnosing cardiogenic pulmonary edema in an emergency situation is chest radiography. In dogs increased heart size, increased perihilar interstitial lung opacity and distended pulmonary vessels are visible. In cats increased lung opacity is often distributed over the whole lung area (more diffusely). Additionally, pleural effusion may be present. As chest radiographs can be stressful for animals in respiratory distress, alternative methods are warranted.

In recent years, chest and lung ultrasound techniques have been developed. Chest ultrasound (T-FAST - thorax-focused assessment with sonography in trauma) aims at detection of free fluid at the dorsal 9th intercostal space and the middle 5th intercostal space. VetBLUE (Veterinary bedside ultrasound examination) seems to be more reliable for detection of pulmonary edema. The dorsal 9th intercostal space, middle 5th intercostal space, ventral 4th intercostal space and middle 2nd intercostal space are evaluated on both sides. Typical rocket signs or a complete wet (grey) lung is visible in the ventral lung areas or at all 8 sites. In cats,

pleural effusion may be visible, which is even more reliable compared to radiographs. Recently studies have evaluated the VetBLUE system and found the sufficient diagnostic accuracy of this techniques to detect cardiogenic pulmonary edema.

Biomarkers have also been evaluated for differentiating cardiogenic and non-cardiogenic dyspnea. N-terminal pro-B-type natriuretic peptide (NT-proBNP) in plasma and pleural effusion are markedly higher in cardiogenic compared to non-cardiogenic respiratory distress. Bedside one-step tests have been established for this diagnostic purpose in cats.

If the emergency work-up supports a high suspicion of cardiogenic pulmonary edema, diuretic therapy should be started. The author used high dose (5-8 mg/kg in dogs, 3-5 mg/kg in cats) intramuscular furosemide as a first line treatment very early in the evaluation process. If needed, this dose can be repeated every hour until the respiratory rate decreases below 40 –50 breaths per minute. If the patient is stable enough to tolerate venous catheter placement, IV medication is more effective. In non-responding or severe cases, an intravenous constant rate infusion of furosemide is often required, at 1-2 mg/kg/h.

As an alternative IV torasemide (0.3 – 0.5 mg/kg) could also be used. It has a stronger and longer lasting diuretic effect, starting 20 min after IV application with a peak at 2-4 hours. Oral medication is not recommended in severely dyspnoeic animals.

The most important clinical monitoring consists of assessment of respiratory rate and respiratory effort. During treatment, respiratory rate and effort should decrease. In severe cases, the respiratory rate may decrease but respiratory effort will increase, leading to development of respiratory fatigue and the death of the animal.

Due to the lack of an adequate probe site and motion artifacts due to panting, pulse oximetry often does not work in dyspnoeic animals. New-generation pulse oximeters, using a reflection probe placed at the tail base, could be used to measure oxygen saturation. In any case, signal quality should be assessed before interpreting the results. Oxygen saturation above 95% is indicative of adequate oxygenation. Regardless of the fact that saturation is satisfactory, if the animal is breathing at a high rate and effort to reach this level, therapy should be intensified. At oxygen saturation below 95%, diuretic therapy and oxygen support should be increased to improve oxygenation.

Pulse oximetry is a surrogate marker of oxygen content in the blood. Optimally, arterial oxygen partial pressure should be assessed with arterial blood gas analysis. As this it is not easy to perform and potentially stresses the patient, it is not often used clinically. In complicated cases, arterial blood gas analysis, using an indwelling arterial catheter, may help to monitor therapy and support decision processes.

As an alternative monitoring tool the VetBLUE technique can be used for tracking the patient. Sometimes changes in the lung tissue can be seen more quickly than clinical or radiographic changes.

In animals not responding to diuretic therapy, advanced therapy techniques can be used. Applying a CPAP (continuous positive airway pressure) helmet may help to keep edematous alveoli open, but it requires high oxygen flow. Positive pressure ventilation, with positive end-expiratory pressure under general anesthesia also helps to improve short-term survival. In cardiogenic pulmonary edema, it requires about 12-48 hours ventilator treatment until the medical therapy dissolves the edema, and the patient can be weaned from the ventilator. Ventilation also offers the opportunity to work with high inspiratory oxygen concentration over a short time, and perform alveolar recruitment maneuvers.

CONGESTIVE HEART FAILURE PATIENT – A CARDIOLOGIST'S VIEW

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Emergency veterinarians commonly care for canine and feline patients with congestive heart failure (CHF). Depending on the primary cause and severity of the cardiac disease, clinical signs can vary from patient to patient and are not pathognomonic for cardiovascular disease. Clinical signs in patients with CHF may include: weakness and exercise intolerance, cough, lethargy, inappetence, vomiting, tachypnea, respiratory distress, syncope, or collapse. CHF is often presumptively diagnosed based on the patient's primary presenting complaints, signalment, a thorough history, and physical examination findings.

In dogs, the most common causes of CHF are acquired heart diseases, in the majority of cases degenerative mitral valve disease and dilated cardiomyopathy. One must not forget that congenital heart diseases, most often patent ductus arteriosus, as well as bacterial endocarditis and myocarditis, can lead to the development of CHF. Also, severe cardiopulmonary dirofilariasis or cardiac tamponade can also potentially lead to the development of CHF.

Chronic valvular disease (CVD) represents the most common, clinically significant acquired heart disease in older miniature and small breed dogs. The degenerative process can affect any of the four valves, but the clinical disease most often affects the mitral valve (chronic degenerative mitral valve disease or myxomatous mitral valve degeneration or mitral valve enocardiosis). CVD is estimated to be the cause of canine cardiac disease in approximately 75% cases. Studies suggest that CVD is an inherited trait, as is the case in Cavalier King Charles spaniels. CVD is characterized by loss of normal function and integrity of the affected valve, with the resulting regurgitation causing the functional inefficiency of the left or right side of the heart (depending on the valve affected by the process). Mitral valve endocardiosis leads to left-sided CHF, with development of pulmonary edema, while tricuspid valve endocardiosis leads to right-sided CHF and resultant accumulation of fluid in the systemic veins and body cavities (ascites and pleural effusion).

In comparison to CVD, dilated cardiomyopathy (DCM) is the most commonly acquired heart disease in medium, large and giant breed dogs. The most commonly affected breed with DCM in both European and North American studies is the Doberman Pinscher. DCM has been described in other breeds, such as Boxers, Newfoundlands, Great Danes, Irish Wolfhounds etc. DCM progresses through three stages. Stage I is characterized by a morphologically and functionally normal heart; there is no evidence/clinical sign of heart disease. Stage II is known as the "occult" or "silent" stage of DCM. The morphological changes consist of left ventricular enlargement and/or arrhythmias. Terms "occult" or "silent" refer to the owner's perspective, since the dog appears normal. Finally, stage III of DCM is characterized by clinical signs of CHF, and is referred to as the "overt" or "clinical" stage of DCM. Compared to stages I and II, which can last for years, stage III is of short duration (several months).

Cardiomyopathies are by far the most common cause of CHF in cats. The most common form of feline cardiomyopathy is hypertrophic cardiomyopathy, followed by restrictive/unclassified cardiomyopathy, dilated cardiomyopathy, and arrhythmogenic right ventricular cardiomyopathy, the rarest form of feline cardiomyopathies. Other possible causes of CHF in cats include hyperthyroidism, myocarditis/endocarditis, and congenital heart diseases. One may suspect cardiac decompensation and CHF in cats with respiratory distress and a history of recent intravenous fluid administration, recent anaesthesia or administration of long-acting glucocorticoids.

Hypertrophic cardiomyopathy (HCM), the most common form of feline heart disease, is characterized by concentric or asymmetric left ventricular hypertrophy, without an identifiable cause. The most common pathomechanism by which HCM leads to development of CHF is diastolic dysfunction, with elevation of filling pressures.

In the emergency setting, the most useful tests are emergency ultrasound and the NT-proBNP (N-terminal pro-B-type natriuretic peptide) test. Thoracic radiography is still the gold standard for diagnosis of cardiogenic pulmonary edema, but is often contraindicated in patients with respiratory distress, since it can worsen the patient's status and lead to death.

Generally speaking, the key elements of successful CHF patient stabilization include avoidance of stressful situations, rest, oxygen supplementation, intensive diuresis and/or paracentesis. Other possible interventions include heart rate control (use of antiarrhythmic drugs), positive inotropic support, and antithrombotic therapy. Fluid therapy is almost never a therapeutic option in a CHF patient, the only exception being a severely dehydrated patient where fluids are used extremely judiciously.

Emergency stabilisation is directed toward management of congestion, and often includes oxygen therapy, minimisation of stress, and aggressive diuretic therapy. Cats in congestive heart failure often present with pleural effusion, so in cases of moderate to large volumes of pleural effusion, thoracentesis is indicated. The goal of thoracentesis should not be complete removal of the effusion but relief of signs of respiratory distress.

Furosemide is probably the most important drug for management of acute CHF, and is currently the diuretic of choice for management of severe pulmonary oedema in dogs and cats. In animals with acute severe CHF, high doses of furosemide are often required, given as an intravenous bolus or continuous rate infusion. The definitive dose of furosemide required by an individual animal is hard to define, but for dogs, doses of up to 4 mg/kg, and cats 3 mg/kg every 1-2 hours are required. Continued use of furosemide commonly causes azotaemia and electrolyte depletion (hypokalaemia).

In animals with CHF and low cardiac output, as is the case in DCM, positive inotropic support is indicated. The most commonly used drugs are dobutamine and pimobendan. Dobutamine, a sympathomimetic and ß1 receptor agonist, is most commonly given as a continuous rate infusion, because of dobutamine's short half-life. In dogs, the infusion rate is adjusted upward from 2.5 µg/kg/min (at 2.5 µg/kg increments) until signs of improved cardiac function are apparent (e. g. increased systemic blood pressure, warm limbs, normal CRT duration). Increases in heart rate greater than 20% above the baseline, heart rates >190 bpm or occurrence of arrhythmia require dose reduction. Pimobendan is a calcium sensitizing drug and phosphodiesterase 3 inhibitor possessing positive inotropic and vasodilating effects. It has been studied in dogs with chronic valvular disease and dogs with dilated cardiomyopathy, where its use results in significant clinical improvement. Pimobendan is also available as a solution for injection (Vetmedin®, solution for injection, 0,75 mg/ml, Boehringer Ingelheim), and as such can be administered in emergency patients. The dosage of Vetmedin® solution is 0.15 mg/kg intravenously. It should be administered only once, and therapy should be continued with peroral administration of pimobendan.

In certain cases, mechanical ventilation is desirable in order to avoid respiratory muscle fatigue, and initiate positive end-expiratory pressure to help mobilize oedema while other therapies take effect.

Most animals with CHF improve markedly within 24 to 48 hours after initiation of stabilization. Hence, if improvement is not detected, both the diagnosis and current therapeutic plan should be reconsidered.

PORTOSYSTEMIC SHUNT - DRUGS OR SURGERY?

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The most common portosystemic vascular anomaly is a congenital portosystemic shunt (PSS), i.e. abnormal communication between systemic and portal blood vessels. Congenital PSS most often involves only one blood vessel (rarely 2 or more blood vessels). Its location is either within the hepatic parenchyma (intrahepatic PSS) or outside the parenchyma (extrahepatic PSS). Blood from the abdominal organs contains toxins and other products absorbed in the intestines. In healthy animals, the liver is the primary site for toxin metabolism. In animals with PSS, portal blood bypasses the liver and enters the systemic circulation. At that moment, the liver is not able to neutralize toxins, and clinical signs of PSS occur. Hepatic encephalopathy is a syndrome associated with a severe hepatic problem (loss of more than 70% of hepatic function) and is caused by ammonia, tryptophan, glutamine, aromatic amino acids, GABA, endogenous benzodiazepines, short chain fatty acids etc.

Congenital extrahepatic PSS is common to a variety of breeds, including Yorkshire terriers, Maltese, shi-tzu, etc. Congenital PSS in large and giant breed dogs (Irish wolfhounds, Australian cattle dog, etc.) is also more often extrahepatic (one study showed that 71% cases of PSS in large breed dogs are extrahepatic), but the incidence of intrahepatic PSS is higher in large breed dogs than in small breed dogs.

Clinical signs observed in dogs with PSS are divided into three groups: neurological, gastrointestinal and urological. The neurological clinical signs are seizures, blindness, circling, ataxia pacing, bizarre behaviour and coma. Gastrointestinal clinical signs (vomiting, diarrhea, anorexia, pica, melena) were observed in 30% dogs in one study. The urinary tract clinical signs are stranguria, urinary obstruction and urate calculi.

Treatment of PSS can be divided into medical and surgical treatment. The medical treatment controls the clinical signs, but does not resolve the underlying reduced hepatic perfusion. Dogs with acute clinical signs of hepatic encephalopathy can be treated with a warm water enema, peroral lactulose administration, lactulose enema, antibiotics to reduce intestinal urease-producing bacteria, and anticonvulsants as needed. The anticonvulsants used are midazolam, phenobarbital, potassium bromide, sodium bromide and levetiracetam. Lactulose is a disaccharide metabolized by colonic bacteria into organic acids. The synthesized acids promote acidification of colonic contents and reduction of intestinal bacterial numbers. The nutritional management is an important part of the PSS patient protocol. Proteins should be moderately restricted and recommended sources of proteins are milk and vegetables. Dogs with intrahepatic PSS are predisposed to develop gastrointestinal erosion/ ulcers. Therefore, antacid drugs (H_2 blocker and proton pump inhibitor) are a constitutive part of medical treatment in these dogs. Hepatoprotective agents (S-adenosylmethionine, ursodeoxicholic acid, silymarin and vitamin E) are currently used to support hepatic therapy.

Surgical treatment is a standard option in PSS treatment. Partial or complete acute occlusion with ligatures was used standardly in history. Complete occlusion of PSS can result in portal hypertension and development of multiple acquired shunts. Currently, gradual attenuation is a standard surgical option and can be performed with cellophane bands, an ameroid constrictor or hydraulic occluder. Intrahepatic PSS can be closed using endovascular embolization, and either lobar hepatic vein ligation or lobar portal vein ligation.

Perioperative mortality rate in dogs with extrahepatic PSS is 2-32% after suture ligation, 7% after ameroid constrictor placement, and 6-9% after cellophane banding. Similar percentages are observed in dogs with intrahepatic PSS. The most common cause of death in the first month after surgery is severe persistent neurological seizure. The median survival time of dogs treated medically was 10 months from the time of diagnosis to euthanasia. After long term medical treatment, owners of 65% dogs with intrahepatic PSS decided for euthanasia due to uncontrolled neurological signs, but only 33% dogs with extrahepatic PSS were euthanised. The longterm survival rate was 52% in dogs treated medically, and 88% in dogs treated surgically. Therefore, it can be concluded that surgery is in the long term a better option, but the perioperative mortality rate should not be forgotten.

MANAGEMENT OF EARLY PREGNANCY AND EARLY PREGNANCY LOSS IN THE MARE

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Diagnosis of pregnancy in a horse

For detection of pregnancy in a horse, transrectal ultrasound is the method of choice from day 10 after ovulation until term. Pregnancy examination by transrectal ultrasound should be scheduled before the next expected oestrus, i.e. on day 17 or 18 after ovulation. If the mare is found not to be pregnant, there is sufficient time to prepare her for rebreeding during the subsequent oestrus that follows immediately. In case of detection of a pregnancy, this has to be definitely recognized as singleton or twin. However, in mares with double ovulation, pregnancy diagnosis is preferably done already on day 14 or 15 after ovulation, allowing for easy reduction of twins into a single conceptus before uterine fixation. Especially in mares with a history of early embryonic loss, a second pregnancy check is recommended around day 35 after ovulation, i.e. before the start of placentation. A third pregnancy scheduled at the end of the 7th month of pregnancy is advisable because abortion before then frequently remains undetected and may result in mismanagement of mares that are no longer pregnant.

Early pregnancy loss in mares

In horses, early pregnancy loss before day 35 after ovulation occurs on average in 10% of pregnancies. In this species, early pregnancy is more prone to conceptus loss than any other phase of gestation. In subfertile and aged mares, the condition is seen even more frequently, and over a longer time period during pregnancy. The major reasons for early pregnancy loss are the retarded growth and development of the conceptus. This may be either caused by factors intrinsic to the conceptus, or insufficient support by the endometrium.

Approaches to support maintenance of early pregnancy in mares

In mares in early pregnancy, maximal concentrations of progestins occur on day 8 after ovulation and subsequently slowly decrease until the formation of the accessory corpora lutea. In cattle, periovulatory progesterone induces down-regulation of endometrial progesterone receptors, which is considered a pre-requisite for the onset of histotrophe production. In this species, a direct relationship between progesterone secretion and conceptus development has been proven. In the horse, although such a relationship has not been proven until now, the significance of progestins for undisturbed conceptus development was suggested over 20 years ago. Therefore, oral progestin supplementation is a routine treatment to prevent conceptus loss in early pregnancy in mares. In older mares, treatment of mares from day 5 after ovulation

with the progestin altrenogest counteracted delayed conceptus growth. As in cattle, progestin supplementation resulted in an early down-regulation of endometrial progesterone receptors, which is suggested to enhance endometrial histotrophe production.

For the same purpose, stimulation of endogenous progestin secretion has attracted interest in several species. In cattle, treatment with hCG (human chorionic gonadotrophin) during the early luteal phase stimulated development of the corpus luteum, and also increased plasma progesterone concentration. In horses this treatment did not influence corpus luteum function, but induction of ovulation with hCG stimulated periovulatory progestin concentration, and was associated with the increased size of the embryo proper. This demonstrates that injection of hCG during oestrus does not only induce ovulation but has also a stimulatory effect on corpus luteum function and progestin release. In a recent study, mares in early pregnancy were treated with altrenogest to increase endogenous progestin concentration, injected after ovulation with the prostaglandin F2α analogue cloprostenol to decrease luteal function, or left untreated. While conceptus size on day 14 after ovulation was not affected, the influence of progestin concentration on endometrial function and gene expression were clearly detectable. These findings support the suggestion that periovulatory progestin concentration in pregnant mares affects endometrial function beyond the time of maternal recognition of pregnancy.

COLIC HORSE AS A DIAGNOSTIC AND THERAPEUTIC CHALLENGE

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Colic is one of the most important medical problems of horses. Helping a horse with colic to recover and the horse owner to regain his companion in good shape remains a challenge for equine veterinarians worldwide.

After taking a good medical history and initial clinical evaluation sometimes mild colic can be managed medically without additional tests and procedures. If not, procedures such as nasogastric intubation, and diagnostic tests, such as rectal palpation, may follow. The primary aim of the initial evaluation of a horse affected with acute colic is to attempt to distinguish horses with mild or uncomplicated disease processes from horses with a potentially life- threatening disease. Early referral is of vital importance and should be considered as an emergency. A delay in making the decision to refer a colic horse is one of the most important factors contributing to the chances of survival of horses requiring surgery or intensive medical care.

After referral procedures such as abdominocentesis, intravenous catheterization, and trocharization, and diagnostic tests such as clinical laboratory data, peritoneal fluid analysis, abdominal ultrasound and radiographic examination, are performed to plan the treatment in the most optimal way. After medical management with analgesics, anti-inflammatory drugs, laxatives, fluids and electrolytes, the decision whether or not to undertake an abdominal exploration must be made. There is no single criterion that can be relied on when deciding about the need for surgery in an individual horse. The horse's pain, response to analgesic therapy, cardiovascular status, rectal examination findings, the amount of gastric reflux, sonographic results, and analysis of blood and peritoneal fluid are to be considered carefully. Surgical lesions include: tympany, impaction and intussusceptions of the caecum, strangulation, volvulus, entrapment, herniation and intussusceptions of the jejunum, ileal impaction and hypertrophy, volvulus, displacement, entrapment, obstruction of the large colon, intraluminal obstruction of the small colon and pedunculated lipomas. The survival of horses after surgical treatment has improved also as a result of careful post-operative management.

Management of colic horses requires the full attention, dedication and cooperation of a medical, surgical and nursing team of an equine clinic.

TICK BORNE DISEASES IN HORSES

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Tick-borne diseases have been a well-known problem in the southeastern region of Europe for a long time, and they are considered endemic in horses. Climate changes and international animal movement enable tick populations and distributions to rise. The best-known tick transmitted diseases in horses are Lyme disease (*Borrelia spp*.), Equine Granulocytic Anaplasmosis (*Anaplasma phagocytophilum*) and Equine Piroplasmosis (*Theileria equi* and *Babesia caballi*).

Clinical signs seen in a horse with **Lyme disease** include stiffness, lameness in more than one limb, joint effusions, lethargy and behavioral changes. Since detection of the pathogen is rarely possible, it is mostly diagnosed by serological testing. The treatment consists of intravenously administered tetracycline for seven to ten days, commonly followed by a one to two month course of oral doxycycline or minocycline.

Equine Granulocytic Anaplasmosis (EGA) is mostly a self-limiting disease, although any concurrent infection can be exacerbating. It causes fever, partial anorexia, depression, ataxia, icterus, reluctance to move, limb edema, petechiae and jaundice.
Diagnosis is confirmed by visualizing inclusions within peripheral neutrophils, PCR or rising serological titers. The treatment with intravenously administered tetracycline antibiotics induces prompt relief of clinical signs.

Most signs of acute **Equine Piroplasmosis** result from haemolytic anaemia or from systemic inflammatory response. Demonstrating the presence of organisms within invaded erythrocytes confirms the diagnosis, while PCR, a more sensitive and specific test, also allows species determination. Intramuscular imidocarb is suggested as the most effective drug for parasite clearance, administered once or twice with a 24-hour interval, depending on clinical evaluation and accompanying laboratory confirmation.

Detecting chronically infected horses without clinical manifestation is important due to their reduced working ability and breeding problems, and because latently invaded horses can serve as tick-borne disease reservoirs.

ADVANCES IN HEREDITARY DISEASES IN DOGS – DIAGNOSIS & MANAGEMENT

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Many of the characteristic breed traits and common and rare diseases seen in veterinary practice have a heritable basis. Recent exciting advances in our current knowledge of the completed dog genome sequence and the molecular genetic tests offer the opportunity to clinicians and clinical pathologists to use these emerging tools in clinical practice and have a positive impact on the health of dogs and in particular the diagnosis, management, and control of hereditary diseases. In this and subsequent sessions, practical aspects on breed structure and characteristics, practical diagnostic clinical, imaging, laboratory and genetic tools and management will be addressed and illustrated with many case examples.

Introduction

There are many unique traits of canine breeds and many hereditary disorders and genetic predispositions to disease have been identified. With the recent completion of the canine and feline genome sequences and molecular techniques these genetic (breed) traits and defects have been and are being characterized from the clinical signs to the molecular defect. Many specific breed traits such as size, chondrodysplasia, brachycephaly and many skin and coat color characteristics have recently been defined. While clinical and routine laboratory and imaging tests are helpful, specific biochemical and DNA tests have become available for >200 single gene defects through various laboratories in dogs. Moreover, with DNA tests it is now possible to determine the ancestry of mixed breed and purebred dogs, a first example of a complex trait. As it is difficult to keep track of all these hereditary diseases, tests, and treatments, a web-based database for available DNA tests on hereditary diseases in companion animals for clinicians is available (http://research.vet.upenn.edu/WSAVA-LabSearch).
Because of the increased awareness of breeders, pet owners, and veterinarians of genetic defects and the improved diagnostic abilities in clinical practice, the number of reported hereditary diseases in small animals is rapidly growing. At present, >900 hereditary diseases in dogs have been adequately documented. For the small animal practitioner, it can be a daunting, nearly impossible task to remember all these diseases and be aware of the many novel tests and their appropriate management.

Diagnostic Clinical Signs

While clinical and routine laboratory and imaging techniques are helpful, specific biochemical and DNA tests have become available for >200 disorders through various laboratories. This session will discuss various diagnostics for hereditary diseases and illustrate these tools with case examples. Simple test sample requirements and result interpretations are presented to use in clinical practice.

Beyond physical examination and imaging tools, genetic, metabolic, and other laboratory techniques are used to diagnose hereditary disorders in companion animals. Most genetic defects cause clinical signs early in life. The term congenital does only imply that the disease is present at birth, and does not necessarily mean it is inherited.

Routine Diagnostic Tests

Diagnostic tests are generally required to further support a genetic disorder in a diseased animal. Radiology and other imaging techniques may reveal skeletal malformations or cardiac anomalies, and an ophthalmologic examination may further define an inherited eye disease, although some are not recognized until several years of age. Routine tests such

as complete blood cell count, chemistry screen, and urinalysis may suggest some specific hematological or metabolic disorders or rule out many acquired disorders. Furthermore, clinical function studies may more clearly define a gastrointestinal, liver, kidney, or endocrine problem. Histopathology and/or electron microscopy of a tissue biopsy from an affected animal or from the necropsy of a littermate or relative may give the first clue to a genetic defect.

Special Genetic Laboratories

A few laboratories provide special diagnostic tests that allow a specific diagnosis of an inborn error of metabolism. Inborn errors of metabolism include all biochemical disorders due to a genetically determined, specific defect in the structure and/or function of a protein molecule. Disorders of intermediary metabolism typically produce a metabolic block in a biochemical pathway leading to product deficiency, accumulation of substrates, and production of substances via alternative pathways. The Metabolic Genetic Disease Laboratory at the University of Pennsylvania offers such tests http://research.vet.upenn.edu/penngen. Once the failing system has been identified, the defect can be determined at the protein level. Homozygously affected animals have very low protein activity and/or quantities (0-10%). These tests may also be used to detect carriers (heterozygotes), who typically have intermediate quantities at the protein level (30-70%), but no clinical signs. Unfortunately, protein assays require submission of appropriate tissue or fluid under special conditions to specialized laboratories along with a control sample, and are labor intensive.

Specific DNA tests

Many DNA screening tests have been developed. These tests are mutation or DNA marker specific and can, therefore, only be used in animals suspected to have the exact same gene defect. Small animals within the same or a closely related breed will likely have the same disease-causing mutation for a particular disease. However, dogs and cats as well as unrelated breeds of a species with the same disorder will likely have different mutations. On the other, hand a few mutations have been found in a few breeds or may be widespread within the canine population. DNA tests have several advantages over other biochemical tests. The test results are independent of the age of the animals, thus, the tests can be performed at birth or at least long before an animal is placed in a new home as well as before clinical signs become apparent. All currently available DNA tests for hereditary diseases in dogs and cats and associated laboratories worldwide can be found at http://research.vet.upenn.edu/WSAVA-LabSearch. Furthermore panel screening for a ll reported mutations has been reported and may be most cost effective as long as one is assuring that the mutation found also causes disease in another breed and genetic counseling is provided.

While the therapeutic interventions for hereditary diseases are somewhat limited, some can be managed successfully with medical and/or surgical interventions. Cures for hereditary diseases are rare unless one considers mostly experimental transplantation and gene therapy. For several hereditary diseases, there are specific therapies available and practical. For others, supportive measures can make the animal more comfortable. Even more important is the control of these diseases in future generations by informed breeding of dogs to avoid the production of any affected animals and still to preserve the gene pool in each breed.

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APPROACH TO THE BLEEDING DOG - DIAGNOSIS & MANAGEMENT

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Bleeding disorders are a common presentation in dogs and less commonly in cats and may be inherited or acquired. Furthermore, thrombotic conditions are being increasingly recognized. This lecture will focus on the clinical diagnostic approach to the bleeding animal focusing on blood loss and bleeding disorders. There are several point-of-care and reference laboratory tests permitting the separation between primary and secondary hemostatic defects as well as a specific diagnosis. Particularly challenging is the diagnosis of Disseminated Intravascular Coagulation (DIC), a syndrome observed with a variety of disorders. Transfusion therapy as well as a few specific agents can greatly improve the patient with blood loss anemia and bleeding.

Bleeding diatheses are generally separated into primary and secondary hemostatic disorders and in some cases both systems are affected, such as in disseminated intravascular coagulation (DIC). Primary hemostatic disorders include not only the common thrombocytopenias but also thrombopathias, vasculopathies, and von Willebrand disease. Secondary hemostatic disorders include all coagulation factor deficiencies involved in fibrin formation and are strictly speaking the coagulopathies. Platelet and vascular problems often present with surface hemorrhage, while coagulopathies generally cause hematomas and cavity bleeds. Excessive hemorrhage at an injury or surgery site and bleeding from multiple places are suggestive of bleeding disorder, and there are a several breed predilections for specific hereditary defects.

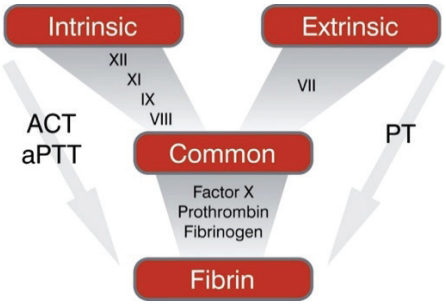
Hemostatic tests are indicated whenever an animal is bleeding excessively, prior to surgery when an increased bleeding tendency is suspected, to monitor therapeutic interventions, and for genetic screening in certain breeds or families with a known bleeding disorder. Hemostatic abnormalities should be assessed prior to instituting therapy whenever possible or at least appropriate blood samples should be collected pretreatment. Excellent venipuncture with discarding of the first few drops of blood (to avoid platelet activation and tissue factor) and extended compression over jugular, saphenous or femoral vein is required. The **cuticle bleeding time** crudely assesses overall hemostasis, but is not standardized and painful and is, therefore, not recommended. A minimal database includes a packed cell volume and total protein evaluation, and **evaluation of a blood smear** can provide a platelet estimate and identify platelet size and clumping as well as schistocytes. The results can also provide some measure of the extent of blood loss and red blood cell transfusion requirement.

TOOLS FOR PRIMARY HEMOSTASTIC DEFECTS

Platelet counts can be estimated on a blood smear or specifically counted by a hematology instrument. Since 8-15 platelets (1 platelet equals 20,000/µl) are normally found per high power oil emersion microscopic field, an absence to low number of platelets suggests a severe thrombocytopenia. Various modern impedance and laser hematology instruments have the ability to count platelets and measure their mean size including platelet size distribution and platelet crit; they may have been validated, but some have difficulties in differentiating large platelets from erythrocytes (particularly in cats). Furthermore, platelets can readily be activated which results in platelet aggregation, hence, platelet counts need to be confirmed by a careful review of a blood smear including the feather edge for platelet clumps (preferably on fresh non-anticoagulated blood). Hemorrhage is generally not observed unless the platelet count is <40,000/µl (normal 150-500,000/µl) or there is also a coagulopathy like DIC.

COAGULATION TESTS

Whereas the whole blood clotting time test is insensitive and mostly inaccurate, there are several standardized coagulation screening tests that are useful to define coagulopathies in clinical practice.



The intrinsic and common pathways are assessed by either the activated coagulation time (ACT) or activated partial thromboplastin time (aPTT or PTT). Factor XII of the intrinsic cascade is activated by diatomaceous earth (celite) in the ACT test and by kaolin or other contact phase substrates in the aPTT test. The extrinsic and common pathways can be assessed by the prothrombin time (PT) test. In these two assays different tissue factors (thromboplastins) are activating factor VII, which in turn will lead to fibrin formation.

HEMOSTATIC SCREENING TESTS AND GROUPS OF BLEEDING DISORDERS

	Platelets	BMBT	PTT	PT	TT
Thrombocytopenia	**D**	**I**	**N**	**N**	**N**
Thrombocytopathia & vWD	**N**	**I**	**N**	**N**	**N**
Intrinsic coagulopathy	**N**	**N**	**I**	**N**	**N**
Extrinsic coagulopathy (FVII)	**N**	**N**	**N**	**I**	**N**
Combined coagulopathies (DIC, liver, rodenticide)	**D**	**I/N**	**I/N**	**I/N**	**I/N**

N = normal; I = increased (prolonged) time; D = decreased

Although hereditary coagulopathies can be suspected based upon the pattern of coagulation test abnormalities, specific factor analyses are needed to confirm a diagnosis. Disseminated intravascular coagulopathies due to many different disorders is associated with variably prolonged coagulation times. More helpful to the diagnosis of DIC are the recognition of schistocytes, thrombocytopenia, low fibrinogen and antithrombin III levels, and increased D-dimers and fibrin split (degradation) products. Finally, thromboelastography (TEG or ROTEM) techniques can now be used in the emergency room, intensive care units, and referral centers to assess overall hemostasis and particularly thrombotic/fibrinolytic tendencies of citrated whole blood.

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IMMUNE-MEDIATED HEMOLYTIC ANEMIAS IN DOGS

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INTRODUCTION

Immune-mediated hemolytic anemia (IMHA) is one of the most common and serious hemolytic anemias in dogs, but occurs rarely in other animal species. In IMHA an immune response, including anti-erythrocytic antibodies, complement and macrophages, targets directly or indirectly erythrocytes and a hemolytic anemia ensues. There are many triggers for IMHA such as infections, drugs and other agents, and cancer leading to secondary IMHA, but in many dogs no cause is identified (so-called idiopathic, autoimmune or primary IMHA) or a genetic predisposition has been observed (Cocker spaniels). Furthermore, alloimmune hemolytic anemias, such as hemolytic transfusion reactions, both acute and delayed, and neonatal isoerythrolysis (only litters from transfused bitches), are caused by specific anti-erythrocytic alloantibodies. In contrast to other species, dogs with IMHA also develop an often overwhelming inflammatory response resulting in thrombosis and necrosis of various organs. And while the anemia can be corrected with transfusions, these complications in dogs are causing severe morbidity and mortality despite aggressive immunosuppression and antithrombotic interventions.

IMMUNE-MEDIATED DESTRUCTION OF ERYTHROCYTES

Regardless of the underlying cause, IMHA results from a breakdown in immune self-tolerance or from a deficit in the control mechanism that regulates B and T lymphocyte as well as macrophage reactivities. Immune destruction of erythrocytes is initiated by the binding of IgG or IgM antibodies to the surface of erythrocytes. Under most clinical circumstances, immune destruction is an extravascular process that depends on recognition of erythrocytes opsonized with IgG, IgM and/or complement by specific receptors on reticuloendothelial cells. Antibody-coated erythrocytes may also be lysed by complement fixation and the membrane attack complex, which is clinically noted as intravascular hemolysis.

A diagnosis of IMHA must demonstrate accelerated immune destruction of erythrocytes. Evidence of a hemolytic anemia is suggested clinically by icterus and a regenerative anemia with hyperbilirubinuria, and hemoglobinemia and hemoglobinuria refers to an intravascular process. However, the erythroid response in the bone marrow may be blunted by the immune, inflammatory and necrotic process or the underlying disease thereby leading to non-regenerative anemias. It is difficult to diagnose a primary immune destruction of erythrocyte precursors and these anemias are not hemolytic, but rather a marrow disorder. Besides documenting a hemolytic anemia for IMHA, one or more of the following 3 hallmarks must be present to support a diagnosis of immune-mediated hemolysis: persistent autoagglutination, marked spherocytosis and a positive direct Coombs' test result.

AUTOAGGLUTINATION

Anti-erythrocytic IgM and in large quantities IgG antibodies may cause direct erythrocyte autoagglutination. The autoagglutination may be seen by the naked eye in an EDTA tube or on a glass slide or may become apparent as small clumps of erythrocytes on blood smears. It is important to determine whether the agglutination persists after "saline washing", which has been coined persistent or true autoagglutination. This is accomplished by adding excessive physiologic saline to the tube containing a small amount of EDTA-anticoagulated blood (>4:1), mixing, centrifuging and removing the supernatant including the plasma and repeating this saline washing 3 times. True or persistent autoagglutination is indicative of an immune process, but precludes the performance of Coombs' test and crossmatching procedures which are

based upon an agglutination reaction as result. If the agglutination breaks up after washing, the Coombs' test is expected to be positive, if it is a case of IMHA.

SPHEROCYTOSIS

If erythrocytes are only partially phagocytized or lysed by complement in circulation, erythrocytes with reduced surface area to volume ratio, known as spherocytes, are formed. They appear spherical and microcytic with no central pallor and are considered fragile. Note proper areas on the blood smear need to be reviewed in order to find spherocytes in between single regular discoid red blood cells. Large numbers of spherocytes (>20/microscopic high power field) are nearly diagnostic for IMHA, whereas small numbers may be seen with other conditions including DIC, endotoxemia and zinc intoxication. In the author's experience, all dogs with marked spherocytosis and suspected to have IMHA also had a positive Coombs' test. However, only 60-80% of dogs with a positive direct Coombs' test or clinically diagnosed with IMHA had marked spherocytosis. Hereditary spherocytosis due to genetic membrane defects has rarely been seen in dogs, but should be considered as a differential diagnosis in dogs with negative Coombs' test results.

Because of the difficulties with the Coombs' test (see below), Slappendale had proposed to use the erythrocytic osmotic fragility test at specific saline concentrations as a mean to diagnose IMHA and this test is currently used in various clinics in Europe. However, there are many other reasons for increased fragility of erythrocytes beside IMHA, including hereditary red blood cell defects. This test is not used in human medicine and has not been shown to be superior to determination of marked spherocytosis and a positive direct Coombs' test in dogs with IMHA. The osmotic fragility test is also a cumbersome and not well standardized technique further limiting its usefulness.

POSITIVE DIRECT COOMBS' TEST

The direct Coombs' test is also known as direct antiglobulin test (DAT) and is used to detect antibodies and complement on the surface of erythrocytes when the anti-erythrocyte antibody strength or concentration is too low to cause spontaneous agglutination (sub-agglutinating titer).

A novel standardized antiglobulin test method has recently been developed by Alvedia (France) similar to the immunochromatographic strip technique for blood typing and crossmatching of dogs and cats. Although many commercial veterinary laboratories offer Coombs' testing for dogs, clinicians have questioned the test's sensitivity and specificity and often forgo the test and/or use response to therapy as a diagnostic. However, negative Coombs' test results may potentially be seen because of technical reasons, insufficient quantities of bound antibodies, the presence of weakly bound antibodies (no data available), or the disease in remission. The Coombs' test stays positive for days to months after initiating treatment. A few days of immunosuppressive therapy will not reverse the Coombs' test result, as unlikely a transfusion would cause a positive Coombs' test result. Also a persistently anemic dog with suspected IMHA even when treated for weeks to months with immunosuppressive therapy should have a positive Coombs' test. Thus, anemic dogs with negative Coombs' test results should be reevaluated for other causes of hemolytic anemia. Conversely, dogs with primary IMHA or with secondary IMHA, when the trigger has not been found and removed, may well have a positive Coombs' test for months to years.

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/ / / / ORAL PRESENTATIONS / / / /

PREVALENCE OF LYMPHOCYTIC CHORIOMENINGITIS ANTIBODIES IN PERSONS WITH RODENT EXPOSURE

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Lymphocytic choriomeningitis virus (LCMV) is a neglected human pathogen associated with aseptic meningitis, fatal infections in immunocompromised persons and congenital infections. The natural rodent host and principal reservoir for LCMV is the house mouse (*Mus musculus*). Humans become infected by inhaling infectious aerosols or by ingesting contaminated food. The incidence of clinically significant LCMV infection in humans is unknown. Seroepidemiological studies in the general population revealed the seroprevalence of 5-10%.

During a one-year period (June 2016-May 2017), a total of 94 serum samples collected from persons exposed to rodents were tested for the presence of LCMV IgG antibodies using indirect immunofluorescence assay (IFA). All participants were asymptomatic and did not report recent febrile disease.

In the tested group, there were 88 (93.6%) males and 6 (6.4%) females aged 25-71 years from continental Croatian counties. In addition to contact with rodents through occupational exposure (forestry workers, hunters, agriculture workers), rodent infestations around house/resting house and cleaning rodent-infested areas, the majority of participants reported some other potential risk factors, such as frequent visits to forest areas and food storage in basements. LCMV IgG antibodies were detected in 9 (9.5%) tested persons at low titers (16 and 32, respectively). The seropositive persons were from northwestern (Zagreb area) and eastern (Slavonski Brod area) regions.

Our results indicate that LCMV is present in Croatia. Further studies on a larger sample of exposed persons, as well as the general population, are needed to determine the prevalence of this neglected zoonosis.

SEROPREVALENCE OF HEPATITIS E IN PROFESSIONALLY EXPOSED GROUPS IN CROATIA: PRELIMINARY RESULTS

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Hepatitis E is becoming an emerging infection in many European countries. The main reservoirs of hepatitis E virus (HEV) are pigs and wild boars, but the virus has been isolated from other animals as well. While some studies showed higher HEV seroprevalence in hunters and forest workers, other did not found significant difference in seropositivity between professionally exposed persons compared to the general population.

We analyzed the prevalence of HEV infection in professionally exposed persons from continental Croatia. From June 2016 to May 2017, a total of 62 serum samples collected from hunters and forest workers were tested for the presence of anti-HEV IgM and IgG antibodies, using a commercial enzyme-linked immunosorbent assay (Euroimmun, Lübeck, Germany). Initially reactive samples were confirmed using a commercial immunoblot assay (HEV Recomline; Mikrogen, Neuried, Germany). The control group consisted of 87 unexposed adult persons (general population). No participants showed symptoms of recent liver disease.

Overall HEV IgG seropositivity in exposed persons was 4/62 (6.5%; 95%CI=0.2-15.7): 3/37 in forest workers (8.1%, 95%CI=0.2-21.9) and 1/25 in hunters (4.0%; 95%CI=0.1-20.4). In the general population, HEV IgG antibodies were detected in 3/87 (3.4%; 95%CI=0.7-9.8) participants.

Our preliminary results indicate that the seroprevalence of hepatitis E among occupationally exposed persons is higher compared to the general population. However, due to the small number of participants, these results should be interpreted with caution. Further studies on a larger sample are needed to confirm this observation, and provide information on HEV prevalence among other professionally exposed groups in Croatia.

EXTENDED SPECTRUM BETA-LACTAMASE (ESBL)-PRODUCING KLEBSIELLA PNEUMONIAE ISOLATED FROM DOGS

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Infections caused by Gram-negative multidrug-resistant bacterial pathogens are being reported with increasing frequency. Special concern is given to carbapenem-resistant and extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* that are, according to the World Health Organization (WHO), on the Global priority list of antibiotic-resistant bacteria that pose the greatest threat to human health. Moreover, the WHO highlights the need for coordination between human and animal surveillance systems to improve the 'one health' approach, to limit the spread of resistant bacteria.

The aim of this study was to determine the susceptibility to cephalosporins of Klebsiella pneumonia isolates originated from dogs. The antimicrobial susceptibility was determined by disk diffusion testing according to CLSI guidelines, and interpreted according to CLSI breakpoints. Double-disk synergy test (DDST) was performed as a confirmatory test for suspected ESBL-production.

Screening testing revealed six strains of *K. pneumoniae* resistant to third generation of cephalosporins. The isolates were identified according to the procedure described by MARKEY et al. (2013) and identification was confirmed with a commercial strip for biochemical identification API 20E (bioMerieux, France). DDST testing was positive for all six isolates and the presence of genes encoding broad and extended-spectrum β-lactamases was examined by PCR. In all isolates PCR testing revealed the presence of bla_{TEM} and bla_{CTX_M} genes.

To our knowledge, this is the first report of ESBL-producing strains of *K. pneumoniae* isolated from animals in Croatia. It is important to monitor the prevalence of such strains and their possible spread because they pose a great threat to human health.

COMPARATIVE PHYLOGENETIC ANALYSIS OF NOROVIRUS FROM SHELLFISH AND PATIENTS WITH GASTROENTERITIS IN CROATIA

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Norovirus, genus of the family *Caliciviridae*, is a leading cause of viral gastrointenteritis in humans and is responsible for many outbreaks worldwide. Among six established genogroups (GI to GVI), GII strains are responsible for almost 90% of clinical cases (with a high prevalence of the GII.4 cluster), while the other 10% are caused by GI strains. The aim of this study was to evaluate the phylogenetic similarity of norovirus obtained from patients with gastroenteritis and norovirus from shellfish collected in Croatian production areas. During March and April 2014, 23 human stool samples were ELISA tested positive. Seven randomly selected stool samples and 99 shellfish samples were analyzed using real-time RT-PCR for NoV GI and GII, with primers directed towards the ORF1-ORF2 junction region for both genotypes. Virus extraction efficiency was calculated with mengovirus, and it was acceptable (>1%). Following the phylogenetic characterization of the human stool samples, five out of seven belonged to GII.4 which confirmed the circulation of the epidemiological strain Hu/GII.4/sydney/NSW05 (100% similarity). One stool sample showed 97.6% similarity to genotype II.2 - Snow Mountain strain and for one we did not obtain a good sequencing fragment. Twelve out of 99 shellfish samples tested real-time RT-PCR positive and two were successfully sequenced clustered to genotype GII.4 and showed 97.1% and 98.5% similarity with Hu/GII.4/sydney/NSW05. Here we describe the emergence of norovirus strains genetically related to Sydney2012 during the 2014 season in Croatia, providing the first evidence of that strain in Croatia. The circulating genotype found in the present study differs from results obtained previously in which GII.4 Lordsdale was the dominant circulating genotype in Croatia during the 2004/2005 season. Norovirus GII.4 in shellfish originating from Croatian production areas was simultaneously determined for the first time in Croatia. This contributes to knowledge of norovirus contamination in shellfish worldwide. Although outbreaks related to consumption of shellfish in Croatia were not notified, it is well known that human sewage is a possible source of shellfish contamination. Understanding the transmission route and vehicle of a norovirus outbreak is of great public health importance, and these results implicate the co-circulation of different strains of NoV GII.4 in Croatia.

COMPARISON OF SAMPLING PROCEDURES FOR THE DETECTION OF CHLAMYDIACEAE IN FREE-RANGE CHICKEN FLOCKS

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Different sampling procedures were employed in evaluating poultry flocks for the prevalence of avian chlamydiosis, based on the detection of *Chlamydiaceae* by real-time polymerase chain reaction (qPCR) assay. The presence and detection of *Chlamydia* in samples can be influenced by the type of avian sampled and the length of time of infection.

This study compared three sampling procedures used for the detection of *Chlamydiaceae* in free-range poultry flocks. Triple swabs (conjunctival, pharyngeal and cloacal), litter-fecal and water samples were collected from 30 free-range chicken flocks in Croatia and the Philippines. Detection of *Chlamydiaceae* was done by qPCR assay, targeting the *Ch23S rRNA* gene. The detection rate from litter-fecal samples (25/30; 83.33% positive) was found to be significantly higher than triple swabs (11/30; 36.67%; $p<0.01$) and water samples (18/30; 60.00%; $p<0.05$). The detection rate in water samples was also significantly higher than triple swab samples ($p<0.05$).

Based on these results, the litter-fecal sample combination may be examined for the presence of *Chlamydiaceae,* especially if it is difficult to collect an optimum number of samples from free-range chickens to represent a flock for epidemiological studies. However, it is suggested that multiple sampling procedures be performed in evaluating flocks for the presence of *Chlamydiaceae,* regardless of whether the infection is acute or chronic.

SEROLOGICAL EVIDENCE OF TICK BORNE ENCEPHALITIS VIRUS INFECTIONS IN DOGS IN CROATIA – IMPORTANCE FOR THE VETERINARY MEDICINE AND PUBLIC HEALTH

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Tick borne encephalitis (TBE) is a disease caused by the tick borne encephalitis virus (TBEV) belonging to the genus *Flavivirus*, transmitted by *Ixodes ricinus* ticks. In human patients TBE has a biphasic course that starts with a fever and may develop into a neurological disease caused by severe meningoencephalitis. Continental parts of Croatia are an endemic area, with the incidence of human clinical cases from 0.2 to 1.90 per 10^5 inhabitants. Dogs may also be infected by TBEV and, since they live in a close relationship with humans, could represent sentinel animals for risk assessment for humans, especially in urban areas. Therefore, the aim of this study was to estimate the exposure of dogs in urban areas to TBEV and the possible correlation with human risk areas in Croatia. Four Croatian cities were screened for the presence of anti-TBEV IgG antibodies in the dog population. Blood samples were collected from six different Veterinary Clinics and the Central Laboratory of the Internal Diseases Clinic, Faculty of Veterinary Medicine in Zagreb, between June and December 2016. Anti-TBE IgG antibody concentrations were determined using an Immunozym FSME IgG all-species ELISA kit (Progen Biotechnik GmbH, Heidelberg, Germany). A total of 356 dog sera samples were tested and thirteen samples yielded positive results (3.65 %). The concentration of IgG antibodies in positive samples was in the range from 141 VIEU/ml to 252 VIEU/ml, and one sample was above the range of quantitation. The majority of positive dogs were found in the area of Slavonski Brod with nine detected cases, followed by four detected cases in the Bjelovar city area. High prevalence of anti-TBE IgG in the dog sera in the Slavonski Brod and Bjelovar city areas is in accordance with the previous confirmation of Brod-Posavina and Bjelovar-Bilogora counties as moderate to high risk areas of TBE infections in humans. These results confirmed the circulation of TBEV in dogs in inland Croatia and the usefulness of this surveillance system as one of the factors that can influence risk analysis for humans.

BRUCELLOSIS IN CATTLE AND HUMANS IN BOSNIA AND HERZEGOVINA

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Brucellosis in Bosnia and Herzegovina began to appear individually and sporadically in 2001, even though the disease occurred as an enzootic disease in the nineteen-seventies and the early eighties, and was successfully eradicated by use of effective measures. New cases of brucellosis in animals and humans started occurring in the period from 2004 to 2006, but the highest number of infected ruminants (especially seropositive sheep and goats) and diseased people was in 2008 and 2009.

In 2009, after the implementation of vaccination of small ruminants with Rev 1 vaccines, the number of infected people was reduced by half and this downward trend continued for the next ten years.

The aim of this study is to show the number of registered cases of brucellosis in cattle in 2016, in which vaccination has not been carried out. These cases are a significant indicator of the presence of brucellosis in Bosnia and Herzegovina because cattle are usually kept together with sheep and goats. In addition, the aim is to present the number of reported cases of brucellosis in humans in the last three years.

As part of the annual brucellosis control programme, in total 96,901 blood serum samples were tested using rapid agglutination and complement fixation test, and 41 samples reacted serologically positive. On the other hand, based on the data of the Institute of Public Health, the number of reported cases of infected people in 2014 was 118 or 5.05 Mb / 100,000, 101 in 2015 i.e. 4.33, and 182 cases in 2016.

Based on the results obtained from blood serum samples originating from cattle, and the registered cases of brucellosis in humans in the Federation of Bosnia and Herzegovina, we concluded that it is necessary to continue with the vaccination of small ruminants and regular annual testing of cattle for the presence of specific antibodies to brucellosis pathogens.

USE OF ANTIMICROBIAL AGENTS IN LAYING HENS

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The use of antimicrobial drugs in laying hens has always been the subject of numerous discussions. While some authors argue that these veterinary medicinal products (VMPs) should not be used in laying hens at all, there are those who are not "a priori" against their ban. The European Union has granted 6 of antimicrobial agents, which may be used in laying hens. These are: colistin, tylosin, neomycin, oxytetracycline, chlortetracycline and erythromycin.

Antimicrobial drugs are used today primarily for the prevention and treatment of diseases in poultry, and often (not in the EU) to stimulate growth. Bearing in mind the fact that these drugs are often used irrationally, there is a good chance that their residues will be found not only in poultry meat, but also in the eggs for a certain period after the cessation of treatment.

In addition to the administration of approved VMPs, the residues in eggs may be the result of erroneously applied medicated food, the contamination of the food with some antimicrobial drug in the mixing unit, as well as "extra-label" use of drugs in poultry.

The antimicrobial agents are distributed in the body and deposited in the eggs, mainly in the yolk, where they also persist longer in relation to the albumen.

Drugs which are pooly absorbed from the gastrointestinal tract (aminoglycosides, aminocyclitols, polymyxins), cannot be determined in the eggs, while the residues of some antimicrobial drugs that are absorbed can be detected for up to two months (e.g. chloramphenicol) after the last treatment.

POLYMORPHISM OF THE MTNR1A GENE IN SEASONAL AND NONSEASONAL ESTROUS SHEEP BREED

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The MTNR1A gene controls the melatonin MT1 receptor and thus participates in the processes which signal changes of the photoperiod. Exone II of the MTNR1A gene is highly polymorphic. Polymorphic variants could influence the organism's different responses to photoperiodism which is particularly interesting in sheep where the short-long daylight rhythm is the main modulating factor of the breeding season.
The aim of this study was to determine polymorphism of the MTNR1A gene in sheep breeds with seasonal and aseasonal reproduction.

The study included 34 Dalmatian pramenka sheep (DP; a predominantly seasonal estrous breed) and 48 Merinolandschaf sheep (ML; predominantly nonseasonal estrous breed). Genotypes were identified after digestion with *AfaI and MnlI* restriction enzymes. Differences in genotype frequency between the breeds were tested using the Hi squared test ($P<0.05$) in *Statistica 64*. The sequencing method revealed the nucleotide sequence and the presence of SNPs.

Digestion of PCR products with *AfaI* revealed three genotypes: CC, CT and TT. The frequencies of the individual genotypes were relatively similar in both breeds, and TT and CT genotypes prevailed. In both breeds, gene T (DP 72%; ML 68%), had higher frequency. The differences in genotype frequency between ML and DP were not statistically significant ($P>0.05$). After digestion with *MnlI,* all three genotypes (GG, GA and AA) were identified in the ML breed, while the AA genotype in DP was absent. The differences in GG and GA genotype frequency between ML and DP were not statistically significant ($P>0.05$). In both breeds, gene G had a notably higher frequency (DP 90%; ML 84%). Nucleotyde sequence analysis, besides mutations at positions C606T and G612A, revealed six additional mutations at G453T, G706A, G783A, G801A, C891T and C893A positions. Only mutations at positions G706A (Val-Ile) and C893A (Ala-Asp) were functional.

These results do not confirm our assumption that sheep with seasonal and nonseasonal reproduction have significantly different MTNR1A genotypes.

IMPORTANCE OF DRY PERIOD FOR THE NEXT LACTATION – REVIEW

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Far from sight far from the heart. This may be applied to care of dry cows in comparison with lactating cows. These cows receive the least attention on the farm. The main reason is because they do not enter the milking parlor and there is no milk from them in the bulk tank. The dry period gives the udder time for rest and regeneration. A rest period of 6-8 weeks is desirable in order to maximize the next lactation performance. The dry period is a critical and highly stressful period with numerous metabolic, hormonal and management changes occurring in less than 2 months. Immune status is often depressed, so the risk of disease increases. The best-known 3M postpartum problems are mastitis, metritis and metabolic disorders.

Treatment of all quarters in all cows with a dry-off intramammary antibiotic has been recommended as a part of a mastitis control program. Prophylactic use of ATB has undergone criticism in the last few years due to increasing concern about the development of ATB resistant bacteria and the high content of ATB residue in milk. Dry cow therapy is part of good management practice in the dry period. Abrupt halting of lactation is common in large dairies due to easier management, and it is less time consuming. According to many pieces of research, gradual milk cessation shows beneficial effects to udder health in the next lactation. Gradual cessation, results in fewer intramammary infections during the dry period, at calving and in the few days after calving, compared to abrupt cessation.

During the dry off time, high producing milk cows often yield high amounts of milk. There is milk leakage, and the teat canal is open for an extended time which leads to an increased intramammary infection rate. If we reduce milking frequency towards the end of lactation, with slight changes in nutrition, milk production can be significantly reduced prior to dry off, as well as intramammary infection.

Alongside the above we must not forget balanced nutrition and clean, dry, comfortable housing for dry cows during the dry period, in order to achieve the best results in the next lactation.

FRESH COWS PROTOCOL

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Focusing on the health of fresh cows helps keep them more productive, healthier and more profitable for cow owners. For this purpose it is essential to develop a proper fresh cow monitoring protocol.

The first step is to identify normal versus abnormal cows. All cows experiencing abnormal calving (dystocia, twins, retained placenta) should be treat with PGF2a once between 14–21 days postpartum.

Step two is daily evaluation of fresh cows from days 1-10 postpartum. Cows should be evaluated from the front: attitude, appetite, ears and eyes, and from the back: temperature, respiration, manure, discharge, udder fill and tail carriage.

After evaluation, cows should be divided into two groups: the first group are cows with fever over 39.5°C and the second group are cows with no fever.

The "fever group" should be divided into two groups, one with and the second without fetid discharge. Cows with fetid discharge on the first day of fever should receive antibiotic treatment (ceftiofur hydrochloride is recommended because of its uterine tissue affinity, in a dose of 4ml/100kg/day). Additionally, they should receive a glucose promoter and anti - inflammatory drugs in order to prevent metabolic disorders, lowering the fever and reducing pain. From days 2–5 antibiotic treatment should continue under the same regimen.

The group with fever but no fetid discharge should be checked for metritis, mastitis and pneumonia, and treated with antibiotics upon a veterinarian's recommendation, and receive anti-inflammatory drugs and glucose promoter.

The group of cows with no fever should also be divided in two: those with or without fetid discharge.

The group with no fever but fetid discharge should be treated in the same way as group with fever and fetid discharge.

The group with no fever and no discharge but a normal attitude and appetite should be monitored for 10 days and eventually treated for any diagnosed conditions.

The group with no fever, no fetid discharge but showing poor attitude and appetite should be examined for metabolic disease and treated as appropriate.

PROTOTHECA CAUSED MASTITIS ON DAIRY FARMS IN SLAVONIJA

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Prototheca is a colorless alga and can cause mastitis in cows. It is widespread in barns, pens and pastures. The most common places where algae can be found are wet areas: water tanks, well water, milking parlor wash water, teat dip containers, milking machine liners, silage water runoff, feed bunk and manure. Algae can be found on farms with or without *Prototheca* mastitis problems. Infections are thought to occur when teat ends are exposed to high populations of algae in the environment, usually between milking. New infections can occur on farms where milking procedures are poor and a high percentage of cows are already infected with *Prototheca*. All lactation stages, including the dry period, are equally susceptible to infections. *Prototheca* quickly invades the udder and develops into a long-term chronic infection. Infections may persist through the dry period and last for several lactations. Most infections are clinical with visible changes in the milk but no severe systemic signs are present. Milk from cows with *Prototheca* mastitis usually has very high SCC so the bulk tank SCC can also be elevated. Protothecal infection is not susceptible to antibiotic treatment so no treatment should be attempted.

On a large dairy farm in eastern Slavonija, during a routine inspection we found a few clinical cases with grossly abnormal milk, which were non-responsive to antibiotic treatment. After milk sampling, the diagnostic laboratory used selective culture media to improve detection of *Prototheca* because it may grow slowly on traditional bacteria culture media. This may result in some false negative culture results.

After positive culture results on Sabouraud-dextrose plate, autopsy was performed of culled cows. Characteristic macroscopic changes and enlarged supramammary lymph nodes were found. On the farm the milking procedures were correct. We could not find an obvious source of infection. Due to disastrous impact of *Prototheca* mastitis on milk quality and non-responsive antibiotic therapy, culling is advised for all infected cows.

INTRAMAMMARY PROPOLIS SOLUTION (APIMAST) FOR SUBCLINICAL MASTITIS TREATMENT IN DAIRY COWS

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Subclinical mastitis is the most important cause of milk production loss, and one of the largest economic problems in dairy farming. Usually, it is not treated, mainly due to the costs of withdrawal time that requires discarding milk. The aim of this research was to investigate the safety and efficacy of intramammary propolis solution (Apimast) on subclinical mastitis in dairy cows, since propolis is effective against many gram-positives and has no withdrawal time.

Apimast was prepared as 1% and 3% propolis in a non-alcoholic carrier. The concentrations of 8 biomarkers of propolis were determined by high performance liquid chromatography with diode-array detection (HPLC-DAD). The *In vitro* susceptibility (MIC) of common mastitis pathogens to Apimast was determined using the agar dilution method. The safety and efficacy study was conducted on five dairy farms with Holstein cattle, and included 86 dairy cows or 339 quarters. The quarters were subsequently classified into groups depending on the SCC (somatic cell count) values (>200.000/ml or <200.000/ml) and the results of the bacteriological finding prior to the application (infected or non-infected).

Eight propolis biomarkers were determined in Apimast; caffeic, p-coumaric, ferulic and cinnamic acid, quercetin, kaempferol, apigenin and chrysin. The *in vitro* antimicrobial effect of Apimast was good; the MIC_{90} for *S. aureus* was 64, and for *S. uberis* it was 32 µg/ml. Apimast increased SCC values in all animals after the second administration, but these values returned to normal after two days. Additionally, after a third administration of Apimast, bacteriological healing was achieved in a total of 92.5 % of cases in only 7 days. The results of this study have shown that Apimast, a solution without a withdrawal period and with good tolerance, could be used to treat subclinical mastitis, and thus represents a significant innovation in dairy production medicine.

EFECT OF AGE AND FOLICLE SIZE ON LIPID PROFILE AND ANTIOXIDANT POTENTIAL OF BOVINE FOLLICULAR FLUID

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This study determined the lipid profile and antioxidative properties of bovine follicular fluid (FF) and also investigated their possible changes during foliculogenesis and reproductive ageing. Ovaries of heifers and cows were collected at a slaughterhouse. Samples of FF were obtained from follicles of three sizes: <5 mm (N=38), >5 mm (N=40) and >10 mm (N=35). Concentrations of triacylglycerol (TAG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), phospholipids (PHL) and activities of glutathione peroxidase (GPx), superoxide dismutase (SOD) and paraoxonase 1(PON 1) were analysed. The concentrations of TC, LDL-C, HDL-C, PHL were significantly higher, while the activities of GSH-Px and SOD were significantly lower in FF of cows compared to heifers. During foliculogenesis in the follicles of the heifers, concentrations of TAG, NEFA and the activity of SOD significantly decreased. Furthermore, during foliculogenesis in the follicles of the cows, concentrations of LDL-C and TAG as well as the activity of SOD significantly decreased, whereas concentration of TC significantly increased.

In conclusion, these findings indicate that age, together with the growth and development of follicles, affects antioxidant enzyme activity and lipid concentrations.

LIPID COMPOSITION OF BOVINE FOLLICULAR FLUID DURING FOLLICULOGENESIS

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Lipids are an important component of the follicular fluid (FF) as a cellular energy source and also have important biological functions in cell membrane biogenesis and signalling. Fatty acids (FAs) in FF are present in esterified form as triacylglycerol (TAG), cholesterol esters and phospholipids (PHL), and as nonesterified FAs (NEFAs). The aim of the study was to determine the lipid composition of bovine FF during folliculogenesis. Bovine ovaries were collected at a slaughterhouse, and follicular fluid samples were obtained from follicles of three different sizes (<5 mm N=19; >5 mm N=22; and >10 mm N=23). Concentrations of TAG, PHL, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), NEFA and FAs in FF were analysed.

The saturated fatty acids were dominant in bovine FF and the most common was myristic acid (C14:0). Polyunsaturated fatty acids were the second most represented, with linoleic acid (C18:2) in the highest percentage. The least represented were monounsaturated fatty acids (MUFA), with oleic acid (C18:1c9) as the most common. The percentage of C14:1, C15, C18:1c9, C20:5n-3, C22:5, MUFA, and concentrations of LDL-C, TAG and NEFA were significantly higher in follicles <5 mm compared to follicles >10 mm. The results also show the significantly lower percentage of C18:0, the ratio of arachidonic acid/eicosapentaenoic acid (C20:4n-6/C22:5n-3) and TC and PHL concentrations in follicles sized < 5 mm compared to follicles more than 10 mm in diameter.

Our data show that differences in the lipid composition of FF are associated with the stage of follicle growth and development.

SPERMATOZOA SUBPOPULATIONS IN BUCK BASED ON HEAD AND TAIL MORPHOMETRIC PARAMETERS

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The knowledge that spermatozoa differ in morphological and functional characteristics has initiated studies in order to isolate a subpopulation of spermatozoa with better functional abilities.

The aim of this study was to determine spermatozoa subpopulations based on the morphometric parameters of spermatozoa head (MPSH) and tail (MPST) in ejaculates of bucks (N=12) over a period of 3 months. Semen smears were prepared and stained by the method of Bloom to establish the proportion of live spermatozoa. By using a computer assisted-image analyses system, morphometric measurements were performed (50 spermatozoa per smear) with the "SFORM" program. Morphometric data were collected for spermatozoa head, mid-piece and tail (area, outline, length, width), and for head shape (regularity, rugosity, ellipticity, elongation). By analyzing the principal components, two values were obtained explaining 85% of variance for MPSH and 78.5% for MPST. For MPSH, the 1st component focused on measurements of size and the 2nd on head shape. The 1st component for MPST focused on length and the 2nd on the width of the mid-piece. Cluster analysis of the most important parameters for each component revealed 4 spermatozoa subpopulations based on MPSH and MPST. The 1st subpopulation of MPSH comprised small sized spermatozoa with high rugosity (17%), the 2nd the largest size (4.8%), the 3rd were elliptical and elongated (24.5%) and the 4th average sized spermatozoa (53.4%). The 1st spermatozoa subpopulation based on MPST comprised spermatozoa with the smallest size of the mid-piece (63.0%), the 2nd spermatozoa with the widest mid-piece but the shortest tail (10.9%), the 3rd spermatozoa with the longest tail (21.6%) and 4th spermatozoa with the greatest length, area and outline of mid-piece (4.5%).

Morphometric analysis of the principal components and cluster analysis, could provide data about semen quality and freezing ability, according to the proportions of the individual subpopulations in the semen.

ASSOCIATION OF SPERMATOZOA MORPHOMETRIC CHARACTERISTICS WITH AGE OF BUCKS AND PROPORTION OF MOTILE SPERMATOZOA IN EJACULATE

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The morphological and functional characteristics of spermatozoa (SP) in the ejaculate of bucks depends on genetic and environmental factors. The assessment of SP morphology is more sophisticated today with the use of systems for computer-assisted morphometric image analysis. Such methods provide more precise and detailed measurements compared to standard subjective methods.

The aim of this study was to investigate the influence of the age of bucks on the morphometric parameters of SP head and tail, and the association of SP tail morphometric parameters with the proportion of motile SP. During the experimental period of 3 months, the ejaculates of French alpine bucks (n=12), aged from 1-4 years, were collected weekly by artificial vagina. The motility of SP was determined in semen samples by microscopy. Morphometric analysis was performed on stained semen smears (n=144) using the method of Bloom, by the computer assisted-image analyses system. The parameters of SP head and tail size were determined, such as: area, outline, length, width, and parameters for head shape: regularity, rugosity, ellipticity and elongation. The bucks were categorized according to their age and the proportion of motile SP in ejaculate, into 3 groups for each category. The youngest bucks (1-1.5 years) had significantly greater outline and length of mid-piece and significantly greater head ellipticity and elongation compared to the other 2 groups (1.5-2.5, 2.5-4 years). The oldest bucks (2.5-4 years) had significantly smaller elongation and greater rugosity of the head. Bucks with the highest proportion of motile SP in the ejaculate (≥85%) had a significantly greater outline, length and area of the mid-piece and length of tail compared to the other 2 groups (75%; 75-85% motile SP in the ejaculate).

The obtained results suggest that the SP of younger bucks had functionally desirable morphometric characteristics, and that the bucks with a larger proportion of motile SP in their ejaculate had a larger mid-piece and a longer tail of spermatozoa.

ON-LINE INFORMATION SYSTEM FOR MONITORING OF PROTECTED SPECIES – AN EXAMPLE OF MARINE MAMMALS AND EURASIAN LYNX

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On the basis of national and international regulations, Croatia is obliged to monitor and report the status of all protected species, including the Eurasian lynx (*Lynx lynx*) and all marine mammals, primarily the only resident species – the bottlenose dolphin (*Tursiops truncatus*). Already in the 1980s the Faculty of Veterinary Medicine, University of Zagreb, started systematic monitoring of marine mammals in the Croatian part of the Adriatic Sea, while lynx monitoring has been implemented since the late 1990s. During this extensive period, many monitoring tools have been used to collect numerous parameters, so an on-line information system was programmed to unite and categorize all the collected data. Sightings of live animals or traces of their presence, mortality, collected samples and photographs used for photo identification are stored with date/location/photography, and can be viewed on the map. Each entry has a different level of reliability, depending on the source of data, and what kind of data was collected. Data bases are publicly available on-line; lynx -http://lynx.vef.hr/public/ and for marine mammals - http://crodolphin.vef.hr/public/. Also a smart phone application has been developed to facilitate and encourage reporting of marine mammal sightings by the wider public. The location and time of sighting are sent to the main database by SMS, and automatically stored. This kind of information system enables systematic archiving of large data collections, which is especially important for photographs. Moreover, they can be used for gathering data collected by the wider public, and for promoting species protection. With different systems of filters, the stored data can be easily analyzed and used for monitoring the distribution, population size and dynamics, mortality, movements and reproduction of protected species.

PREVALENCE OF *MACRORHABDUS ORNITHOGASTER* IN PET BIRD BREEDERS AND PET SHOPS FROM 2010 TO 2015 IN CROATIA

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Infection with the ascomycete fungus *Macrorhabdus ornithogaster*, usually called megabacteria, causes serious disease in different species of pet birds. Chronic infection and inflammation of the proventriculus causes consecutive bleeding in the digestive tract, emaciation and death of the bird. It easily spreads to other susceptible birds by feces. Even if diagnosed on time, therapy using antimycotics or antiseptics is long and usually doubtful. The incidence of megabacteriosis in pet bird patients in Croatia is high.

The aim of this research was to detect the prevalence of this fungus in pet bird breeders and pet shops, as places with a high transmission rate. Detection of megabacteria was performed by PCR using specific primers. DNA was isolated from feces collected from pet shops or from breeders in the period from 2010 to 2015. Altogether 97 samples were analyzed, around half from pet shops and half from breeders. The results showed that 22 (22.68 %) samples were positive for megabacteria, of which 15 (15.4 %) were from pet shops and 7 (7.2 %) from pet bird breeders. These results showed the high prevalence of megabacteria from both examined sources of samples, which confirmed the massive spread of megabacteria in pet birds that had already been noticed. Due to the high incidence of megabacteriosis in pet bird patients, continuous monitoring is needed, as well as significant improvement of biosecurity in pet shops and in pet bird breeders' facilities

ANALYSIS OF EFFICIENCY OF BROWN BEAR HUNTING FROM HIDES AT FEEDING SITES

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Large carnivore management has many challenges, especially when a species is both protected by law and declared as a game animal, as is the case with the brown bear (*Ursus arctos*) in Croatia. The annual harvesting quota, equaling 10 - 15% of the total estimated population size, is decided each year and up to 120 bears a year are commercially hunted in Croatia. Bear hunting is performed at feeding sites from elevated hides, so we surveyed the efficiency of this hunting method. In the period from 2006 to 2015, a total of 233 questionnaires on brown bear hunting events were collected. The questionnaire consisted of 31 questions, divided into two groups. The first one included questions about the circumstances in which the bear was shot, while the second one covered the types of rifle calibers and cartridges used in the shooting. After the statistical analysis of the circumstances, the effectiveness of hunting patterns was evaluated by comparing the conditions of hunting the bears that died immediately at the shooting location (45%), the ones who fled from the shooting location (50%), and those who fled and were never found (5%). The results showed that the body mass of the bear significantly ($p<0.05$) influenced the efficiency of the first shot. Smaller bears (58%) fell significantly more at the first shot compared to larger and heavier bears (41%). Females (52%) were more likely to fall from the first shot than males (43%). Surprisingly, the efficiency of the first shot was greater with a distance over 50 m from the animal (56% to 41%), maybe because the hunters were able to concentrate better and were calmer. The position of the bear at the moment of firing, the location in the body where the bear was shot, and the choice of caliber had significant impacts on the effectiveness of hunting. By analyzing the collected data, we wanted to contribute to greater efficiency in hunting for the hunter's content and for the welfare of the harvested animals. We conclude that bear hunting over bait from elevated hides ensures the required efficiency and safety, and minimizes the suffering of the animal. The alternative method of bear hunting by stalking, practiced in some countries, is probably not as efficient.

FIBROPAPILLOMATOSIS IN A MALE MOUFLON (*OVIS MUSIMON*) – A CASE REPORT

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Tumorous skin lesions associated with papillomaviruses were described previously in various wildlife species. Depending on the nature of its surface (smooth or cauliflower-like), they were usually described by practitioners as fibromas or papillomas. Later, in various literatures these lesions were named fibropapillomatosis. Family *Papillomaviridae* consists of a diverse and rich group of small viruses that infect different vertebrate species including humans. Within Cetartiodactyla 15 species are infected with 39 papillomaviruses. For a long period of time it was speculated that papillomaviruses are highly species-specific, however, recent findings of concurrent subclinical infections with different types of virus in wild ruminants from Italian Alps oppose this hypothesis. In this study we present a case of severe fibropapillomatosis in a 4 yr. old male mouflon from the island of Rab. The head of the respective mouflon was transported to the Faculty of Veterinary Medicine following regular execution of the game management plan. Cauliflower-like lesion was present on the upper and lower lips, covering majority of the muzzle. Lesion was reddish in a colour and partly wet. On the histological section thickened hyperkeratous epithelium with finger-like projections was visible. Connective tissue (tumour stroma) was present in the centre of these projections. Sporadic haemorrhages and mixed inflammatory infiltrate (lymphocytes, plasma cells, neutrophils, and macrophages) was also present. Presence of koilocytes was suggestive for viral aetiology. Ulcerations and bacterial colonization were present on the surface of the lesion.

Ovine papillomaviruses belong to 2 different genera, *Deltapapillomavirus* and *Dyokappapapillomavirus*. A similar nasal neoplasia was recently described in a free-ranging chamois (*Rupicapra rupicapra*) in Italy with the isolation of novel papillomavirus RrPV1. However, to the authors' knowledge there are no descriptions of clinical fibropapillomatosis in a free-ranging mouflon.

ARTIFICIAL INSEMINATION OF CAPERCAILLIE (*TETRAO UROGALLUS L.*)

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Capercaillie (*Tetrao urogallus* L.) is an endangered bird species from the group of forest grouses (*Tetraoninae*). Despite still being present in the majority of its previous habitats, the population is declining rapidly. Therefore, measures aimed at habitat analysis, understanding of factors with negative influence, and establishment of artificial breeding are very important in preserving this species. Capercaillie inhabits mountainous areas of Lika, Gorski kotar and Velebit, above 1000 m.a.s.l. The mating season begins at the end of the March and early April, with the environmental temperature reaching 8 to 17 ° C. Out of sixty days of display, actual mating lasts for approximately only 8 days. Furthermore, in artificial breeding, problems such as failing to copulate, aggressive behaviour of males and females, avoidance of certain males, etc., were observed.

The study was carried out in the Prezid capercaillie breeding centre. The centre consists of 2 males and 5 females. At the time of the study they were at the end of the mating season, but without a successful mating. We took sperm from the males by the lumbar massage method in an Eppendorf tube. Immediately after ejaculation, the sperm was diluted with a diluent (ASG medium) in a ratio of 1:2. Prepared diluted sperm was administered through the cloaca to the initial part of the uterus by a pipette. On the fifth day after artificial insemination, one of the eggs was cracked during the laying and analysed. On the surface of the egg yolk, we identified the embryo plate, and confirmed successful fertilization.

Artificial insemination is a reserve method when natural fertilization fails, but also in the case of delayed egg production by females, when males are already non-responsive.

FATTY ACID COMPOSITION OF DIFFERENT TISSUES OF FREE - RANGING GRAY WOLF (*CANIS LUPUS*) IN CROATIA

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Analysis of the fatty acid (FA) composition of tissues is a research tool which can give information on the foraging patterns of free-living animals. In Croatia, research of gray wolf nutritional ecology performed so far has been based on faeces and stomach analysis, which can only show the composition of relatively recent meals.

The objective of this study was a better understanding of the foraging patterns and physiological status of free ranging gray wolves, which is essential for management and conservation strategies of wildlife populations. The study was conducted on 36 animals (female, F=14; male, M=22) legally harvested during the years of legal quota cull from 2009 to 2012. In Lika and Gorski kotar (LGk), 19 animals were sampled, while in Dalmatia 17 animals were sampled. Samples from *m. gluteus superficialis* (MG), *m. vastus lateralis* (MV), *m. semitendinosus* (MS), subcutaneous adipose tissue (SAT), perirenal adipose tissue (PAT) and liver were collected. After homogenization and lipid extraction, the FA composition of tissues was determined by gas chromatography with regard to gender, age, body mass, sampling season and sampling location. Age had a significant influence on the FA composition of MV, MS, both adipose tissues and liver. In the PAT older F (+2 year) had a higher percentage of C16:1t compared to younger F ($p=0.04$), as well as a lower percentage of C18:1t compared to older M ($p=0.01$). Body mass showed a significant influence on FA composition of MV, MS and both adipose tissues. In MV heavier M (+25 kg) had a higher percentage of C18:0 and C18:3 ($p=0.02$, $p=0.04$, respectively) compared to lighter M. The sampling season had a significant influence on the FA composition of all investigated tissues. Males sampled in winter had a higher percentage of C20:4n-6 in PAT ($p=0.03$) compared to winter F. Finally, location had a significant influence on the FA composition of all investigated tissues. Males from LGk had a higher percentage of C18:1t ($p=0.01$) in SAT than M from Dalmatia.

It may be concluded that the composition of FAs, for both F and M, differs according to gender, age, body mass, sampling season and location, and probably reflects different foraging patterns.

MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF GOLDEN JACKAL INTESTINAL PARASITE

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In recent years there has been growing research interest in the golden jackal (*Canis aureus*), as this medium sized wild canid is spreading rapidly across Europe. Historically in Croatia this species was present in southern Dalmatia and occasionally individuals were seen and hunted in parts of Slavonia, but nowadays the population is present almost along the entire Adriatic coast, and in the continental part of the country. The goal of our research was to investigate the gastrointestinal parasitic fauna of golden jackals in Croatia, so we performed necropsy on 30 adult animals, collected by hunters following regular hunting operations. Intestinal content was examined and the collected parasites were identified under a stereomicroscope, based on their morphology. On the basis of morphological identification, for each sample species or genus, specific primers were used to amplify DNA regions appropriate for molecular identification. The obtained DNA sequences were compared to those available in GenBank. Due to the significant degradation of collected material, identification to the species level was not possible for some samples. The following intestinal parasites were found in 16 (53,3%) examined jackals (% prevalence in all analyzed samples): *Taeniaserialis* (26,7%), *Uncinaria stenocephala* (16,7%), *Alaria alata* (16,7%), *Opistorchiidae spp.* (16,7%), *Toxocara canis* (10%), *Mesocestoides litteratus* (6,7%), *Strongyllida spp*. (6,7%), *Toxascaris leonina* (3,4%) and *Echinococcus multilocularis* (3,4%). All the identified parasites were previously described in jackals in Europe, but *E. multilocularis* was only recently identified in Croatia, for the first time in red foxes. So, this is an important indication that this dangerous zoonotic parasite might be more common in Croatia than previously thought.

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ANAESTHETIC MANAGEMENT OF UNILATERAL ADRENALECTOMY IN A EASTERN QUOLL (*DASYURIUS VIVERRINUS*) - CASE REPORT

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Quolls are carnivorous marsupials and popular zoo-animals. A four-year-old female eastern quoll weighing 0.98 kg was presented with a history of lethargy, weight loss and inappetence lasting for five days. Abdominal radiography noted signs of ileus, which was confirmed by contrast radiography the following day. Exploratory celiotomy was undertaken.

The quoll was premedicated with an intramuscular injection of medetomidine 0.025 mg/kg, ketamine 5 mg/kg, butorphanol 0.1 mg/kg and midazolam 0.3 mg/kg. General anaesthesia was induced by propofol 3 mg/kg, intravenously given to facilitate endotracheal intubation. The animal was intubated with a 3.5 mm internal diameter cuffed tube. Anaesthesia was maintained with 2% sevoflurane in a mixture of oxygen and air. During surgery, hydration was maintained with an intravenous infusion of Lactated ringer solution 10 ml/kg/h. Anaesthesia monitoring included electrocardiography, pulse oximetry, capnography and measurement of oesophageal temperature and noninvasive blood pressure. During the operation the quoll was normotensive with short periods of hypertension. Intra abdominal inspection revealed no signs of intestine pathology, however left adrenal enlargement was identified. Left adrenalectomy was performed. The anaesthesia recovery was quick and uneventful. The quoll was placed in a cage and heated with a conductive heater. The next day, a decreased appetite was noted and feeding was supplemented with Emeraid Critical care food for Carnivores. Postoperative analgesia was provided using meloxicam 0.1 mg/kg and continued for three days after surgery. From the third day the follow up was unremarkable. Histopathological analysis defined the resected adrenal mass as adrenal hyperplasia.

This is the first report of anaesthesia in a quoll that underwent adrenal gland surgery. Anaesthesia of most dasyurids includes tiletamine/zolazepam, but recovery can be quite long. This report presents a suitable anaesthesia combination with rapid recovery.

ATTITUDES AMONG CROATIAN VETERINARY STUDENTS TOWARD WELFARE OF GAME ANIMALS

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There is a growing concern for animal welfare worldwide, and veterinarians are increasingly expected to ensure and promote high standards of welfare of all animals. The aim of the present survey was to examine the attitudes of veterinary students in Croatia towards game animal welfare.

The study included students of all years of the integrated undergraduate and graduate studies at the Faculty of Veterinary Medicine, University of Zagreb, in their first to sixth study years, whereby first-year students were surveyed twice, i.e. before and after attending the course on animal welfare (total sample 513 (93%) and 505 (91%) students, respectively). Student attitudes were assessed using a questionnaire containing a series of 5-point Likert scale questions.

Study results showed no differences in first-year student attitudes after attending the course on animal welfare. However, first- to third-year students generally expressed significantly ($P<0.05$) more concerned attitudes toward game animals as compared with fourth - to sixth - year students, including more negative attitudes toward hunting and hunters, breeding and production of game animals and compromise of their welfare.

The results obtained may be explained by the fact that with the progression of their study, veterinary students actually perceive hunting, breeding and production of game animals as activities that at the same time protect animals, their health and welfare, as well as the environment and biodiversity, in a specific manner, by management of hunting grounds, rather than as activities primarily focused on killing animals.

ANALGESIC ACTIVITY AND MECHANISM OF ACTION OF THE MONOTERPENE p-CYMENE IN THE RAT MODEL OF INFLAMMATORY PAIN

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p-Cymene is a monoterpene phenol, an active ingredient of essential oils extracted from various plants (Cumin, Thyme...). In addition to its anti-inflammatory and anti-oxidant activities, the analgesic activity of p-Cymene has recently been considered..

The aim of this study was to examine the analgesic effect of p-Cymene, and to compare it with the same effect of standard analgoantipyretic diclophen, and especially to examine the possibility of the interaction of p-Cymene and non-selective and selective NO-synthase inhibitors L-NAME and aminoguanidine (AG) on carrageenan-induced hypernociception in female rats.

Inflammatory pain (hypernociception) was induced by intraplantar (i.pl.) administration of carrageenan (500μg) into the rat hind paw. Electronic von Frey apparatus (ELUNIT, Belgrade) was used to determine paw withdrawal threshold induced pressure as the painful stimulus, and the effect was measured in grams (g).

p-Cymene (5-50 mg/kg,p.o.), given 50 min before i.pl. injection of carrageenan, produced significant ($p<0.01$,$p<0.001$) dose-dependent antinociception. p-Cymene (25 mg/kg,p.o.) and diclophen (10 mg/kg,p.o.) exhibited a similar antinociceptive activity in intensity and duration. p-Cymene (5 mg/kg,p.o.) coadministered with L-NAME or AG (5 mg/kg and 0.3 mg/kg,i.p.) caused a significantly higher ($p<0.01$, $p<0.001$) antinociceptive effect compared to the effect of p-Cymene alone. Also, in the presence of NO donor L-arginine (10 mg/kg,i.p.) the antinociceptive effect of the combination of p-Cymene + L-NAME and p-Cymene + AG, showed significant attenuation ($p<0.05$,$p<0.01$,$p<0.001$) throughout the whole measurement (1-6 hours).

p-Cymene leads to a dose-dependent antinociceptive effect in carrageenan-induced hypernociception, with intensity and duration similar to the antinociceptive effect of diclophen. p-Cymene, non-selective and selective NOS inhibitors (L-NAME and AG) administered together have a synergistic effect in carrageenan-induced hypernociception which is significantly reduced in the presence of L-arginine. On the basis of these results, we conclude that p-Cymene has analgesic activity based on the modulation of the L-arginine-NO system.

SPINAL TRAUMA IN DOGS IN EMERGENCY RESCUE PROGRAM „NOINA ARKA"

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Spinal trauma is a condition with devastating effects in dogs. It can damage the vertebra, disc, meninges, spinal cord or any combination of these. In abandoned dogs the history of trauma is usually unknown. The aim of this study is to investigate the incidence and outcome of spinal trauma in dogs admitted through the "Noina arka"emergency rescue program for traumatised stray dogs.

The medical records of dogs with spinal trauma admitted through the emergency rescue program over a period of 7 years were reviewed. Spinal trauma inclusion criteria consisted of: vertebral fracture, luxation or subluxation, prolapsed intervertebral disc, or coccygeal vertebrae amputation. A total of 44 dogs qualified under the inclusion criteria (22 males and 22 females). Their age was evaluated according to the available data, and the dogs were divided into age categories. Thirteen dogs (29.54 %) were younger than 1 year old, while 6 dogs (13.63 %) were geriatric, older than 10 years. Most of the injuries were anatomically localized in the thoracolumbar region (T11 – L3) and the lumbosacral part of the vertebral column (L6 – S1). There was only one cervical spine trauma. The most common findings included intervertebral disk protrusion (44.44%) and vertebral fracture (39.68%). Vertebral luxation (4.76%), subluxation (3.17%) and coccygeal amputation (4.67%) were found in fewer cases. More than one injury was present in 34% of dogs. Only two dogs were surgically treated due to the uncertain prognosis and outcome of treatment. The serious clinical condition and neurological damage in 20/44 dogs (45.45%) resulted in euthanasia, 8/44 dogs (18.18%) died, while 16/44 (36.36%) successfully recovered from the spinal injury.

Vertebral trauma outcome was associated with the localisation, severity of injury and method of treatment. The high mortality rate in abandoned dogs with clinically and radiologically diagnosed vertebral trauma additionally increased due to the long recovery period, dubious outcome and costs.

IMPACT OF MISSING CAT FEATURES ON THEIR RETURNING TO OWNERS

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Nowadays, pets play a very important role in human lives and many people consider them true friends, even family members. When a pet goes missing, it provokes emotional stress for the owner, while missing pets are scared, hungry and thirsty, exhausted, and may become victims of poisoning, traffic accidents, other animals or malevolent people. The aim of this study was to investigate whether and to what extent the features of missing cats, as one of the most popular pet animals, influence their return to their owners.

Data were collected from the Suza Info database referring to cats missing and found in the City of Zagreb during the 2011-2016 period. The following features were investigated: breed, sex, age, colour, hair length, castration and microchipping.

During the study period, 946 cats were missing, of which 372 (39%) were returned to their owners. The features that significantly contributed to finding them were breed (purebred cats were more frequently returned to their owners, $P<0.01$), castration ($P<0.05$), microchipping ($P<0.05$), and hair length (long haired and semi-long haired cats were more often returned to their owners, $P<0.05$). Unlike these, sex, age and colour had no significant impact on finding and returning missing cats to their owners.

PREVALENCE AND CLINICAL CHARACTERISTICS OF CONGENITAL HEART DISEASES IN DOGS EXAMINED AT THE CLINIC FOR INTERNAL DISEASES, FACULTY OF VETERINARY MEDICINE, UNIVERSITY OF ZAGREB IN THE PERIOD FROM 2013-2017

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Congenital heart diseases/defects (CHDs) encompass a large group of complex malformations of the heart. These malformations most often involve valves, cardiac septa or large vessels, and lead either to pressure or volume overload of the heart. The precise etiology of CHD is seldom known, but it can generally be said that they are caused by genetic and environmental factors. Depending on the type of defect and its impact on cardiovascular hemodynamics, clinical signs of CHD are wide-ranging from asymptomatic or mild to life threatening. In the setting of clinical examination, the vast majority of CHDs are accompanied by a loud heart murmur, which makes heart auscultation the most important screening test. Treatment of CHDs depends on the type of defect, and can be conservative (symptomatic), minimally invasive or surgical. Early and accurate diagnosis and successful management of CHD has an impact on patient morbidity and survival.

The main objective of this study was to assess the prevalence of CHDs in the population of dogs examined at the Clinic for Internal Diseases of the Faculty of Veterinary Medicine in Zagreb, in the period from January 1st 2013 to September 30th 2017. Additionally, the main clinical characteristics of the diagnosed CHDs are described. All dogs included in the study (n=25, 15 females and 10 males) underwent a full physical examination and complete echocardiography. In one of the dogs studied necropsy was performed.

In the current study, CHDs had an overall prevalence of 0.25% with reported frequencies of diagnoses as follows: 24% patent ductus arteriosus, 20% pulmonic stenosis, 16% subaortic stenosis, ventricular septal defect and tricuspid valve dysplasia, and 8% right persistent aortic arch.

CENTRAL VENOUS PRESSURE MEASUREMENT FOR INTRAOPERATIVE DETECTION OF PORTOSYSTEMIC SHUNT – A CASE REPORT

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A one year-old intact Great Dane female was presented with clinical signs of stunted growth, intermittent anorexia and vomiting, polyuria and mild neurological signs such as lethargy. The clinical signs had been present for 7 months.

Laboratory tests revealed a low urea serum concentration, as well as mild hypoproteinemia and mild hypoalbuminemia. Elevated preprandial and postprandial serum bile acid concentrations were also noted.

A portosystemic shunt was suspected, although not definitively confirmed by ultrasonography. An exploratory celiotomy was planned.

Once the animal was anaesthetized, a central venous line was placed in the left jugular vein to assist with blood sample collection, fluid administration and for central venous pressure (CVP) measurement. A 7Fr, double lumen, wire-guided line (Seldinger technique) was aseptically placed and connected to the anaesthetic monitor, via a pressure transducer chamber.

Since the shunt was hard to locate due to the severely changed anatomy of the abdominal vasculature, the CVP monitoring was used to detect the shunt by applying manual pressure for one minute onto the suspected shunting vein. This induced a drop in the CVP by 1 cmH_20. With the release of the occluded vein, the CVP increased by 1cmH_20. The procedure was repeated three times, to ensure the correct blood vessel was being occluded. A splenophrenic shunt was detected just caudal to the diaphragm, and it was attenuated with celophane tape. The dog recovered quickly with all the clinical signs disappearing within two weeks of surgery.

While the central line was originally placed for frequent blood sample collection for glucose monitoring and large volume administration, it was also helpful in locating the shunt due to CVP monitoring. This technique may be used to detect a portosystemic shunt when ultrasonography findings are inconclusive and a computed tomography scan is not available.

ANAL SAC APOCRINE ADENOCARCINOMA OF DOGS – A HISTOLOGICAL, IMMUNOHISTOCHEMICAL AND CLINICAL CHARACTERIZATION

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Anal sac apocrine adenocarcinoma (ASAA) is the most common malignant tumor of the perianal region of dogs. Its malignancy is reflected in common metastases to distant sites and usually apparent paraneoplastic syndrome. In this study, the incidence, biological behavior, histologic features and proliferation index of anal sac apocrine gland adenocarcinoma are investigated.

The study included 17 samples of ASAA, which were retrieved from the archives of the Department of Veterinary Pathology of the Veterinary Faculty, University of Zagreb, in 2008-2016. All the samples were thoroughly examined microscopically. The samples were also analysed using immunohistochemical Ki-67 marker (MIB-1, Dako). Finally, a data search, which included a survey of the owners, was conducted to gain clinical data on the biological behaviour of each particular tumor.

The results showed a greater incidence of ASAA in golden and Labrador retrievers (seven tumors, 41.2%). The average age of the dogs was 9.22 years, 12 samples (70.6%) were from male dogs, and 5 (29.4%) from female dogs. There were 12 samples of tumors of a solid subtype and 5 of a tubular subtype. Tumor recurrence was noted in 55.5 % of dogs, and metastases in 44.4% of dogs. The average value of Ki-67 expression in all the samples was 24.5% (25.7% in the solid subtype of tumors, and 21.7% in the tubular subtype). The average value of Ki-67 expression in the group of small tumors (diameter less than 2 cm) was 18.65%, while in the group of large tumors (diameter of tumor equal or more than 2 cm) it was 32.88% - this difference is also statistically significant ($p = 0.0112$).

In conclusion, larger tumors had a significantly higher expression of Ki-67, which could point to their later phase in the process of carcinogenesis. We believe that a larger number of samples would also show significant differences in the expression of Ki-67 according to the different histological tumor subtype.

SKIN CYTOLOGY - USEFUL DIAGNOSTIC TOOL FOR CUTANEOUS LESIONS AND LUMPS

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The range from different studies and different practices shows that approximately 65% of cases relate to dermatology problems, of different etiology.

Cutaneous lesions and lumps were observed in 23 companion animals: 21 were dogs and two cats. Information obtained from anamnesis and physical examination was recorded for each patient. Superficial and deep skin scrapings were performed and analyzed using a microscope. Skin tape cytology was used for exfoliative lesions. Samples were stained without further fixation. Impression cytology and fine needle aspiration (FNA) were used for local or diffuse skin lesions and lumps. Cytology skin smears were stained with Diff Quick, o-Toluidine blue, Lactophenol cotton blue staining (LCB). The obtained results for skin cytology revealed high diversity of diagnosis in companion animals. *Scabies spp*. and *Demodex spp*. were found in hairless pruritic areas, combined with an inflammatory condition of the skin. Skin tape cytology revealed *Malassezia* overgrowth, as superficial dermatitis. Pyoderma was also diagnosed. Malignant tumors in cytology are divided according to their origin and morphology, into three groups: epithelial tumors, mesenchymal tumors (carcinomas and sarcomas), and round cell tumors. Cytology in one young dog revealed a T-lymphoma. Adenocarcinoma of the perianal sac was found in one dog. Lipoma was found in two dogs with benign features of adipocytes. Fibrosarcoma, as an exfoliative tumor was found with FNA with less differentiated mesenchymal cells. Benign features were only found in lipoma lumps. One of the cats presented with dermatophytosis of *Microsporum spp*. but another cat presented with Injection Site Sarcoma as a result of vaccination.

Skin cytology can be used as a routine diagnostic procedure in daily practice for skin lesions and lumps in companion animals, because evaluation of the obtained material is easy, non-invasive, quick and inexpensive. This diagnostic tool gives useful information for further prognosis and treatment of various diseases.

COMPARISON OF DOGS AND CATS GUNSHOT INJURIES IN URBAN AREAS

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Gunshot injuries in veterinary practice present a relatively uncommon cause of trauma. The incidence of projectile injured patients in a veterinary clinic depends whether the clinic is situated in an urban or a rural area. Treatment varies on the basis of radiographic evidence of the metal projectile, injury location and type of firearm. The aim of the present study was to compare gunshot injuries in dogs and cats recorded in urban areas, due to their different behaviour and living habits.

The medical records were studied of dogs and cats with gunshot injuries treated at the University small animal hospital in a two year period. Signalment, time of trauma, number of projectiles and projectile type (air gun projectile, unknown projectile fragments, shotgun projectile), distribution of projectiles and bone fractures were investigated. In this time period 28 cases of gunshot trauma in cats (16 males and 12 females) and 35 cases in dogs (26 male and 9 female) were recorded. The incidence of gunshot injuries in cats was 1.03% and in dogs 0.27%. The mean age was 6.8 years in dogs and 6.6 years in cats. There was a significant difference in projectile number, in 15 dogs and 17 cats only one projectile was found, while 20 dogs and only 11 cats had more than one radiographically evident projectile. Most of the recorded projectiles were air gun pellets (82.1% in cats and 68.6% in dogs), followed by unknown projectile fragments (14.3% in cats and 20% in dogs) and shotgun (3.6% in cats and 8.6% in dogs). Fractures in dogs were mostly recorded on appendicular skeletal bones, while in cats most of the fractures were located in the thoracic rim skeleton. There was no congruence in the distribution of dogs and cats cases in terms of the months of the year.

The results indicate that gunshot injuries are still a relevant cause of trauma in urban areas, especially in cats and dogs. Therefore, this finding should be taken into account in all cases of unknown cause of trauma in dogs and cats.

ISOLATION AND SPECIES IDENTIFICATION OF *CAMPYLOBACTER* SPP. FROM HEALTHY AND DIARRHEIC DOGS

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Campylobacter spp. is a leading bacterial cause of human diarrheal illness worldwide and one of the most common enteric bacterial pathogens in dogs. The exact role of *Campylobacter* spp. as an obligate enteric pathogen is sometimes considered difficult to assess because it can also be isolated from healthy animals. However, it is isolated significantly more often from diarrheic dogs, and also excreted in much higher quantities. Research into *Campylobacter* spp. in healthy and diarrheic dogs suggests that different species may have a different clinical significance in dogs. The aim of this study was to compare isolation rates of *Campylobacter* spp. in healthy and diarrheic dogs, and identify the species of the isolated bacteria. Fecal samples of 190 (105 diarrheic and 85 healthy) dogs were tested for *Campylobacter* spp. Species was determined for twenty isolates, using MALDI TOF mass spectrometry. The dogs were divided into two groups: younger and older than 12 months. Statistics were performed using the chi square test. *Campylobacter* spp. was isolated from 44 (23.2%) dogs and significantly more frequently detected in diarrheic (36.2%) than in healthy (7.0%) animals ($p<0.01$). In healthy dogs, *Campylobacter* spp. was more frequently isolated from dogs younger that 12 months ($p<0.01$). In diarrheic dogs, there was no difference in isolation rates between the age groups. All isolates were identified as *C. jejuni*. The results of this investigation clearly demonstrate that *Campylobacter* spp. has a significant role in the pathogenesis of gastroenteritis in dogs. The higher isolation rate in diarrheic dogs is probably a result of the dominance of *C. jejuni*. In studies reporting similar isolation rates in healthy and diarrheic dogs, *C. upsaliensis* was the most prevalent species, which points out the importance of *Campylobacter* species identification. In the population of healthy dogs, young animals are more likely to shed *Campylobacter* spp. while in diarrheic dogs there is an equal possibility of campylobacteriosis in all age groups.

DIVERSITY OF PIROPLASMS DETECTED IN TICKS: INDIVIDUAL SEQUENCING APPROACH

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Piroplasms are haemoprotozooan parasites that include two major genera: *Babesia* and *Theileria*. So far ticks are the only known vectors, and the worldwide distribution of piroplasms depends on the presence of a specific tick vector. Frequent use of molecular methods has allowed increasing knowledge of the real diversity of the *Babesia*/*Theileria* species. The same approach has improved knowledge of tick species diversity and their capacity to be vectors of specific *Babesia*/*Theileria* species.

Ticks were collected from animals and from the environment by flagging and dragging. Each tick was morphologically analyzed and confirmed by sequencing of mitochondrial 16S rRNA. 18S rRNA of *Babesia*/*Theileria* species was sequenced to obtain a deeper insight into the diversity of species present in Croatian ticks.

For the first time we have confirmed presence of *Ixodes rugicolis* carrying *Babesia* sp. Badger type A, while closely related *Babesia* sp. Badger type B was detected in *I. hexagonus*, both collected from red foxes. Another species "*Babesia vulpes*" was present in *I. hexagonus*. Detection of *Babesia* cf. *crassa* represents the first detection in Europe. Parasite was present in *Rhipicephalus sanguineus* from Pelješac and questing *Hamaphisalis parva* from Slavonia. *Theileria* cf. *buffeti* and *Babesia* sp. isolate from Angola were detected in *I. ricinus* tick from Cres and *Rhipicephalus turanicus* from Pelješac. In *R. turanicus* and *R. bursa* ticks *Theileria ovis* and *Babesia ovis* have been confirmed from Southern Croatia. One of the unexpected findings represents presence of *B. canis* DNA in *R. sanguineus* s.l. and *R. bursa* from Southern Croatia collected from cows and goats and expected in questing *Dermacentor reticulatus* from Central Croatia. Two zoonotic species *Babesia venatorum* and *B. microti* have been found in unique genotype of *I. hexagonus* and questing *I. ricinus*, both from inland.

The results from the current study clearly show the high diversity of piroplasms from different Croatian regions, as well as conformation of ˝new˝ tick species such as *H. parva* and *I. rugicolis,* together with various genotypes of *I. hexagonus*. Further studies are needed to evaluate the vector capacity of different tick isolates and the distribution of piroplasms all over Croatia.

CANINE OCULAR DIROFILARIASIS - A CASE REPORT

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Dirofilaria repens is highly prevalent in Croatian dogs. Infection is asymptomatic but dermatitis may appear. Eye lesions have not been described so far.

Ophthalmological examination of the left eye in a four year old, mixed breed, male dog from Vukovar revealed a single nonpruritic nodule. Located episclerally, close to the limbus, was a pink tumor of elastic consistency and botryoidal surface, measuring 8 mm in diameter. Complete excisional biopsy and tissue formalin fixation were performed.

On the surface of the tumor there was an irregular pseudo cyst wall covering a coiled white nematode. The tissue was processed for routine histopathology and a few nematode segments 2 mm in diameter were microscopically identified as immature female *Dirofilaria* sp. according to their outer cuticular longitudinal ridges and lack of microfilaria. *Dirofilaria repens* was confirmed on the basis of the morphology of the anterior part and longitudinal stripes.

Histology showed the concentric 3 mm thick outer layer of the collagenous wall. The inner layer consisted of loosely arranged collagenous fibers, and a network of capillaries and fibroblasts, surrounded by macrophages, eosinophils, neutrophils and plasma cells. Eosinophilic proteinaceous fluid filled the pseudo cyst space and surrounded fragments of the coiled nematode. On transversal sections of fragments evenly spaced cuticular ridges were present over pronounced muscular layer, lateral chords and segments of a digestive and an immature reproductive systems. This finding confirmed dirofilariasis as a cause of granulomatous episcleritis.

Clinical, hematological, biochemical, radiological as well as cardiac sonographic and modified Knott's tests excluded the presence of other adult nematodes, microfilaria or lesions consistent with canine dirofilariasis, respectively.

In cases of nodular ocular lesions except neoplasms, *Dirofilaria repens* has to be considered as a possible etiology in Croatian dogs. In this unusual case an immature adult caused chronic inflammation, despite the lack of previous descriptions of microfilaria as a cause of strong inflammatory response in ocular lesions.

BACTERIA AND PROTOZOA IN FLEAS AND TICKS FROM DOGS AND CATS DIED DUE TO HEMOLYTIC ANEMIA

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Fleas and ticks are important vectors of vector-borne pathogens that affect cats and dogs. In the present study, ticks and fleas collected from dogs and cats were investigated for the presence of vector-borne pathogens and the endosymbionts they harbor.

The ectoparasites were collected from 14 deceased dogs and 22 deceased cats with gross findings of hemolytic anemia. After the morphological determination of ectoparasites, DNA was extracted for molecular detection of *Babesia*/*Theileria* sp., *Anaplasma*/*Ehrlichia* sp., *Hepatozoon* sp., *Mycoplasma* sp. and *Bartonella* sp.

Dermacentor reticulatus (4/7, 57%), *Ixodes ricinus* (4/7, 57%) and *Rhipicephalus sanquineus* s.l. (1/7, 14%) ticks were collected from 7 of 14 dogs. Fleas were present in 64% of dogs: *Ctenocephalides felis* in 67% and *C. canis* in 33%. In three dogs co-infestation with more than one ectoparasite was detected: (i) *C. felis* and *I. ricinus, (ii) C. canis* and *D. reticulatus, (iii) D. reticulatus*, *I. ricinus* and *R. sanquineus. Babesia canis* was detected in four ectoparasites collected from four dogs (4/14, 29%): *D. reticulatus* (2/4, 50%), *I. ricinus* (1/4, 25%) and *C. canis* (1/4, 25%). *Rickettsia raoultii* was found in a single *D. reticulatus* (1/14, 7%). *Candidatus* Midichloria mitochondrii was detected in all *I. ricinus* collected (4/14, 29%). Two groups of endosymbionts were detected in canine fleas: (i) identical to *Wolbachia pipientis, endosymbiont* found in *C. felis* (4/14, 29%) and *C. canis* (2/14, 14%), (ii) identical to *Wolbachia endosymbiont of Xenopsylla* sp. found in *C. felis* (2/14, 14%) and *C. canis* (1/14, 7%). In two cases co-infections were detected (i) *B. canis* and *W. pipientis* in *C. canis*, (ii) *B. canis* and *Can. Midichloria mitohondrii* in *I. ricinus.*

C. felis fleas were present on all the examined cats, as well as a single *I. ricinus* tick. Sequences revealed the presence of two endosymbiont groups in *C. felis*: one identical to *W. pipientis* (9/22, 41%) and the second to *W. endosymbiont* of *Xenopsylla* sp. (13/22, 59 %). *Anaplasma phagocytophilum* was detected in *I. ricinus* (5%, 1/22), *Bartonella clarridgeiae* in four (4/6, 67%) and *B. hensellae* in two fleas (33%). In six cases co-infections were detected (i) *W. endosymbiont of Xenopsylla* sp. and *B. clarridgeiae* (4/6, 67%), (ii) *W. pipientis* with *B. hensellae* (33%, 2/6).

SEROPREVALENCE OF VECTOR - BORNE PATHOGENS IN DOGS FROM CROATIA

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Vector-borne pathogens are widely distributed and represent an emerging group of diseases in dogs. For veterinary practitioners they represent a substantial diagnostic challenge because clinical signs might often be concealed and overlapping. Although the prevalence of antibodies against vector-borne pathogens has already been reported in apparently healthy dogs from Croatia, here we report the results of a survey of blood samples with SNAP®4Dx®Plus collected from three groups of dogs: (i) apparently healthy (52%, 753/1,448), (ii) dogs with suspected clinical signs of the disease (44%, 632/1,448), (iii) deceased dogs with a history of anemia and thrombocytopenia (4%, 63/1448). In total 1,448 dogs were tested for the presence of *Dirofilaria immitis* antigen, antibodies to *Anaplasma phagocytophilum*/*Anaplasma platys*, *Borrelia burgdoferi* and *Ehrlichia canis*.

The highest prevalence was detected in the group of apparently healthy dogs with 6% (47/753) serologically-positive. Seroprevalence was: *A. phagocytophilum*/*A. platys* 5% (41/753), *B. burgdoferi* 0.2% (2/753), *E. canis* 0.6% (5/753) and *D. immitis* 0.3% (3/753). Within the group of dogswith suspected clinical signs a slightly lower prevalence of 5% (30/632) was noted, with similar distribution of pathogens: *A. phagocytophilum*/*A. platys* in 3% (21/632), *B. burgdoferi* 0.4% (3/632), *E. canis* 0.6% (4/632) and *D. immitis* in 0.6% (6/632). Deceased dogs showed identical seropositivity (6%, 4/63) as the apparently healthy group: *A. phagocytophilum*/*A. platys (3%,* 2/63), *B. burgdoferi* (2%, 1/63) and *D. immitis* (3%, 2/63). Co-infections were found in 0.5% (4/753) of apparently healthy dogs, 0.3% (2/632) within the group of dogs with suspected clinical signs, while in deceased dogs only one case was registered (2%, 1/63). In all tested groups *A. phagocytophilum*/*A. platys* was the most prevalent pathogen, followed by *E.canis* in apparently healthy and dogs with suspected clinical signs, while the lowest prevalence was for *D. immitis* and *B. burgdoferi*.

These findings raise question regarding the role of selected vector borne diseases in the development of anemia and thrombocytopenia, since the prevalence among the groups was almost identical.

VECTOR-BORNE INFECTIONS IN CROATIAN CATS: PATHOLOGIC AND MOLECULAR STUDY

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Fleas and ticks represent common ectoparasites of cats. However, the pathogens they transmit have not been investigated in Croatian cats. The aim of this study was to investigate vector-borne pathogens from Croatian feline patients, and to determine their pathogenicity on the basis of pathology and molecular findings.

The study included 37 cats with gross findings of anemia and/or jaundice, submitted for necropsy upon the owners' request over a period of two years. During necropsy, representative tissue samples were collected for histopathology and molecular analyses. The blood, bone marrow, liver, lungs, myocardium and spleen were screened by conventional PCR for the presence of *Anaplasma/Ehrlichia, Babesia/Theileria, Bartonella, Hepatozoon, Mycoplasma* and *Rickettsia* DNA. All amplified samples were sequenced in both directions and assembled. The cause of death was determined following the results of pathology and molecular investigations.

In 25 cats out of 37 investigated (68%) at least one pathogen was detected: *Candidatus* Mycoplasma haematoparvum (64%), *Can.* M. haemominutum (32%), *Babesia canis* (16%), *Can.* M. turicensis (12%), *M. haemofelis* (8%) and *Bartonella clarridgeiae* (4%). Hemoplasmas were detected in different organs, while *B. canis* was detected only in the blood and *B. clarridgeiae* in one liver. Co-infections were found in nine cats (9/25=36%). In three cats (3/25=12% of positive cats and 3/24=13% of cats with hemoplasmas) causes of death were hypoxia and circulatory collapse, linked to hemoplasma infection: *M. haemofelis* infection, *Can.* M. haemominutum and *M. haemofelis* co-infection, and *Can.* M. haemominutum and *Can.* M. haematoparvum co-infection. In the remaining 23 cats the cause of death was not associated with the detected pathogens, which raises the question of their pathogenicity in these animals.

Although vector-borne pathogens are common in Croatian cats, it seems they mostly present incidental findings, but not the primary causes of death. However, in the minority of animals infected with one or more hemoplasmas, hemolytic anemia can cause death as a consequence of massive erythrocyte destruction.

METABOLIC AND OXIDATIVE STRESS IN ENDURANCE HORSES DURING RACING

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Endurance riding is the most physically demanding equine sport discipline. Being a relatively young discipline, the full impact of endurance racing on horse metabolism has not yet been thoroughly researched.

The study included 53 horses that successfully completed 40 km and 80 km endurance races. The average speed and recovery time were compared to age, gender, breed, experience and length of the race track. Two blood samples were taken from the horses for metabolic and oxidative stress research, one before and one after the race. The analysis consisted of several serum biochemical parameters, as well as determination of reactive oxygen metabolites (d-ROMs), malondialdehyde (MDA) and biological antioxidant potential (BAP), as indicators of oxidative stress.

Values of renal and muscle damage parameters increased after the races, with the degree of change more significant at longer racing lengths. Electrolyte loss was more pronounced during longer races. Females had significant increases in urea, creatinine kinase, aspartate aminotransferase and lactate dehydrogenase values compared to males, while older horses had significantly higher values of creatinine. Significant increases in all observed oxidative stress markers were recorded after the races. Higher values of BAP were recorded during longer races. A positive correlation was recorded between urea and triglyceride concentrations, while both parameters positively correlated with BAP concentration. MDA and blood glucose increased proportionally, indicating that hyperglycemia affects cell lipid peroxidation.

Endurance riding causes increased body requirements in horses that are manifested by increased electrolyte loss, muscular and urinary system activity, and oxidative stress. Longer races are characterized by increased metabolic demands and oxidative stress, emphasizing the need of prompt veterinary intervention in cases of exhausted horse syndrome expected during longer races.

BILLATERAL GUTTURAL POUCH MYCOSIS WITHOUT EPISTAXIS - A CASE REPORT

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Guttural pouch mycosis is a rare but serious disease in horses, with epistaxis as a most common clinical sign, caused by fungal erosion of the carotid and maxillary arteries. The prognosis for guttural pouch mycosis is questionable due to slow recovery, long therapy and a high mortality rate, despite treatment.

An eleven-year-old Croatian Warmblood stallion was presented with a history of swollen neck lasting for almost two months. Four weeks previously bilateral nasal discharge, together with fever and dysphagia were also noted. The horse was treated with broad spectrum antibiotics and analgesics without any improvement. Interestingly, the owner did not report any sign of epistaxis during the whole period. At admission the horse was lethargic with mild edema in the upper region of the neck, bilateral mucopurulent nasal discharge and dysphagia. Airflow from the left nostril was significantly decreased. Haematological and biochemistry blood tests were within referent limits. Endoscopy revealed the collapsed roof of the pharynx, with the nasopharynx containing foot material. The whole upper third of the left guttural pouch was covered with a white, brown and black lesion with a diphtheritic membrane. The findings in the right guttural pouch revealed a slightly smaller lesion with the same characteristics. A biopsy of the suspected fungal mass in the guttural pouch was taken for cytology and fungal culture. Cytology revealed hyphal structures compatible with *Aspergillus* species. Fungal testing yielded abundant growth of pure culture of *Aspergillus* sp. The recommended therapy included daily guttural pouch irrigations, with antimicotic medications coupled with oral antifungals. However, the owners rejected the therapy due to financial concerns and the horse was euthanized.

This report presents a rare case of bilateral guttural pouch mycosis without signs of epistaxis. It is therefore important to consider guttural pouch mycosis as a differential diagnosis in every dysphagic horse.

ENUCLEATION WITH OPTIC NERVE LIGATION IN A HORSE - A CASE REPORT

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Various diseases can cause the need for enucleation to be performed in a horse. Two preferred methods are transpalpebral and subconjunctival enucleation. As the optic nerve is very short in horses, its ligation is not advised due to the danger of impairment of vision in the other eye.

A 12 year-old Arabian stallion was presented with eyelid tumour regrowth. A suspected sarcoid was surgically removed from the upper eyelid 8 years before, using H-flap and local implantation of cisplatin beads. The owner noted significant tumour regrowth after 6 years. The tumour was extirpated along with ¾ of the upper eyelid, leaving it strained dorsocaudally. Blepharoplasty with a sliding graft from the temporal region was performed and carboplatin chemotherapy was introduced. Histopathology showed a multifocal peripheral nerve sheath tumour of the eyelids. Since the horse was unable to move the upper eyelid, it developed keratoconjunctivitis sicca with secondary chronic corneal ulcers. Enucleation was planned.

Three months later, transpalpebral enucleation of the right eye was performed under general anaesthesia. Care was taken not to damage the conjunctival sac and to avoid traction to the optic nerve. Precise dissection of the periocular muscles was performed to enable optic nerve visualisation. A hemostat with a curved tip was placed on the optic nerve, then it was ligated using a Polysorb 0 suture and transected. A tension-releasing incision was made ventral to the enucleation incision, prior to wound reconstruction.

The horse recovered quickly with no vision loss to the other eye. No haemorrhage or haematoma formation was noted at the place of enucleation. Although it is usually not performed, the optic nerve was ligated in this case, in order to prevent haemorrhage and minimize the possibility of infection, so chemotherapy could be continued as planned. The present case shows that a ligature placement on the optic nerve can be safe if it is performed carefully, avoiding traction to the optic nerve.

NONSURGICAL REMOVAL OF UROLITHS IN HORSE

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Horses with uroliths are most commonly admitted to hospital because of the inability to pass urine, and the treatment of choice is ultrasonic fragmenting, lithotripsy and surgical removal.

A 19 year-old castrated gelding was admitted to the clinic due to histhe inability to urinate for 24 hours, but still in a generally good condition. The horse was straining to urinate and would pass a few drops of urine. On rectal palpation a distended bladder was found. The results of blood analysis indicated renal failure. Urethroscopy was preformed and the first stone was found 30cm into the urethra, wedged in the urethra obstructing the whole lumen. Surgical removal of the stone was suggested to the owner, but he wanted to euthanise the horse.

Urethroscopy was then repeated and the removal of the stone was attempted with a small animal gastroscope, grasping forceps, stone retrieval baskets and retrieval snare. The plan was to attempt to crush the edges of the stone so as to unwedge it, and the snare or basket could then be positioned for retrieval. The animal was sedated and kept on analgesics during the whole process. Upon removal of the stone the mucous membrane of the urethra was severely damaged. Multiple stones were removed from 4 locations in the urethra and the two final ones were removed from the bladder. The largest removed piece was 2.5 cm long and 1 cm in diameter. Antibiotics were given order to prevent urinary infection. On the second day the urethra was examined by endoscope and multiple adhesions were found, some of them almost completely obstructing the lumen. The adhesions were torn with the endoscope and flushed with saline. After a few days the owner reported normal urination without straining.

Reevaluation was performed two months after the procedure and minimal scaring with two minor strictures in the urethra were found, but no signs of inflammation or new stones.

Endoscopic urethral stone removal with stone crushing by forceps is not a treatment of choice for urethrolits and uroliths, but with euthanasia being the only alternative it is worth a try.

/ / / POSTER PRESENTATIONS / / /

MERCURY DETERMINATION AND SENSORY ANALYSIS OF FISH FROM SERBIAN MARKET

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The aim of the study was to determine the freshness of the fish on the market and also their mercury content, applying a reliable chemical method in different samples of fish and fish products purchased in markets, and to compare the obtained values with the values prescribed by legislation.

The concentration of mercury in samples of fish and fish products taken from Belgrade grocery stores was determined applying a simple, rapid and accurate method. A mixture of HNO_3 and H_2O_2 was used for complete oxidation of organic tissue, and mercury was detected by the cold vapor atomic absorption spectrometry technique. Sensory analysis was carried out using the quantitative descriptive analysis method.

In total, 30 samples of fish and fish products are analyzed for total mercury and also observed by a group of trained people for sensory characteristics. It was found that the concentration of mercury in the edible muscle tissue of these fish and their products was less than the limit of quantification (LOQ) of the method and within the range of good quality, as described in legislation.

The low concentrations of mercury detected in the samples do not constitute any significant mercury exposure to the general population through fish consumption. The overall score obtained by the sensory evaluation of the working group was very high.

ISOLATION AND IDENTIFICATION OF *YERSINIA ENTEROCOLITICA* STRAINS FROM FOOD PRODUCTION CHAIN

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Human foodborne yersiniosis is mostly associated with consumption of raw or undercooked food of animal origin. During a three-year period, we tested 91 thermally processed and fermented meat products, 32 fresh meat and meat preparations at the end of their shelf-life, 295 raw milk and 9 unpasteurized milk cheeses sampled at retail level, and 78 pig tonsils sampled during slaughter processing, for the presence of *Yersinia enterocolitica*. Samples were enriched in PSB and ITC broth, and then streaked on CIN agar and CHROMagar™ Y.enterocolitica. Presumptive colonies were identified by MALDI TOF-MS.

Pathogenic *Y. enterocolitica* was absent in raw milk, unpasteurized milk cheeses, thermally processed and fermented meat products. *Y. enterocolitica* was recovered from 26 tonsils (33.33 %) and two fresh pork meat samples from retail (6.25 %). A total of 80 presumptive colonies were selected from CIN/CHROMAgar and all were identified as *Y. enterocolitica* by means of MALDI TOF-MS.

The results demonstrate the low occurrence of pathogenic *Y. enterocolitica* in dairy products and processed meats on the market. On the other hand, the persistence of pathogen in the pig population is a risk factor for meat contamination during slaughter processing and meat cutting. The combination of chromogenic culture media and MALDI TOF-MS may be used as alternative method for faster isolation and confirmation of pathogenic *Y. enterocolitica* from food chain samples.

ANTIMICROBIAL RESISTANCE OF *ESCHERICHIA COLI* ISOLATED FROM POULTRY AND PET BIRDS

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Antibiotics were often used in intensive poultry production not just to treat disease, but also to prevent bacterial infections. Uncontrolled usage of antibiotics has therefore led to the development of antimicrobial resistance. The spread of resistant bacteria such as *E. coli* through the food chain can represent a risk for public health. *E. coli* bacteria represent normal microflora in a bird's intestine, although some strains can cause avian colibacilosis, a systemic fatal disease and one of the major infectious diseases in birds of different species.

The aim of this study was to compare the level of antimicrobial susceptibility of *E. coli* found in intensive and extensive poultry production, and in pet bird patients. Samples from poultry were taken during necropsy from different organs, while samples from other birds were taken during clinical examinations. Samples were cultured using a standard bacteriological examination, and antimicrobial susceptibility was determined using the disk diffusion method. Antimicrobial susceptibility tests were performed on 43 *E. coli* isolates. The results showed a high level of antimicrobial resistance (68.93 %), with the majority of resistant strains being isolated from intensive poultry production (44.6 %), 26.3% from free range poultry, 11.3 % from pet birds, and 17.8 % from pet bird breeders. These results are expected, since in intensive poultry production the use of antimicrobial therapy is frequent, which results in resistance development, especially in cases of underdosing and short therapy periods. Such resistant strains could significantly compromise production, so other methods should be used to defeat them, such as autogenous vaccines, probiotics etc.

OVSYNCH EFFICIENCY IN ANOESTROUS DAIRY GOATS

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Oestrus induction and synchronization protocols in small ruminants are mainly based on the use of progestagens and eCG, comprising AI, during and out of the sexual season. These methods result in acceptable kidding rates, but there is a worldwide intention of avoiding the use of steroid hormones for oestrus synchronization, due to the micro residues in the food chain. At the same time, there is a tendency to restrict the use of these hormones in the EU (Directive 96/22/EC), and new protocols, that allow the use of AI and result in sufficient kidding rates are being investigated.

The aim of our research was to establish the efficiency of a modified Ovsynch protocol with TAI in dairy goats. The modification of the standard Ovsynch included a longer interval between PG and second GnRH application, and we expected a decrease in the percentage of goats with a short luteal phase.

The research was conducted on 20 Saanen goats during September in northern parts of Croatia, when all the goats were expected to be in full season. The animals received a GnRH analogue, 7 days later $PGF_{2\alpha}$, and 64 h later a second GnRH. TAI with deep frozen semen was performed 16 h later, with intracervical deposition of semen. Six weeks later all goats were checked for pregnancy. In order to establish synchronization success by analysing progesterone concentration, blood was drawn from all animals from the jugular vein on the days of GnRH application.

Conception rate was 0%, and the progesterone concentration at the first GnRH administration in Ovsynch was 0.318 ± 0.13 ng/ml.

Although the protocol was conducted in September, when the goats in the NW area of Croatia are supposed to be in full season, the progesterone concentration suggests that there was no luteal activity in the high yielding goats' ovaries, thus they were still in anoestrus. Therefore, the conception rate of 0% indicates that Ovsynch is an inadequate protocol for use in anestric goats.

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IMPACT OF FEEDING 10 % FERMENTED BIOPRODUCT ENRICHED WITH BIOLOGICALLY ACTIVE COMPOUNDS ON FATTY ACID PROFILE AND QUALITY OF CHICKEN BREAST MEAT

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A biotechnological approach based on solid state fermentations (SSF) is one of the most perspective techniques to enrich cereals with the desired metabolites. The main challenge of our research was to determinate the possibility of the use of fungal SSF bioproducts enriched with PUFAs (mainly gamma-linolenic acid), beta-carotene, coenzyme Q10, ergosterol and amylase, as supplements for broiler chicken feed.

The aim of this study was to analyse the effect of addition of 10% (w/w) fermented bioproduct into commercial broiler feed on the fatty acid profile, cholesterol content and lipid oxidative stability of chicken breast meat. The fermented bioproduct was prepared by fermentation of corn scraps by filamentous fungi (*Umbelopsis isabellina* CCF 2412) in fungal SSF and gamma-linolenic acid, and beta-carotene pigments were developed in this process. In the experiment, 80 one-day-old COBB 500 chickens were used. Half of them (control group) were fed with only commercial feed. The chickens in the experimental group were fed with commercial feed, and from the 10th day of age until the time of slaughter (39th day), 10% of the commercial feed was replaced by fermented bioproduct.

The addition of fermented bioproduct to the commercial feed mixture positively influenced the fatty acid profile in breast meat. The amount of gamma-linolenic, alpha-linolenic and oleic acids in the fat of the breast muscles increased and the n-6/n-3 ratio was also changed. The profile and content of PUFAs did not change after thermal treatment of the meat. The cholesterol content and oxidative stability of fat during the storage (4°C, 7 days) of meat were not affected by the fermented bioproduct.

The conclusion is that addition of 10% (w/w) fermented bioproduct to commercial broiler feed is an easy way to increase the content of important PUFAs in meat, and to produce functional food.

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THE USE OF HUMIC SUBSTANCES IN BROILER CHICKENS DURING THE FATTENING PERIOD

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Humic substances seem to be a suitable alternative to antibiotic growth promoters for use in chicken meat production. Currently they are used in horses, ruminants, swine and poultry in the treatment of many disorders, such as acute intoxication, dyspepsy and diarrhea for their antiphlogistic, adsorptive, detoxifying and antimicrobial properties. In broilers during the fattening period they positively affect production parameters, growth rate, carcass yield and also decrease mortality.

The aim of this study was to monitor the effect of humic substances on total feed consumption, feed conversion, and weight gain. At the end of the trial we evaluated the impact on body weight and carcass characteristics: breast and thigh muscles yield.

A total of 80 1-day-old COBB 500 chickens were randomly divided into two groups. The control group (C) was fed with the basal diet, whereas those in the experimental group (E) were fed with the same diet, supplemented with 0.8% of humic substances (Humac Natur, Slovakia) from the third day until the end of the fattening period (39th day).

The addition of humic substances did not affect feed intake and feed conversion ($P > 0.05$). Nevertheless, the average weight of the chickens, and the carcass and thigh muscle yield in the experimental group were higher at the end of the experiment ($P < 0.05$). In the experimental group, we also noted a positive impact on the health status of the broilers and a significantly lower mortality rate ($P < 0.05$).

Preventive administration of humic substances ensures improvement in production parameters due to their antimicrobial, anti-inflammatory, antiviral and detoxifying properties. The great benefit is that the animal products obtained through the use of natural substances such as humic acids are without residues of antibiotics and other drugs that require withdrawal periods.

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EFFECT OF FEEDING 10 % FERMENTED FEED ENRICHED WITH GAMMA - LINOLENIC ACID AND BETA - CAROTENE ON BLOOD BIOCHEMICAL PARAMETERS OF BROILER CHICKENS

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One of the most important techniques used to enrich cereals and by-products with essential polyunsaturated fatty acids is a biotechnological method called Solid State Fermentation (SSF). In principle it is the ability of microorganisms to transform the substrate (cereals are often used) into products with a higher content of n-6 and n-3 polyunsaturated fatty acids (PUFAs) and pigments. Our research focused on the effect of bioproducts produced by fungal SSF on the quality of broiler meat, and also blood parameters. Blood parameters indicate the clinical and nutritional status of the animals.

The aim of this study was to investigate the effect of the addition of fermented bioproduct produced by *Umbelopsis Isabellina* CCF 2412, mixed into the commercial feed of broiler chickens, on the biochemical parameters in their blood (Ca, P, Mg, AST, ALT, ALP, CHOL, HDL, LDL). A total of 80 1-day old, COBB 500 chickens were randomly assigned to two treatments. Birds in the control group (C) were fed the basal diet, while those in the experimental group (E) had 10% of basal diet replaced by fermented bioproduct from the 10th day of fattening until the time of slaughter (39th day). Feeding fermented bioproduct increased triglyceride ($P<0.05$) and decreased LDL ($P<0.05$) levels in the blood of the experimental chickens compared to the control. No significant difference ($P>0.05$) was observed in plasma Ca, P, Mg, AST, ALP, ALT, CHOL and HDL levels between the control and experimental groups.

On the basis of our results, it may be concluded that feeding fermented bioproduct has an effect on lowering LDL-cholesterol particles, but it also increases the levels of triglycerides in the blood of broiler chickens. The increased values of triglycerides were the result of their increased content in the fermented bioproduct produced.

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FATTY ACID COMPOSITION IN SEMEN OF BOARS

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Spermatozoa reproductive function depends on the composition and ratio of lipids which are essential for membrane fluidity, acrosome reaction, and thus sperm fertility competence.

The objective of this study was to determine boar seminal plasma and spermatozoa fatty acid composition (FA). The study was performed on two boar breeds: German Landrace (N = 7) and Pietrain (N = 5). Samples of semen (sperm rich fraction) were taken using a single phantom. The semen samples were centrifuged in iodixanol density solution to obtain two sperm fractions. The two fractions included sperms with motility greater than 90% (live) and less than 20% (dead), respectively. Total lipids in the seminal plasma and two subpopulations of spermatozoa were extracted and, after transmetilation, the composition of FA methyl esters was determined by gas chromatography.

The seminal plasma and spermatozoa of boars were dominated by saturated fatty acids, and the most common was palmitic acid (C16). Monosaturated fatty acids were the second most represented, with oleic acid (C18:1c11) in the highest percentage. The least represented were polyunsaturated fatty acids with α-linoleic acid (C18:3n3) in seminal plasma, and linoleic acid (C18:2c/t) in spermatozoa as the most common. The fatty acid compositions in seminal plasma showed no significant differences between breeds ($P>0.05$). In the German Landrace subpopulation of live spermatozoa a higher percentage of C14 was determined in comparison to the Pietrain boars ($P<0.05$). Also, a lower percentage of C18:3n3 was measured in the population of dead German Landrace spermatozoa ($P<0.05$). The subpopulation of live spermatozoa in the Pietrain breed showed a higher percentage of C16 and C18, and a lower percentage of C16:1c and C17 FA, compared with the dead spermatozoa ($P<0.05$). The subpopulation of live German Landrace spermatozoa showed a higher percentage of C16, C18, C18:3n3 and C24:1 compared to the dead spermatozoa ($P<0.05$). Furthermore, in live spermatozoa a lower percentage of C16:1, C18:2, C20:1, C20:4n6 was determined compared to the dead spermatozoa ($P<0.05$).

On the basis of these results it may be concluded that lower sperm motility and thus fertility competence may be caused among other things by the composition of fatty acids.

FASCIOLOSIS IN ALBANIA

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This study includes the results from several years (1990-2017) of fecal examination, carcass inspection and environmental observation. The main infected species are cattle and sheep, rarely goats, and infection may be occasionally encountered in dogs and humans. The main parasite in Albania was shown to be *Fasciola hepatica,* and in very rare cases *Fasciola gigantica* (0.1%). No other species of Fasciolidae were observed. According to fecal examination a 90-100% infestation rates was observed in sheep and 40-60% infestation rates in cattle. The seasonal infestation rates vary from 20-100% in the animals tested. Long term data from slaughterhouses reveal an infestation rate of 30 to 50 % of slaughtered animals.

The prevalence rate of fasciolosis increases greatly in years with high humidity, especially when there are high levels of rainfall during the summer. In sheep, an egg count of 50 eggs/g feces in 1/3 to 1/2 of the flock is considered to be an alarm signal, 80-150 eggs/g feces in ½ of the flock is considered serious and 200-500 eggs in ½ of the flock is considered severe and very severe, if the load is over 500 eggs/g of feces in more than ½ of the flock. Lambs were negative for egg count until 6 months of age, but within 10 months of age 1/3 of the lambs will be positive for egg counts in feces, and at 1 year of age 2/3 of the lambs will be positive. Cattle in Albania are considered at risk when a 50-60 egg count per gram of feces is observed. Fasciolosis in Albania is usually controlled by treating animals twice a year. The first treatment is done from 1-15 March and the second one from 15-30 October.

RUMINANTS' ENVIRONMENTAL ENRICHMENT

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Environmental enrichment usually refers to modification of the animals' environment, thus improving biological function by increasing the number and range of the type of normal behaviour, preventing the occurrence and development, the frequency and severity of abnormal behaviour, or increasing the available space and reducing stress.

"Social" enrichment involves contact with individuals of the same species or humans. "Occupational" enrichment challenges animals and gives them an opportunity to experience control, as well as encouraging them to exercise; "Physical" enrichment involves changing the size or complexity of the animal's enclosure, or adding objects or substrates to the structure; "Sensory" enrichment implies visual, auditory, or other forms of enrichment (olfactory, tactile, tastes) and "Nutritional" enrichment involves giving new, different types of food, or changing the method of food delivery.

Poor environment, qualitatively and quantitatively inadequate nutrition and lack of movement may be triggers for behavioral changes.

In cattle, tongue rolling, licking and biting the stall equipment, non-nutritional suckling and extensive urine drinking may occur. Cattle should preferably be provided with visual or auditory contact with a conspecific or human, daily pasture, different scratching roller brushes, etc.

The abnormal behaviour of sheep could be wool-sucking, intersucking, and self-sucking, and in goats mostly self-sucking. It is not recommended to keep sheep and goats separate from the herd. Environmental enrichment for sheep could be provided by hanging ceiling chains over the pens, as well as by giving them a basketball ball or increasing the amount of fiber food. Goats prefer different types of objects that can be climbed on.

Objects for enrichment should not be dangerous to the animal (poisonous, sharp edges, etc.) and should be in accordance with the environmental enrichment strategies, based on an understanding of the highly specific behaviour and physiology of each species.

QUALITY OF HONEY BEE (*APIS MELLIFERA CARNICA*) ATTENDANTS IN TRANSPORT QUEEN CAGES AND *NOSEMA* SPP. PREVALENCE

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Queen breeders insert the mated queen and a few young worker bee attendants from the mating nucleus into the queen transport cages, with 6 or 7 workers on average. The workers feed the queen in the cage until the introduction to the new honey bee colony. This is a critical time when a young mated queen must survive the transport from the queen breeder to the beekeeper. The queen is fed quality food by the attendants through secretion of the food glands, and the bees are fed candy of differing variety inserted into the transport cage. The bees should not be infested by spores of *Nosema* spp., the microsporidum that can be transmitted to the young queen already in the nucleus.

In our experiment we tested the survival of the young bees in the queen cage, feeding them different types of sugar candy, and measured the size of their food glands. The bees and transport cages were weighed and the candy analysed during the experiment. At the end of the trial the bees were individually examined for the presence and number of *Nosema* spp. spores. The importance of *Nosema* free workers and the size of the food glands is discussed.

ANALYSIS OF SERUM ENZYMES IN *DICROCELIUM DENDRITICUM* INFECTED SHEEP

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Dicrocelium dendriticum, also known as lancet liver fluke, is a parasite that infects the liver of various mammals. It has worldwide distribution and it causes significant liver damage and weight loss. The liver plays a central role in the physiology of the body, and it performs over 500 functions. A healthy liver is important for the health of flocks and herds. Liver diseases are an important segmentt of diseases in the veterinary domain. Among other things, liver pathologies can cause animal production decline and shorten the productive life of animals. A specific characteristic of liver diseases is the fact that in most cases they are not manifested clinically, and the consequences for animal production resulting from liver damage are great. The studies on sheep liver pathological processes in Albania, were grouped as follows: processes with weak clinical signs; processes caused by pathogens (parasites, viruses or bacteria); and others (metabolically based). Clarification of these could be a concrete step forward for veterinary practicianers in combating liver pathologies.

This study provides evidence of parasite presence in sheep livers and biochemical values of 39 sera samples in naturally infected sheep. From 224 sheep examined, 39 of them were infected with *D. dendriticum*. Infection was proved by macroscopic examination of the livers at slaughterhouses, and through microscopic examination in the laboratory. By both examinations the presence of minor or large fibrosis around the bile ducts was noticed. The biochemical indicators analyzed in this study were alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, γ-glutamyl transferase and lactate dehydrogenase. The results showed different values compared with the reference values, but lactate dehydrogenase (LDH) had a significantly higher value (702.92 U/l).

As a conclusion of this study, we underline the fact that the sheep liver is affected by many pathological processes, especially those of parasitic character. Changes in hepatic enzyme level can serve to monitor the progress of parasitic infections. The activities of hepatic enzymes, and especially LDH, are reliable indicators of liver damage and liver parasitosis severity in naturally infected sheep, and constitute an important diagnostic tool in establishing the official diagnosis and effective treatment process.

VENTRAL ABDOMINAL HERNIAS IN BIRDS; DIAGNOSTIC AND SURGICAL REPAIR – REVIEW PAPER

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An abdominal hernia is a split in the abdominal wall muscles which ends with prolapse of abdominal organs. Patients are usually presented with a painless, soft, reducible swelling in the ventral abdominal region, near the cloaca, and lethargy.

Abdominal hernias can be congenital or acquired and are more frequent in female birds. The etiology of hernias in birds is related to hyperestrogenism, obesity, trauma, egg laying or masses in the abdominal cavity which cause a lack of space for other organs. Therefore, the abdominal muscle often stretches and becomes thin, and the end result is rupture of the abdominal wall. Ventral abdominal hernias occur most in middle-aged or old female birds. Some cases may not be true hernias, but only an extended abdominal wall due to malnutrition, lack of exercise or other reasons.

Blood profiling is useful and x-rays or ultrasound are of particular value in diagnosing a hernia. Contrast x-ray is a particularly useful diagnostic tool. In some cases, surgery can be avoided with a change of diet and weight loss. A second option includes general anaesthesia and surgical repair, which is very stressful for birds.

Surgical repair of hernia includes ventral midline celiotomy and herniorraphy. This approach provides access to both sides of the coelomic cavity. After skin incision, the abdominal cavity is opened, cutting the linea alba over the hernial sack. The contents of the hernia are usually small intestine loops, liver or fatty tissue. Corrective surgery includes returning the organs into the abdominal cavity and suturing the abdominal wall with a simple interrupted suture pattern, in two layers.

The postoperative period includes administration of antibiotics and non-steroide anti-inflammatory drugs for several days. Also a diet change is necessary. After approximately four weeks the birds are fully recovered with no further problems.

BACTERIAL AND FUNGAL FLORA OF WESTERN CASPIAN TURTLES (*MAUREMYS RIVULATA*)

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The Western Caspian turtle (*Mauremys rivulata*), also known as the Balkan pond turtle, from the family *Geoemydidae*, is a turtle species spread throughout the world. Due to habitat loss and competition with other turtle species, the western Caspian turtle is one of the most endangered and rarest reptile species in Croatia. Since information about its health and pathogens which may drive this species even closer to extinction is very scarce, we have examined and swabbed a group of animals confiscated at the Croatia-Bosnia & Herzegovina border, and housed in the quarantine facility of Zagreb Zoo.

Oral cavity and cloacal swabs were taken from six animals after manual restraint, and the samples were plated on different non-selective and selective agars, and incubated under aerobic conditions at 37°C for 24 hrs for detection of aerobic bacteria. Cloacal swabs were additionally enriched with Selenite cysteine broth for *Salmonella* detection, while the cloacal swabs for *Campylobacter* detection were plated on modified Charchoal Cefoperazone Deoxycholate (mCCDA) Agar, and incubated under microaerophylic conditions for 48 hrs. For fungal detection, oral cavity and cloacal swabs were plated on Sabouraud dextrose agar, and incubated at room temperature for five days.

In total, seven bacterial species were isolated from the oral cavity, with one isolate from each animal. Eight bacterial species were isolated from the cloaca, with the predominance of *Escherichia coli*, isolated from all tested animals. All the animals were negative to *Salmonella* spp., while one animal was suspected for *Campylobacter* spp. so that further tests are under way. Regarding fungal isolates, only Asp*ergillus fumigatus* was isolated from one cloacal sample. The results obtained are similar to those previously reported for bacteria and fungi in reptiles. Due to the small sample size and scarce information for this particular species, further research is needed.

DIVERSE MORPHOLOGY OF CHELONIAN BLOOD CELLS

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The morphology of blood cells, particularly erythrocytes and leukocytes, differs among the same order of reptiles, and even within the same species. Understanding this diversity is crucial for laboratory evaluation of blood films in reptiles contributing to appropriate diagnosis and therapy. The purpose of this study was to evaluate the diversity of blood cells in some species of turtles.

Archived turtle blood films, stained with Romanowsky stain were evaluated. There were four different species of turtles: Red-eared slider (*Trachemys scripta elegans)*, Western Caspian turtle (*Mauremys rivulata)*, Chinese softshell turtle (*Pelodiscus sinensis)* and Loggerhead sea turtle (*Caretta caretta),* and three blood films from different animals of similar age were used for each species. Differential blood count and evaluation of blood cell morphology were undertaken and the cells were subjectively compared for shape, length, nucleus and colour.

In Loggerhead sea turtles, the reticulocytes contained basophilic stippling and in the erythrocytes there were round, dot-like intracytoplasmic inclusions. Chinese softshell turtles had the largest basophiles with clearly visible granules and two types of thrombocytes, round and elongated. Lymphocytes and azurophils were similar in all the examined species. The greatest diversity of blood cell morphology between species was noted in eosinophils, and the most specific and different from the others were eosinophils of the Loggerhead sea turtles.

Knowing the blood cell morphological diversity in different species of turtles and the specificity for each species is necessary for accurate performance of differential blood count and therefore valid estimation of an animal's health condition.

EVALUATION OF BLOOD PARAMETERS IN WESTERN CASPIAN TURTLE (*MAUREMYS RIVULATA*)

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The Western Caspian turtle (*Mauremys rivulata*) is one of the rarest and most endangered species of reptiles in Croatia. The distribution of this freshwater turtle in Croatia is limited to the narrow southeast coast. The greatest threats for its extinction are the loss of habitats due to the drainage of water areas, the excessive use of pesticides and artificial manure, traffic and smuggling. Through proper animal management, we can contribute to their protection, welfare and health. One of the useful tools in assessing the physiological and health condition of the animals is hematology. Studies on Western Caspian turtle hematology are very scarce and all information on the hematological parameters of this species is valuable for a better understanding, protection and veterinary care.

Six Western Caspian turtles (three male and three female), confiscated at the Croatian-Bosnian border, were situated in the quarantine facility of Zagreb Zoo. Clinical examination and blood sampling were performed on all turtles, and tested for hematological and parasitological analyses. The blood analysis included determination of hematocrit, red blood cell count (RBC), white blood cell count (WBC), differential cell count and morphological assessment of blood cells.

The results showed very low hematocrit (10-20%) and low RBC (0.1-0.42x10^6/μL) levels in all animals. WBC and differential blood cell count varied among the turtles. A large number of degenerative polycromatophiles were found in five turtles as well as an elevated number of immature and changed azurophiles and lymphocytes. The hemoparasite genus *Haemogregarina* was also found in all animals.

In conclusion, all the animals were anemic, with signs of chronic and degenerative impairment of hematopoiesis. These results will benefit further researchers in a better understanding of the physiological and pathophysiological variations of hematological parameters in Western Caspian turtles, and also in turtles in general. Considering the small number of animals in this study, further research on the topic should be undertaken.

METASTASING SCAPULAR OSTEOSARCOMA IN A BROWN BEAR (*URSUS ARCTOS*)

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Osteosarcomas are the most common primary neoplasm of the appendicular skeleton in dogs and cats, but are rare in other animal species. Only a few reports of osteosarcoma in the Ursidae family can be found in the scientific literature. In this study, a case of metastasing scapular osteosarcoma in a brown bear (*Ursus arctos*) is described.

A male, 18-years and 8 month-old brown bear *(Ursus arctos)* born and housed at Zagreb Zoo, developed a swelling in the left scapular region. Clinical diagnosis was trauma. The swelling enlarged gradually. One month later, the animal was submitted for surgery. A subcutaneous mass weighing 11 kg was removed. The mass was composed of coagulated and uncoagulated blood, mixed with small bone fragments. Part of the mass was submitted for histopathological examination, which revealed only a hematoma within the submitted sample. One month after the first surgery, there was local recurrence of the swelling. A second surgery was performed when complete destruction of the scapular bone was noted. Due to the poor prognosis, the bear was euthanized and necropsy performed.

At necropsy, a large friable mass measuring 61x60x22 cm was found in the left scapular and left lateral thoracic region that had completely destroyed the scapular bone. On the cut surface the mass was predominantly brown-red in colour with gray-white, partly friable and partly hard areas. There were cyst-like spaces filled with blood. Similar masses were disseminated throughout the lung. Tissue samples from the described mass and other representative parenchymal organs were submitted for histopathological examination. Histopathological examination revealed osteosarcoma (compound form) of the scapula with metastases to the lung. The histological appearance of the metastases was similar to the primary tumor.

In conclusion, bone tumors are rare in ursids and this is the first recorded case of metastasing osteosarcoma in bears in Croatia.

ORCHIECTOMY OF THE LOWLAND TAPIR (*TAPIRUS TERRESTRIS*) IN FIELD CONDITIONS

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The Lowland tapir (*Tapirus terrestris*), is a large mammal from the order *Perrisodactyla*. Since their population in nature is declining, this species is listed as "Vulnerable" by the IUCN, and captive programmes in Zoos could serve as a good way of species protection in future.

A 14-year-old, 198 kg, intact, otherwise healthy male from the local Zoo was presented for orchiectomy, for population management reasons. Food was withheld for 24h prior to the procedure, and water for 6h. The animal was excluded from the group, but kept in its own habitat to minimize stress.

General anesthesia was accomplished with a combination of detomidine 0.1 mg/kg, butorphanol 0.1 mg/kg and ketamine 0.5 mg/kg, and administered with a blowpipe.

The animal was placed in dorsal recumbence after 7 minutes. The eyes and ears were covered with a towel to minimize the surrounding stimuli. ECG electrodes were placed on the feet for heart rate monitoring. Venous blood samples were drawn from the saphenous vein for complete blood count and serum chemistry.

The surgical area was cleaned with an antiseptic, and covered with sterile drapes. The incision sites and both funiculi were infiltrated with 1% lidocaine. Two parallel incisions were made just cranial to the scrotum.

Orchiectomy was performed with a closed vaginal tunic. Each testis was dissected from the surrounding tissue and excorporated. Circumferential and transfixational ligatures were placed on each funiculus with a resorbable multifilament suture material (Polysorb 1). The surgical wounds were not reconstructed, allowing them to drain and prevent abscess formation.

Antibiotic therapy with benzylpenicillin+streptomycin was continued for two weeks, together with meloxicam 0.5 mg/kg for pain relief. After the procedure, the animal received 3 ml of atipamezol and recovered from anesthesia uneventfully. He began to eat and drink quite soon. In the following two weeks the wounds were washed daily with tap water, and healed by secondary intention.

FATTY ACID COMPOSITION OF DIFFERENT TISSUES OF INDIAN SMALL MONGOOSE (*HERPESTES AUROPUNCTATUS*) IN CROATIA

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Indian small mongoose (*Herpestes auropunctatus)* is an invasive alien species in Croatia. The mongoose was introduced to the Island of Mljet at the beginning of the 20th century as a biological control of the snake population. Today it is present on five islands: Mljet, Korčula, Hvar, Čiovo and Škrdi, and it was also introduced to the Pelješac peninsula from where it started to spread onto the mainland.

Analysis of fatty acid (FA) composition is a technique used to examine the foraging habits of free-ranging animals. The study was conducted on 10 male mongooses legally harvested on the Pelješac peninsula. We collected *m. gluteus superfitialis* (MG), *m. vastus lateralis* (MV), *m. semitendinosus* (MS), liver around *v. portae* (L) and perirenal adipose tissue (PAT). After homogenization and lipid extraction of different tissues, FA composition was determined by gas chromatography. The results show a significantly higher ratio of arachidonic to docosahexaenoic acid (AA/DHA, C20:4n-6/C22:6n-3) found in L in comparison to other tissues, with the exception of MS ($p=0.01$; $p=0.04$; $p=0.01$ for MG, MV and PRM, respectively). A significantly lower percentage of C14:1 was found in the L compared to other tissues ($p=0.03$; $p=0.04$; $p=0.02$; $p=0.03$ for MG, MS, MV and PAT respectively). A significantly higher percentage of AA was found in the L compared to all other tissues ($p=0.01$ for all tissues). We found significant differences in FA composition among different muscle tissues. Percentages of C14:0 and C15:0 were higher in MS compared to both MG and MV ($p=0.01$ for all comparisons). Also, the percentage of C16:1c was lower in MS compared to MG and MV ($p=0.01$). The perirenal adipose tissue had significantly lower percentages of AA and DHA compared to all three muscle tissues ($p=0.01$ for all tissues). Furthermore, PAT had a significantly higher ratio of monounsaturated FA ($p=0.01$; $p=0.01$; $p=0.02$ for MG, MS and MV, respectively), as well as a significantly lower ratio of polyunsaturated FA, in comparison to muscle tissues ($p=0.01$ for all tissues).

It may be concluded that the composition of FAs differs according to the different tissues, probably reflecting different metabolic pathways.

ARTERIAL VASCULATURE OF THE BOTTLENOSE DOLPHIN HEART

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Whales (*Cetacea*) are adapted to life in water. Their blood volume is 2-3 times larger within their body mass compared to terrestrial mammals, and serves as an oxygen reservoir. Bradycardia, peripheral vasoconstriction and redistribution of oxygenated blood into the brain and heart increase the diving ability of whales. The whale heart, as the central organ of the cardiovascular system, has specific anatomical and functional characteristics that maintain the body balance while diving. We studied the anatomical characteristics of the cardiac arteries (a. coronaria dextra, a coronaria sinistra) of a juvenile male bottlenose dolphin (*Tursiops truncatus*) found dead on Hvar in 2017.

The heart was removed from the specimen and washed with water to remove blood clots. Both coronary arteries where injected with a mixture of polyurethane and red pigment in order to improve the visibility of the arteries. The heart was preserved in 4% formaldehyd water solution for 24 hours. The coronary arteries were dissected and their position, course and branches were studied.

The major cardiac arteries were distributed in the heart grooves and followed the basic mammalian pattern. They gave off numerous branches that ramified into vessels for the cardiac atria and interventricular septum. Subepicardial vessels and a network of underlying branches supplied the ventricles. All branches were notably sinuous, which is a distinct cardiac characteristic of several whale species. The interventricular paraconal branch of the left coronary artery formed an unusual subepicardial vascular network at the heart apex, whose function remains unknown. Macroscopic interarterial anastomoses were not observed.

The sinuosity of the coronary arteries in the bottlenose dolphin heart increases the blood storage capacity of the heart, which is necessary during diving.They serve as oxygen reservoirs for the myocardium and should be considered as an adaptation for diving.

HAEMATOLOGICAL AND BIOCHEMICAL VALUES IN CROATIAN FREE-RANGING BROWN BEARS OR HOW TO SAMPLE A BEAR

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Haematological (H) and biochemical (B) values are important to assess the health of animals on individual and population levels, as well as nutritional status, environmental stressors and habitat quality. In Croatia, capturing and radio-collaring of brown bears have been part of brown bear research since 1981. In this study we present the results of H and B values in 13 captured and radio collared brown bears in 2015-2017.

The aim of this study was to assess the health status of the animals by determing H (14 values) and B (20 values) indicators, and to compare them with the available literature. In Gorski kotar, 5 bears (males) were captured and radio-collared as part of the LIFE DINALP BEAR project activities. Eight bears (3 females, 5 males) were captured and radio-collared in the Plitvice Lakes National Park as a part of the Park's bear project activities. Bears were captured using spring activated foot snares, baited with corn, apples and roe deer carcasses, and they were immobilized by a combination of tiletamine and zolazepam hydrochloride with medetomidine hydrochloride. Bears were fitted with GPS-collars with a time (3 years) and remote controlled drop off system. The bears were clinically healthy. The study was conducted on 13 blood samples. Within H values, only the white blood cell count (WBC) was higher than that reported in the available literature (23.2 $x10^9$/L vs. 12.9 $x10^9$/L). Results for 7 B values were higher than those reported in the literature (urea: 7.7mmol/L vs. 0.4-4.0 mmol/L; aspartate aminotransferase, AST: 368 U/L vs. 101 U/L; alanine aminotransferase, ALT: 68 U/L vs. 12-41 U/L; creatinine kinase: 5078 U/L vs. 342 U/L; lactate dehydrogenase: 1893 U/L vs. 421-1060 U/L; α-amylase: 300 U/L vs. 22.6 U/L; lipase: 170 U/L vs. 9-158 U/L). Females (N=3) had a significantly higher number of erythrocytes and AST, ALT and γ-glutamyl transferase activities than males (N=11).

The obtained differences may be due to differences in bear habitats. According to the results, we recommend taking all possible measures to shorten the time from capture by snare until immobilization of the bears.

FIRST IDENTIFICATION OF THE NEMATODES *SETARIA TUNDRA* IN ROE DEER IN CROATIA BY MOLECULAR METHOD

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The genus *Setaria* includes 43 species of parasites that infest the peritoneal cavity of ungulates. Vectors such as mosquitoes (*Culicidae* spp.) and flies (*Haematobia* spp.), transmit the disease through blood infected with microfilaries. Adult parasites are mostly non-pathogenic, and cause mild fibrinous peritonitis. However, the outbreak of epidemic peritonitis in reindeer from Finland emphasizes the necessity to monitor the occurrence of the species *Setaria tundra* in wild ungulates. In Croatia, the last published data about the genus *Setaria* date back to 1958, when *Setaria labiatopapillosa* was identified in roe deer (*Capreolus capreolus*), so the aim of this study was to analyze the presence of parasites from the genus *Setaria* in roe deer in Croatia today. We conducted necropsies on 45 roe deer killed during regular game management activities, and *Setaria* were found in the abdominal cavity of 11 animals (22.2%). For molecular identification part of the cox1 gene was multiplied by PCR and sequenced. The sequences found in this research were identical to *Setaria tundra* sequences deposited in the Gene Bank, so this is the first confirmation of this parasite in Croatia.

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COMPARISON OF FATTY ACID COMPOSITION OF SUBCUTANEOUS ADIPOSE TISSUE OF GRAY WOLF (*CANIS LUPUS*) AND SMALL INDIAN MONGOOSE (*HERPESTES AUROPUNCTATUS*) IN CROATIA

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Analysis of fatty acid (FA) composition is a method often used in research of the foraging habits of free-ranging animals. Depending on the species, many of the FAs contained in adipose tissue are derived directly from the diet.

The objective of this study was to gain a better understanding of the foraging patterns and physiological status of free ranging gray wolves and small Indian mongooses in Croatia. The study was conducted on 44 individual gray wolves (female: n=19; male: n=23; samples collected during the years of legal quota cull, 2009-2011) and 10 male small Indian mongooses (samples collected after legal harvest on the island of Mljet, 2014). After thawing, homogenization and lipid extraction, the FA composition was determined by gas chromatography. The subcutaneous adipose tissue (SAT) of both species is dominated by FAs with one double bond (38.6% in gray wolf vs. 49.3% in mongoose). Saturated fatty acids constituted 38.4% in gray wolf vs. 38.6% in mongoose. Polyunsaturated fatty acids were found in 7.1% in gray wolf vs. 12.1% in mongoose. We found significantly higher percentages of C16:1c, C18:1t6, C18:1t9, C18:1t11, C20:4n-6, MUFA and PUFA in the SAT of the gray wolf compared to the SAT of the mongoose. Furthermore, we found that gender had a significant effect on the FA composition of the SAT of the two carnivores. Higher percentages of C16:1c, C18:1t9 and MUFA were found in the SAT of the gray wolf males compared to mongoose samples.

The FAs of the SAT of the gray wolf and mongoose showed significant gender and species related differences. Similarities in the FAs of gray wolf and mongoose are derived from the phylogeny of carnivores, while diferences are the result of different physiological status and foraging patterns.

MONITORING HEALTH STATUS IN A GROUP OF CAPTIVE NUTRIAS (*MYOCASTOR COYPUS*)

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The nutria (*Myocastor coypus*) is a semiaquatic, omnivorous mammal. It belongs to the order *Rodentia* and is the only member of the family *Myocastoridae*. This species is native to South America but has been introduced to North America, Europe, Africa and Asia. Owing to the increasing population and the fact that these animals cause damage to water control structures, crops, and marsh systems, they are considered pests, and in some habitats are subject to eradication measures. Nutrias have been introduced to Croatia, especially in the area of the Danube basin where they cause economic damage and significantly affect water ecosystems.

To learn more about the health status of captive animals and their potential to spread disease, we clinically examined and swabbed 15 captive nutrias of different ages and genders from Zagreb Zoo. After sedation prior to orchiectomy, animals were weighed and clinically examined, with special attention to oral cavity inspection. Ear swabs were taken for standard microbiology and external parasite detection, nostril swabs for *Mycoplasma* spp., and anal swabs for standard microbiology. The animals were then microchipped and the males were orchiectomized.

The examination revealed no deviation from normal in most of the animals. Two animals were anorexic, with probable bite wounds. In two animals with excessive incisor growth the teeth were corrected. All the animals were negative for *Mycoplasma* spp., *Salmonella* spp., and *Campylobacter* spp. The ears of all the animals tested negative to *Malassezia* spp., *Pseudomonas aeruginosae* and ear mites. The most frequent bacteria isolated were *Staphylococcus sciuri* from ears and *Escherichia coli* from anal swabs.

These results show that captive nutrias are not a significant source of disease, and that good housing conditions allow them to be kept in a good and healthy condition.

TOTAL ORAL/DENTAL TREATMENT IN A SERVAL (*LEPTAILURUS SERVAL*) - CASE REPORT

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The serval is a slender, medium-sized cat that weighs 9–18 kg. Their lifespan ranges up to 21 years in captivity. A 14-year old, intact male serval was presented with a history of inappetence, lethargy and weight loss. The serval was premedicated with an intramuscular application of medetomidine 37 µg/kg, ketamine 6 mg/kg and butorphanol 0.2 mg/kg. A complete physical examination revealed cachexia, hypersalivation and a mandibular fistula.

Clinical and radiological changes in the form of oral lesions demonstrated involvement of bone and teeth, and included periodontitis, bone expansion, tooth resorption and osteomyelitic changes. Radiography showed Stage 3 (PD3) periodontal disease with involvement of both mandibular canines. A mandibular fistula with purulent discharge was present on the right canine tooth. Stage 2 (PD2) periodontal disease was found on the 2nd right mandibular premolar and the 4th right maxillar premolar.

Samples were taken for complete blood count (CBC) and serum chemistry through a catheter in the cephalic vein. While the CBC was unremarkable, blood chemistry showed elevated blood glucose (14.1 mmol/L), blood urea nitrogen (75.8 mmol/L), creatinine (1080 µmol/L), ALT (155 U/L) and CPK (1379 U/L).

The animal was intubated. General anaesthesia was maintained with 2% sevoflurane. Monitoring included electrocardiography, pulse oximetry, oesophageal temperature, non-invasive blood pressure measurement and capnography.

A complete oral exam was performed with a periodontal probe. Lidocaine was used for infraorbital and mental blocks. An ultrasonic probe was used for dental scaling. Open method tooth extraction was performed on all affected teeth with mucoperiostal flaps used to reconstruct the gingival integrity.

Postoperative analgesia included carprofen and a fentanyl patch. Clindamycine was administered i/v during the procedure and continued perorally for 14 days.

This is the first report of a serval that underwent a complete oral hygiene and dental treatment procedure.

POSTOVULATORY EGG RETENTION (*DYSTOCIA*) IN BEARDED DRAGON (*POGONA VITTICEPS*) – A CASE REPORT

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Postovulatory egg retention (egg binding/dystocia) is one of the most common reproductive disorders in captive female lizards, resulting in difficulties or complete inability of oviposition. Mechanical obstruction of the oviduct, large number of eggs or insufficient calcification of eggshell and *Metabolic Bone Disease* are the most common causes of egg retention in reptiles. A 2 year-old bearded dragon (*Pogona vitticeps*), weighing 300g was admitted to the Faculty Clinic due to interrupted oviposition. Four days before admission, the lizard layed one egg with an insufficiently calcified eggshell. From that time the lizard had been lethargic, without appetite and had been digging holes in substrate of terrarium. The animal is kept in adequate conditions. The owners did not add calcium supplement to its diet, so that may be a possible reason for unsuccessful oviposition. During clinical examination, hard consistency formations were palpable on ventral side of the distended celom. The lizard did not respond to pain during palpation. Ultrasound and X - ray findings confirmed the presence of 14 calcified eggs in the caudal region of the celom. There was no presence of free fluid in the coelomic cavity. Due to the fact that the eggs were well calcified, the diagnosis suggested post-ovulatory dystocia. Therapy included rehydration and stabilization of the patient (warm Ringer lactat+ 5% glucose 2% body weight s/c) and induction of oviposition. Calcium borogluconate at a dose of 35 mg/kg was administrated i/m. After 30 minutes, a second dose of Ca-borogluconate (50mg/kg i/m) was administrated and 5 IU of oxytocin was given i/m. The animal was released from the Clinic to proceed with oviposition in her own terrarium. Within the next 20 hours the patient laid 14 calcified eggs. On X-ray follow up 24 hours after therapy, no presence of retained eggs were observed. The owners were advised that the current diet should be supplemented with calcium in the prescribed daily dose, to prevent future dystocia.

RADIOLOGICAL FINDINGS OF MUCOPOLYSACCHARIDOSIS IN DOGS AND CATS

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Mucopolysaccharidosis is a rare autosomal-recessive genetic disease, characterized by deficiencies of lysosomal enzymes, leading to accumulation of glycoaminoglycans (GAG) in many tissues, resulting in distorted growth or function. Storage diseases may, however, occur in any breed, and new mutations may be identified at any time, so the appearance of clinical signs in an unusual breed should not preclude diagnosis. Clinical signs are closely related to the type of missing enzymes and the tissues where GAG deposition occurs. This disease affects many body organs, but preferentially affects the nervous, musculoskeletal, cardiovascular and respiratory systems, eyes or liver. Of cats, the most likely breeds to be affected are the Domestic Short Hair, Somali, Siamese, Persian, Burmese and Korat, and of dogs the Alaskan Malamute, Field Spaniel, Komondor, Mastiff, Miniature Poodle, Pinscher and Schnauzer, German Shepherd and Labrador Retriever. Radiological changes in patients with mucopolysaccharidosis are observed throughout the locomtor system. The generalized epiphysal dysplasia of long bone and vertebrae is a typical finding. The delayed or incomplete mineralization of cartilage epiphysis is a very common radiological finding. The bone regions of the epiphys are smaller than normal and have non-uniform opacities with a granular appearance. Morphological changes may occur on the maxillar and frontal bones. The vertebral bodies appear cuboid, are shorter than usual, and the articulate processes are misformed. As a result of epiphysal malformations, progressive degenerative joint swelling occurs. Subluxation or luxation of the coxofemoral joint may result from the remodeling of the femoral head epiphysis. The affected animals could be osteopenic. Through radiological modalities, it is possible to discover specific findings that enable the veterinarian to recommend more specific diagnostic tests.

LC-MS/MS ANALYSIS OF DOG SERUM PHOSPHOPROTEOME REVEALS NOVEL PHOSPHORYLATION SITES AND DIFFERENTIAL PHOSPHOPROTEIN PATTERNS IN BABESIOSIS CAUSED BY *B. CANIS*

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Phosphorylation is the most commonly studied protein post-translational modification (PTM) in biological systems, due to its importance in controlling division, survival, cell growth etc. Over the last few years, many large-scale profiling studies of phosphoproteins and mapping of phosphorylation sites of proteins from human and animal cell lines or tissues have been published. Nevertheless, there is little information on general phosphoproteomic characterization and description of the content of the circulating phosphoproteins (present in serum, plasma and other biofluids). We performed a gel-free analysis of dog serum samples using LC-MS/MS, supported by phosphopeptide enrichment. We compared serum from healthy dogs and dogs affected by *B. canis*-caused babesiosis. After digestion, phosphopeptides present in the samples were enriched using TiO_2 magnetic beads. Our approach was both qualitative and quantitative, using TMT-labeled serum pool samples in combination with LC-MS/MS. The results show a moderate number of phosphorylated proteins (45-50), with around 85 phosphorylation sites, many of which have homologous phosphorylation sites in databases for other animal species, but have never been published for dogs. 75.5 % of the detected phosphorylated sites were phosphoserine, 15.9 % were phosphothreonine and only 8.6 % were phosphotyrosine residues. We detected a higher number (300-400) of non-phosphorylated peptides in enriched samples, although a high percentage of them are considered to be phosphoproteins in the databases. Quantitative analysis of pools containing 10 control samples vs. 10 babesiosis samples confirmed the differences observed for individual samples. Differences in phosphorylation of proteins involved in blood coagulation pathways could reflect the adaptation of hemostasis to thrombocytopenia and the variable degree of hemolysis observed in babesiosis. This study represents the first characterization of phosphorylation site mapping of dog biofluids.

MASTICATORY MUSCLE MYOSITIS IN MALTESE

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Masticatory muscle myositis (MMM) is an inflammatory myopathy in which patients most commonly present with jaw pain, trismus, and swelling or atrophy of the masticatory muscles, with subsequently lower food intake and weight loss. The average age of onset for MMM is 3 years of age, and large canine breeds are mainly affected. Dogs generally demonstrate no other neurological or physical abnormalities, which may help differentiation from other causes of trismus.

It is an autoimmune process in which circulating antibodies specifically target the masticatory muscles. It may be an acute or, more commonly, a chronic disease. Complete physical and neurologic examinations are important to confirm that the clinical signs are restricted to the masticatory muscles. MMM requires early detection and immunosuppressive therapy to improve the prognosis.

A 9 year-old neutered male Maltese dog was presented for evaluation of a 4-month history of inability to open the mouth. Trias was within normal ranges. The jaws were painless. Neurological examination excluded trigeminal nerve paralysis and central neurological lesions. Laboratory findings revealed eosinophylia, elevated serum globulins, and creatine kinase (CK). Radiographs of the temporomandibular joints (TMJ) excluded ankylosis, osteoarthritis, luxation and jaw fracture or neoplasia.

The basic diagnostic tools are radiography and ELISA, which is highly specific (100%) and sensitive (85- 90%). Biopsy, MRI, and CT imaging are useful, but require general anaesthesia. Biopsed masticatory muscle submitted for histopathology may reveal lymphocitic-plasmocytic cellular infiltrats, muscle atrophy and, in the end stage, fibrosis.

The only treatment is an immunosupresive dose of corticosteroids. The chronic phase of the disease has a more uncertain prognosis. Extensive fibrosis results in persistent jaw dysfunction. Possible complications are cahexia and lingual venous congestion, which could be life threatening, and exophtalmos and strabismus.

FOUNDING THE ANIMAL EYE BANK AT THE FACULTY OF VETERINARY MEDICINE OF THE UNIVERSITY OF ZAGREB

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The cornea covers the iris, pupil and anterior chamber. It is one of transparent parts of the eye. Transparency, avascularity, corneal thickness, polymegathism, pleomorphism, the presence of immature immune cells and its immune privilege make the cornea an organ of great interest for veterinary medicine. Therefore, the main purpose of this paper was to determine the morphological characteristics of the cat cornea in order to standardize a method of hypothermic corneal storage. This would help found an Animal Eye Bank to ensure the availability of cornea tissue for corneosclerotic reconstruction after eye trauma, or for other pathological conditions.

The research is financed within a short-term financial programme supported by the University of Zagreb for scientific research in 2017 entitled "Quality Control of Donor Cornea Before and After Storage in an Animal Eye Bank". Corneas were collected within 24 hours of death from three cats euthanized for medically justified reasons, or that died of a trauma at the Surgery of the Orthopaedics and Ophthalmology Clinic, Faculty of Veterinary Medicine, University of Zagreb. Morphology of the corneas was studied according to differential histological methods (staining with alizarin red, and haematoxylin and eosin stain). The cornea layers were studied under a stereomicroscope and a light microscope. The quality of the layers and cornea thicknesses were observed. The corneal endothelium was studied for its thickness, transparency and density under the light microscope.

The results will contribute to comparative morphology studies of the cornea and the eye in general. The special importance of our research is the development of methods of hypothermic storage of corneoscleral tissue, which is important for penetrating keratoplasty and corneoscleral reconstruction in veterinary medicine.

DERMATHOPHYTOSIS IN CLINICALLY SUSPECTED DOGS DURING 2014 - 2015 IN SPLIT

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Dermatophytes constitute a group of taxonomically related filamentous fungi, which are able to colonize the keratinized tissues (skin, fur and nails) in humans and animals, thus causing dermatophytic lesions, popularly known as ringworm. They have an enzymatic system which is essential to metabolize the keratin, hence using it as an energy source. Dermatophytes belong to three genera: *Microsporum, Trichophyton* and *Epidermophyton*. Visible skin lesions are variable and do not necessarily form a ring. Often hair loss is observed, usually in small patches at first, but this might progress to pruritus with scratching lesions. Dermatophytes are significant due to their zoonotic potential and the concern of pets owners for their own health. Pet animals usually acquire infection from the soil or from direct contact with infected animals. The incidence of human to animal transmission has not been reported. The present work aimed to evaluate the infection rate of dermatophytosis in dogs from the Split city area. Samples were collected from January 2014 to December 2015. Skin scrapings and fur samples were collected from 154 dogs (different breeds, ages and clinical conditions) with characteristic skin lesions. The samples were recieved and evaluated by the Croatian Veterinary Institute, Split Department, for the presence of dermatophytes. Each sample was inoculated and cultured on Sabouraud Dextrose Cycloheximide agar (SDA) and on Dermatophyte test medium (DTM). The inoculated plates were incubated at 25°C and examined daily for 21 days. Dermatophytes were isolated in 30 dogs (19.48%). Infection with *Microsporum* spp. was observed in 25 out of 30 positive samples (83.33%), whereas infection with *Trichophyton* spp. was observed in 5 out of 30 positive samples (16.67%). Out of 154 dogs with various skin lesions, suspected dermatophytosis, 30 (19.48%) cases were confirmed. Due to the risk of transmission to other animals and humans, it is important to screen periodically for dermatophytosis in order to fully understand the epidemiology of this disease. Confirmed cases and screening can also help to establish future preventive measures and strategies for control.

ANALYTICAL VALIDATION OF CANINE KIDNEY INJURY MOLECULE-1 (KIM-1) IMMUNOASSAY IN URINE SAMPLES

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The kidney injury molecule-1 (KIM-1) is a novel biomarker for prediction, diagnosis and prognosis of acute kidney injury. The aim of this study was to evaluate the commercially available canine-specific KIM-1 ELISA (Immunology Consultants Laboratory, Inc., Portland, USA) for use in canine urine samples.

For assay precision, two pools of urine with different concentrations of analyte, corresponding to low and high ranges of the assay, were prepared from samples obtained from dogs. The intra-assay coefficient of variation (CV) was calculated, after analysis of the high and low pool, six times in a single assay run. Inter-assay CV was determined by analysing the same sample in five separate runs, carried out on different days. Two samples with different amounts of analyte were mixed in different ratios to perform a spiking recovery test. Test recovery, expressed as a percentage, was calculated for each dilution, for comparison of the expected versus the measured concentrations. To investigate the effects of haemolysis, lipiduria and bilirubinuria, the canine pools were mixed with different concentrations of haemoglobin, lipid or bilirubin solution.

Intra-and inter-assay coefficients of variation (CV) for the assay were both below 10%, i.e. for the low range pool CV was 9.98% (mean ± SD: 12,03 ± 1,2 ng/ml); for the high range pool 8.83% (62.57 ± 5.53 ng/ml); and for the inter-assay 8.39% (13.81 ± 1.16 ng/ml). The recovery between the observed and expected concentrations ranged from 94 – 108%, with a mean of 101%. The different degrees of bilirubinuria tested in this study did not affect the measured concentrations of KIM-1 in the canine urine samples, while a significant increase in KIM-1 concentrations was found in the presence of severe haemolysis (> 5 g/l), and also in marked lipiduria (triglycerides > 5 g/l).

This method exhibited acceptable analytical characteristics, allowing its use in the laboratory with adequate precision and recovery, with the exception of highly haemolysed and/or lipuric urine samples.

URINARY IMMUNOGLOBULIN G AND RETINOL BINDING PROTEIN AS BIOMARKERS OF RENAL DYSFUNCTION IN CANINE BABESIOSIS

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Renal dysfunction is often identified in canine babesiosis as minimal renal damage, but acute renal failure may also occur. The aim of this study was to assess the renal damage in dogs with babesiosis, using urinary markers for glomerular (immunoglobulin G, IgG) and proximal tubular dysfunction (retinol binding protein, RBP).

In the study, 42 dogs naturally infected with Babesia canis and 14 healthy dogs were included. The dogs with babesiosis were divided into 3 groups: the first group consisted of 9 non-azotemic dogs (serum creatinine < 140 μmol/L) with a normal urine protein to creatinine ratio (UPCR < 0.5), the second group of 27 non-azotemic dogs with UPCR > 0.5 and the third group of 6 azotemic dogs (serum creatinine > 140 μmol/L) with UPCR > 0.5. The urinary concentrations of IgG and RBP were measured by ELISA assays (ICL, Portland, USA) previously validated for use in canine urine. Statistical analysis was performed using GraphPad Prism 5 and the differences between healthy and diseased dogs were assessed by the Kruskal-Wallis test, with a P-value < 0.05 considered as statistically significant.

Concentrations of both urinary IgG and RBP differed significantly among these four groups (P < 0.0001). For IgG, concentrations were significantly higher in the second (median, Q1-Q3 range: 129.9 μg/ml, 60 – 238.7 μg/ml) and third groups (181.4 μg/ml, 19.23 – 495.9 μg/ml) compared to the healthy dogs (0.57 μg/ml, 0.49 – 1.01 μg/ml), as well as in the second group compared to the first group (10.05 μg/ml, 3.73 – 18.98 μg/ml). Similarly, RBP concentrations were significantly higher in the second group (312.5 ng/ml, 276.9 – 367.5 ng/ml) and the third group (275.4 ng/ml, 196.5 – 325.9 ng/ml) compared to the healthy dogs (13.19 ng/ml, 8.43 – 29.25 ng/ml), as well as in the second group compared to the first group (47.16 ng/ml, 23.79 – 128.2 ng/ml).

These findings indicate the utility of urinary RBP and IgG in the assessment of the level and location of renal damage in canine babesiosis.

EVALUATION OF RECOVERY AFTER CRANIAL CRUCIATE LIGAMENT RUPTURE SURGERY IN 60 DOGS – USING A QUESTIONNAIRE AND CLINICAL EXAMINATION

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Cranial cruciate ligament rupture is the most common orthopedic problem and cause of lameness in dogs. In order to examine the success of recovery after surgical treatment for this orthopedic problem, we compared two operating methods: cranial tibial tuberosity transposition and lateral suture method. Participants were divided into 4 groups, each containing 15 dogs of different ages, sex and breed. The first group consisted of dogs with one stifle joint treated by the modified retinacular imbrication technique (MRIT) method, while the contralateral leg was intact. The second group consisted of dogs with one stifle joint subjected to the tibial tuberosity advancement (TTA) method and the other leg was intact. The third group included dogs with bilateral rupture where one stifle joint was treated with MRIT and the other with TTA. The fourth group included dogs diagnosed with a fresh, untreated rupture.

By conducting a survey among the owners of the first three groups we obtained their subjective impressions and assessments of the recovery of their pets after surgery. Their observations were compared with the clinical examination we performed of each patient. Over 90% of owners graded the outcome as excellent and very good. The results of the measured flexions showed that there was a statistically significant difference between the intact knee and the knee subjected to MRIT. In the TTA group the difference in stifle circumference was significant when compared with the intact knee. Clinical examination showed that the function of the operated stifle joint was not significantly decreased compared to the healthy stifle joint. Treated stifles in all groups had radiograph signs of some progression of osteoarthritis.

We established that the assessment of the owners did not differ notably from the findings of the clinical examination, and that there is no significant difference in the success of recovery between the two specified surgical methods. The questionnaire proved to be a good method for long-term outcome assessement.

IDENTIFICATION OF POTENTIAL SERUM BIOMARKERS FOR KIDNEY DYSFUNCTION IN CANINE BABESIOSIS BY LABEL-BASED QUANTITATIVE PROTEOMIC APPROACH

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Proteomic technology is a promising tool for the identification of diagnostic biomarkers. Clinical proteomics mostly focus on body fluid proteome studies because of the non-invasive collection procedure. Furthermore, blood contains tissue leakage proteins which could indicate different pathological processes. These potential biomarkers are present in low concentrations, making the necessity for depletion of high abundant serum proteins. The aim of this study was to employ high-resolution quantitative shotgun proteomics using Tandem Mass Tag (TMT) isobaric labeling for proteomic profiling of depleted canine serum samples in order to gain an insight into the pathophysiology of kidney dysfunction in babesiosis.

Serum samples from 8 healthy dogs, 6 *Babesia*-infected dogs (UPC <0.5) and 6 azotemic dogs with babesiosis (serum creatinine >140 µmol/L) were pooled, subsequently. After depletion using protein equalization technology (ProteoMiner), the samples were subjected to desalting, reduction, alkylation, digestion and TMT labeling. The labelled peptides were analyzed using nanoLC System and Q Exactive Plus mass spectrometer. Identification and relative quantification were performed using a Proteome Discoverer. Cytoscape and DAVID were used for functional annotation analyses.

As a result, 49 proteins showed a statistically significant difference ($p<0.05$) in deregulation with a log2 fold change more than ±0.5 in azotemic *Babesia*-infected v. healthy dogs. Deregulated proteins are involved in calcium ion and acute phase responses, myofibril assembly, proteasomal ubiquitin-independent degradation and interleukin-8 production. Furthermore, some of deregulated proteins in the azotemic *Babesia*-infected dogs revealed kidney injury when compared to *Babesia*-infected dogs.

In conclusion, shotgun TMT-based high-resolution proteomic profiling, combined with protein equalization technology, allowed identification of proteins that could present potential serum biomarkers for diagnosis of kidney dysfunction in babesiosis.

RADIOGRAPHIC AND PATHOLOGIC PATTERN OF CANINE PRIMARY LUNG TUMORS

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Primary lung tumors (PLT) are relatively rare tumors in dogs. They usually occur as a solitary mass in the lungs of older dogs. The aim of this study was to determine the presence of PLT and to compare radiographic with pathological findings.

Five mixed breed dogs and one Bernese Mountain Dog were necropsied at the Veterinary Faculty, University of Zagreb between July 2015 and September 2016, with the diagnosis of PLT. Gross examination was performed, and tissue was sampled for histopathological examination. Clinical manifestations were mostly a cough and dyspnea, so thoracic radiographic imaging was undertaken, using dorso-ventral and lateral projections.The longest time interval between radiographic imaging and necropsy was two months.

In four cases, necropsy and radiography revealed a neoplasm as a large solitary mass (> 2cm) at the periphery of the caudal lung lobe (3/4), or the cranial lung lobe (1/4). In one case, there was a solitary mass at the hilum of the lung, with no radiographic evidence of the mass due to massive pleural effusion. Also, the diffuse pattern of a tumor was revealed in one dog, and pulmonary metastases were suspected radiographically. Bronchoalveolar carcinoma was the most common diagnosis (3/6). Likewise, papillary adenocarcinoma (1/6) and anaplastic (undifferentiated) carcinoma (1/6) were determined. In one dog, histopathology was not performed due to autolysis.

In conclusion, the later stages of PLT correlate with the presence of clinical signs, and radiographic detection is possible in most cases due to the large size of the tumor. However, gross examination with a histopathological analysis is the diagnostic gold standard for canine PLT.

ESTABLISHMENT OF A DNA-BASED TEST FOR QUICK DIAGNOSTICS OF CANINE BABESIOSIS

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Canine babesiosis is a tick-borne disease caused by the parasite *B. canis canis* (most prevalent in Croatia) and *B. canis vogeli*. Blood smear observation is the routine procedure for diagnostics, but could lead to false negative results and it is unable to discriminate subspecies. A new diagnostic test based on qPCR has been developed.

Two genes were used to set up the PCR: 18SrRNA and HSP70, 18SrRNA being partially available on NCBI. For both *B. canis canis* and *B. canis vogeli* 18SrRNA genes, all sequences (96 and 253 respectively) were assembled to generate the most complete gene sequence. The sequence of HSP70 for both subspecies was already available.

Primers were designed and tested with Primer3. 18SrRNA was used to discriminate the 2 subspecies (92.9% homology), with 2 couples of primers (amplicon lengths of 153 and 163 bp). HSP70 was used to design one couple of primers to detect each *Babesia* genus species (82.7% homology among *Babesia* genus, amplicon length of 164 bp). A patent is still in negotiation for this original work.

First, PCRs specificity was checked on gel electrophoresis, and confirmed on high resolution (0.2°C) qPCRs, which demonstrated that the amplicon melting curves (depending on sequences) are dynamically in accordance with in silico simulations (uMelt). Then, for routine diagnostics qPCR was used to give an estimation of *Babesia* per erythrocyte. Each sample was characterized by the presence/absence of the *Babesia* genus (HSP70), *B. canis canis* (18SrRNA), and *B. canis vogeli* (18SrRNA) with dedicated qPCRs.

So far, 29 samples diagnosed for *B. canis canis* by blood smear have been analysed, 28 were confirmed, and 1 was diagnosed for *B. canis vogeli*. From 8 samples with a negative blood smear, 1 was diagnosed for *B. canis canis*, which was confirmed by a second blood smear.

This diagnostic tool is able to answer veterinarians' needs (fast, specific, cheap). The next step aims to transfer the technique, and we invite veterinarians to use it, through education and presentations.

AGGRESSIVE DOGS: ASSESSMENT AND TREATMENT CONSIDERATIONS

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A dangerous dog may be defined as: one that threatens the safety of people and other animals by showing aggression with little or no provocation. Of the many recognised types of aggression, dominance and fear are the two most likely to be encountered.

In general, animals that are aggressive to their owners pose a greater risk of causing harm. Any assessment of aggression is partly subjective, but consideration of the following will make the examination more objective. A common error is to assume that dominance is the underlying motivation for aggression. True dominance aggression directed toward humans is, in fact, very rare. It is slightly more common toward other dogs, but interdog aggression occurs for many reasons other than dominance issues, and should not be assumed to be the underlying cause of dog fights. Fear is a much more common cause of aggression, especially toward humans. Dogs may also be afraid of other animals or inanimate objects, which can cause confusion and an incorrect diagnosis in some cases. Other possible types of canine aggression include territorial, redirected, sexual, protective, possessive, and predatory aggression. In all cases, it is important to identify the circumstances in which the dog is aggressive, to help diagnose the motivation for aggression. A careful history may reveal that aggression is associated with a specific place or item (e.g., the house, a toy, food). Animals may have more than one type of aggression. The veterinarian should perform a thorough physical examination and conduct tests as indicated by the dog's age, behavior and clinical signs, to rule out medical causes of aggressive behavior, such as pain, infectious disease or neurological disease.

Animals do not bite for no reason, and any good behaviorist can determine what provoked a specific bite. The owners of dogs that have a history of aggression need to be aware of the many types of aggression, and the risks factors that must be considered before initiating treatment.

ACTIVITY OF SALIVARY ENZYMES AND LEVEL OF SALIVARY UREA IN GINGIVITIS OF DOGS

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Clinically relevant salivary inflammatory indicators of gingivitis that have a role in diagnostics, therapy, and prognosis, must have the ability for disease classification and treatment follow-up. To date, no research has been conducted that could determine if salivary enzymatic activity and urea level in dogs can serve as a diagnostic tool for gingivitis. The aim of this study was to measure the activity of salivary enzymes: alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), as well as urea in the saliva, in groups of dogs assigned according to the presence of gingival inflammation.

The study was conducted on 20 otherwise healthy dogs that were divided into two groups according to the clinical presence (G+) or absence of gingivitis (G0, control group). The activity of enzymes ALT, AST, ALP, and the level of urea in the saliva was determined using a VetTest® Chemistry Analyzer (Idexx, USA).

In G+ group we determined the significantly higher activity of enzymes (mean ± SD) (U/L): ALT (630.3 ± 178.9) ($P < 0.01$), AST (544.8 ± 285) ($P < 0.001$), and ALP (62 ± 33.74) ($P < 0.05$) in comparison to the G0 group: ALT (296.2 ± 297.3), AST (150.6 ± 230.2) and ALP (40.91 ± 21.79). On the other hand, the level of urea in saliva (mean ± SD) (mmol/L) did not differ significantly between the two groups ($P = 0.699$); (G0 = 1.895 ± 1.35; G+ = 1.167 ± 0.626).

The results of this preliminary study imply that the activity of the salivary enzymes ALT, AST, and ALP in dogs could serve as a potential diagnostic tool for gingivitis of dogs. Nevertheless, we have to take into account the great variability of salivary enzyme levels, as well as their activity being susceptible to reflecting other pathologies, which could classify them as non-specific indicators that cannot serve as a precise diagnostic method. Further research is warranted to confirm the reliability of these parameters for screening for gingivitis, as well as other periodontal pathologies in dogs.

LACTATE CONCENTRATION CHANGES DURING TREADMILL EXERCISE IN CATS

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Lactate is an indicator of glycolytic activity in skeletal muscles. The degree of lactate increase during exercise is related to the intensity of activity, as well as to the stress caused by exercise.

Sixteen untrained Maine Coon cats, mean age (± SD) 4.0 (± 2.4) years, mean body mass (±SD) 6.4 (± 2.0) kg, participated in the study. For testing purposes, a VEF-Fe protocol was used; it included the initial speed of 0.6 km/h, increased by 0.2 km/h each minute of test duration. It was considered that the cat reached maximum exhaustion when open-mouth panting started and/or the cat showed signs of uncoordinated gait.

The average (±SD) duration of the exercise was 7.8 (± 2.9) min, in which the cats covered an average distance of 0.2 (±0.1) km. The average maximum speed was 2.0 (±0.6) km/h. Blood lactate concentration was determined by a portable Scout analyser (LS, SensLab GmbH, Germany) at 5 measuring points: prior to the exercise, immediately after exercise and 15, 30 and 60 minutes after the exercise. The mean (± SD) concentration prior to the exercise was 1.41 (± 0.67) mmol/L; immediately after the exercise 1.53 (± 0.68) mmol/L; 15 minutes after the exercise 1.25 (± 0.74) mmol/L; 30 minutes after the exercise 0.78 (± 0.36) mmol/L and 60 minutes after the exercise 0.78 (± 0.25) mmol/L. There was a statistically significant ($p<0.05$) difference between lactate concentrations measured both prior to and after the exercise, compared to 30 and 60 minutes after the exercise.

The results of this study indicate that in untrained cats the stress related to either exercise anticipation or exercise itself can result in an increased level of lactate concentration. Further studies in trained cats are necessary to determine the sole effect of exercise on lactate levels.

ECG MONITORING DURING ELECTIVE SURGICAL PROCEDURE IN CAT

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Castration in cats is a short surgical procedure that rarely requires intubation. In young and healthy cats, a combination of injectable anesthetics is recommended. There are several anesthetic cocktails ("kitty magic") that usually include a combination of opioids (butorphanol), sedatives (medetomidine/dexmedetomidine) and dissociative anesthetics (eg. ketamine). Arrhythmia is often present in the anesthetized animal, either from the effect of anesthetic and analgesic, or as a preexisting cardiovascular disease or arrhythmia.

The aim of the study was to determine the incidence of arrhythmia in cats anesthetized by a combination of dexmedetomidine, ketamine and butorphanol anesthetics. The study was conducted on 20 domestic crossbred cats, 10 males and 10 females, with ECG monitoring at 15, 30, 60 and 120 minutes after anesthesia administration. Comparing the ECG of non-sedated and anesthetized animals, a continuous decrease in heart rate was observed of 37% on average. The heart rate decrease was most pronounced 60 minutes after anaesthesia administration. The decrease was the most pronounced in females and males, mainly after 60 minutes of anesthesia administration ($p<0.05$). During anesthesia, respiratory sinus arrhythmia and increased variability of the R-R interval occurred. Sixty minutes after the anesthetic application, the prolongation of the P-R interval was observed, i.e. in 75% of cats there was a first-degree atrioventricular (AV) block, which can be attributed to anaesthetic activity. After 120 minutes from the anesthetic administration, the P-R interval remained extended, but 2nd and 3rd degree AV blocks were not established. There were no differences in the configuration and appearance of the P wave and QRS complex between non-sedated and anesthetized cats.

On the basis of the ECG parameters of anesthetized animals in relation to the parameters of alert, non-sedated animals, we concluded that the combination of butorphanol, dexmedetomidine and ketamine is safe for short surgical procedures (castration in cats, abscess drainage), dental procedures and otoscopic examination in unsociable cats.

CHRONIC ULCERATIVE STOMATITIS IN MALTESE DOG - A CASE REPORT

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Canine chronic ulcerative stomatitis (CCUS) or chronic ulcerative paradental stomatitis is a painful and often debilitating disease, with focal to diffuse ulcerations in mucous membranes, mucosal inflammation and, in some severe cases, mucosal necrosis. Changes usually appear on mucosal surfaces opposite plaque on dentition, lateral surfaces of the tongue, the glossopalatine fold and the mucocutaneous junctions of the lips. A higher prevalence has been noted in middle-aged male castrated dogs especially in Maltese dogs, Cavalier King Charles Spaniels, Labrador Retrievers, Greyhounds and Scottish Terriers.

A five-year-old female Maltese dog was presented for a history of six months of halitosis, ptyalism, bloody saliva and oral pain. Complete clinical examination, complete blood count and serum biochemistry was conducted and revealed mild neutrophilia and leucocytosis. A thorough oral examination, with X-rays under anaesthesia, was performed, with no signs of calculi due to regular dentistry cleaning. Moderate stomatitis with vesicles and ulcerations were observed on the gingiva of the canine teeth, both maxillar premolars and on the lateral aspects of the tongue, with necrosis. Periodontal probing was normal in all teeth (0.5 mm) without signs of periodontitis. Microscopic findings of three mm punch biopsies revealed increased thickness of the epithelial mucosal layer with subepithelial lichenoid infiltrate that mainly consisted of plasma cells, lymphocytes and neutrophils, with multifocal necrosis up to subepithelial area.

CCUS is a disease that is poorly understood and needs a multidisciplinary approach. Differential diagnoses for canine chronic ulcerative stomatitis include autoimmune subepidermal blistering diseases, epitheliotropic T-cell lymphoma, and uremic stomatitis. Biopsy with pathohistological diagnosis is inescapable. Treatment usually consists of systemic glucocorticoids, antibiotics and local antiseptics, with the tendency to relapse.

PRELIMINARY EVALUATION OF IDEXX COMBO SNAP® TEST FOR USAGE ON POSTMORTAL SAMPLES

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Retrovirus infections caused by feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) are common in domestic cats in Croatia. The IDEXX SNAP Combo FeLV Antigen/ FIV Antibody Test® is used in routine diagnostics from whole blood, serum or plasma (clinical samples), but not from cats presented for necropsy. We therefore performed a study to investigate the usefulness of the SNAP test on blood and body fluids obtained postmortally.

Samples were collected from two groups of cats: (A) cats with known status of FeLV/ FIV-N=12; (B) cats without a known history of FeLV/FIV testing-N=19. In group A the results from live cats were compared to the test results from postmortally collected blood. In group B, FeLV/FIV status was determined from the postmortal results of blood samples and compared to results obtained from body fluids including lung juice, ascites, and thoracic or pericardial effusion.

In group A, tests performed during life and after death gave the same result in 67% (8/12) of animals. In four cats from same group the results did not match: i) two cats showed FIV infection on clinical samples and FeLV/FIV coinfection on postmortal samples; ii) a single cat was positive for FeLV and FIV during life, while it was only positive for FeLV after death; iii) a clinical sample from a cat was negative, while postmortal blood was positive for FeLV. In group B, results from postmortal blood matched with body fluids in 94% (16/17) of tested cats. Lung juice from one cat was negative, while postmortal blood showed infection with FeLV.

This study presents preliminary results which indicate that the IDEXX SNAP Combo test could be useful in postmortal diagnostics of FeLV/FIV status. However, systematic research which includes live and dead, positive and negative cats (with viral status determined by serological and molecular methods) should be performed for a definite conclusion on the accuracy of this SNAP test on postmortal samples.

THE PREVALENCE OF GASTROINTESTINAL PARASITES IN DOGS FROM ZAGREB AREA

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Dogs can harbor a variety of parasites in the digestive tract, which cause gastrointestinal disturbances. At the same time, several species have zoonotic potential. In the current study fecal samples from 2,335 dogs were analyzed in the period from 2012 to 2015. The fecal samples were collected by the dog owners and delivered to the Croatian Veterinary Institute Laboratory for parasitology, through veterinary clinics, due to gastrointestinal disorders, mostly from the Zagreb area. Coprological examinations were conducted with the flotation technique, using magnesium sulfate with direct immunofluorescence for detection of *Giardia* cysts and *Cryptosporidium* oocysts. The results are presented by year for better visibility. The overall prevalence in 2012 was 48.1%: *Giardia duodenalis* (23.8%), *Cryptosporidium* spp. (8.0%), *Toxocara canis* (4.5%), *Isospora* spp. (4.7%), *Toxascaris leonina* (0.7%), *Ancylostoma/Uncinaria* (0.5%), *Trichuris vulpis* (5.2%), *Sarcocystis* sp. (0.5%), *Capillaria* spp. (0.2%). A single-pathogen was detected in 55.2%, and co-infection with two and more than two pathogens in 44.8%. The overall prevalence in 2013 was 57.7%: *Giardia duodenalis* (26.5%), *Cryptosporidium* spp. (10.8%), *Toxocara canis* (5.0%), *Isospora* spp. (5.3%), *Toxascaris leonina* (0.9%), *Ancylostoma/Uncinaria* (12.0%), *Trichuris vulpis* (5.3%), *Sarcocystis* sp. (0.5%), *Capillaria* spp. (1.1%), *Strongyloides stercoralis* (0.5%). A single-pathogen was detected in 60.4%, and co-infection with two and more than two pathogens in 38.6%. The overall prevalence in 2014 was 64.9%: *Giardia duodenalis* (26.7%), *Cryptosporidium* spp. (18.7%), *Toxocara canis* (4.7%), *Isospora* spp. (5.0%), *Toxascaris leonina* (1.5%), *Ancylostoma/Uncinaria* (3.2%), *Trichuris vulpis* (3.9%), *Sarcocystis* sp. (0.2%), *Capillaria* spp. (0.7%), *Strongyloides stercoralis* (0.1%). A single-pathogen was detected in 51.8%, and co-infection with two and more than two pathogens in 48.2%. The overall prevalence in 2015 was 56.6%: *Giardia duodenalis* (26.3%), *Cryptosporidium* spp. (16.4%), *Toxocara canis* (3.6%), *Isospora* spp. (4.8%), *Toxascaris leonina* (0.6%), *Ancylostoma/Uncinaria* (1.6%), *Trichuris vulpis* (1.4%), *Sarcocystis* sp. (1.8%), *Capillaria* spp. (0.2%). A single-pathogen was detected in 45.1%, and co-infection with two and more than two pathogens in 54.9%. The most common co-infection in all years was with *Giardia duodenalis* and *Cryptosporidium* sp.

The results of the current study clearly show the need for regular fecal analysis to exclude parasites as common agents causing GI problems. Also, it is quite important to reconsider routine dechelminisation programs since protozooan parasites were most frequently detected.

CHEWED BONES IN CANINE STOMACH – TREATMENT OPTIONS AND OUTCOME

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Gastrointestinal foreign bodies are commonly encountered in veterinary practice and may present with a variety of clinical gastrointestinal signs. The most common clinical signs of gastric foreign body are vomiting and sometimes diarrhea. Dog owners are often not sure if bones are food, part of the usual diet, a titbit or strictly forbbiden. The aim of the study was to show the incidence and importance of bone findings in canine stomachs.

This retrospective study included dogs treated at the Small Animal Clinic, Faculty of Veterinary Medicine, University of Zagreb, during a four year period. The inclusion criterion was a radiologically visible shadow of bones in the stomach. Foreign bodies were observed as a single bone shadow and as chewed bones with larger number of sharp fragments. The results showed 105 dogs with bones in their stomachs of which 63 (60%) had chewed bones and 42 (40%) had a single bone. In 39% of the cases dogs were of mixed breeds and a high incidence was recorded in: Golden Retrievers, Pekingese, Shi Tzu, Labrador Retrievers, German Shepherds and Cane Corso. In 44 dogs (41.9%) bones in the stomach were an incidental finding, while 61 dogs (58.1%) presented with gastroinestinal symptoms. Conservative treatment was used in 54 (88.5%), surgery was performed in 5 (8.2%) and endoscopic removal was administered in 2 dogs (3.3%). Complications after treatment appeared in 6 dogs. Lethal outcome due to bones in the stomach was not reported, although 6 dogs that were involved in the study died from some other cause unrelated to bone findings.

An additonal retrospective study about the incidence, treatment and outcome of bones as foreign bodies in canine intestines is needed. However, veterinarians should pay attention to bones presenting as foreign bodies in the stomach, considering the frequency of occurence even if in these cases complicatons are negligible and the outcome is usually favorable.

HEMATOLOGICAL AND SERUM BIOCHEMICAL PARAMETERS IN SEARCH AND RESCUE DOGS BEFORE AND AFTER A WHOLE DAY FIELDWORK

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The aim of the study was to evaluate the changes in serum biochemistry and haematological values in Croatian Mountain Rescue Service rescue dogs after a whole day of simulated fieldwork.

There were 19 healthy dogs of 4 different breeds included in this study, 13 males and 6 females, with a mean age of 3.3 years (±SD 2.0 years). Samples were obtained twice, the baseline early in the morning before the dogs were fed, and the second one at the end of 12 hours of fieldwork. Blood was collected from saphenal vein, in EDTA and serum tubes, kept in a refrigerator and analysed on the day of collection.

Serum biochemistry demonstrated a statistically significant increase in albumine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK) and lactate dehydrogenase (LDH) from baseline values. Significant ($p<0.05$) decreases in magnesium (Mg), triglyceride, potassium (K) values were also noted. Haematological parameters: mean corpuscular volume (MCV), red blood cell (RBC) and haematocrit (HCT), were significantly ($p<0.01$) decreased post fieldwork compared to the baseline values before fieldwork.

The decreased values of MCV, RBC and HCT after fieldwork were unexpected since it is known that in dogs stored erythrocytes are released during exercise by splenic contraction. A possible explanation for this result is in the pre-exercise elicited stress and dehydration due to transport, resulting in higher HCT baseline values before blood sampling. It is important to note that values of MCV, RBC and HCT before and after fieldwork were both within the reference ranges.

The present study has shown that rescue dogs have a greater requirement for energy during fieldwork exercise. Energy is provided through anaerobic glycolysis, followed by an increase in AST and LDH levels. Muscle damage indicators suggest that long periods of different intensity level workload in search and rescue dogs result in overall increased muscle activity demands.

In conclusion, the physical activity of search and rescue dogs during prolonged fieldwork is similar to changes described in endurance activity.

SERUM BIOCHEMICAL PARAMETERS IN CLINICALLY HEALTHY ADULT BOSNIAN AND HERZEGOVINIAN MOUNTAIN HORSES

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The Bosnian and Herzegovinian mountain horse is the only autochthonous horse breed in Bosnia and Herzegovina. It is a Warmblood horse. No data are available in literature on the serum biochemical parameters of the Bosnian and Herzegovinian mountain horse breed. For this reason, the aim of the present study was to investigate the values of biochemical parameters in Bosnian and Herzegovinian mountain horses under local conditions.

Blood samples were collected on the "Borike" stud farm from 30 clinically healthy adult horses of both genders. Samples for biochemical analysis were processed using standard procedures with a Catalyst OneTM Chemistry Analyzer.

The following mean values were comparable to previously published reference intervals in horses: glucose 4.96 ± 1.30 mmol/L; urea 7.83 ± 2.18 mmol/L; creatinine 112.05 ± 29.75 μmol/L; phosphorus 0.91 ± 0.22 mmol/L; calcium 2.93 ± 0.18 mmol/L; total protein 72.45 ± 5.75 g/L; albumin 28.91 ± 3.27 g/L; globulin 43.50 ± 5.49 g/L; alkaline phosphatase 170.90 ± 54.70 U/L; total bilirubin 22.00 ± 11.01 μmol/L; cholesterol 2.54 ± 0.28 mmol/L and amylase 33.50 ± 12.77 U/L.

Mean values of urea and total protein were higher than those found in any other investigation of Warmblood horses. Also, a higher mean value of globulin and a lower mean value of alkaline phosphatase were noticed. However, all mean values were within reference intervals for adult horses in general, adult Warmblood horses, and previously published values for other horse breeds. The values of amylase were much lower than the recommended reference intervals for adult horses in general. The results obtained in this study confirm the need for further investigation to establish reference values of serum biochemical parameters in the Bosnian and Herzegovinian mountain horse breed, in line with appropriate sample size, age, gender, nutrition, breed and season.

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EQUINE METABOLIC SYNDROME IN A PONY – A CASE REPORT

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The term "equine metabolic syndrome" (EMS) was first introduced to veterinary medicine in 2002 when it was proposed that obesity, insulin resistance and laminitis are to be considered the typical clinical manifestations of this complex metabolic disorder. This report describes a case of equine metabolic syndrome in a 12 year old male pony.

The pony was admitted with a history of lameness and prolonged periods of recumbency lasting for two years. His diet was predominantly based on grazing, but his owner additionally fed him bread, pastry, oats, corn, and sometimes even dog food and goulash. Physical examination showed a BCS of 8/9 and a prominent nuchal crest subcutaneous fat deposition ("cresty neck") of 20 x 50cm. Divergent growth rings on the hooves were present on all extremities. The pony showed typical signs of laminitis, he was reluctant to move and tended to land the heels first, with a marked shortening of the caudal phase of the stride. An increased digital pulse, together with positive hoof testers at the toe region, were noted on all limbs. Radiographs showed mild rotation of the coffin bone. Laboratory tests showed hyperglycaemia (7.0 mmol/L) and hyperinsulinemia (550.24 pmol/L) with hypertriglyceridemia (1.9 mmol/L). Gamma glutamyl transferase levels (30 U/L) were also elevated, possibly indicating hepatic lipidosis. The level of cholesterol was decreased (0.4 mmol/L). Based on physical examination, laboratory tests and radiography, the pony was diagnosed with EMS. The suggested treatment plan included dietary management, physical activity and corrective hoof trimming.

Veterinarians play a key role in educating pony owners about proper nutrition, since sometimes owners forget that a pony is also a horse, which requires a diet regime adjusted to his needs, and overfeeding can have serious and even fatal consequences. Equine metabolic syndrome is a disease induced by diet and management factors, and appropriate dietary restriction is paramount in its prevention.

SKIN NODULES PREVALENCE IN GREY AND WHITE HORSES

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Skin tumours are common in horses of all breeds and ages. The three most common skin tumours in horses are sarcoids, melanomas and squamous cell carcinomas. Melanocytic neoplasms have been reported to represent up to 18.7% of all equine cutaneous neoplasms. Most melanomas occur in aged, grey horses, primarily in the skin of the perineum and ventral tail regions, but have also been reported in the parotic region and within the skeletal muscles.

In this study, a population of 69 Warmblood, grey and white horses were examined for the presence of cutaneous nodules. Data regarding their age, gender and time spent outdoors were also recorded. The study included 30 male and 39 female horses, four to 24 years old (average age 10.4±5.2). All the horses were carefully inspected and palpated in daylight conditions, and all the nodules were recorded, describing the quantity and region of their presence. Horses were divided according to age into three groups: up to 10 years old, 10 to 15 years old and 15 years and older.

Skin nodules were recorded in 39.13% of the examined horses. The most common regions with skin nodules were the ventral tail, perineal, perianal and parotic regions. Skin nodules were recorded in 16 mares (23.19%) and 11 male horses (15.94%). The most cutaneous nodules were noted among the oldest age group ($p<0.01$). Taking into account the horses with skin nodules, most of them (77.78%) were kept outside a stable for more than 4 hours a day. A single skin nodule was noted in 37.04%, whereas 62.96% of the animals had multiple nodules. Of the recorded nodules, 29% were covered with hair and the rest had dark grey to black colour.

Most skin nodules were noted in older horses, indicating the possible effect of melanoma formation in older grey and white horses. Although there were more horses with skin nodules in the group that spends most time outdoors, no significant association was noted. However, larger studies are warranted to investigate this hypothesis.

WHAT SHOULD BE CONSIDERED WHEN INTERPRETATING BIOCHEMISTRY BLOOD RESULTS IN HEALTHY ENDURANCE HORSES?

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Horse athletes, competing in endurance riding, are trained for long-distance races demanding a high level of performance and altered baseline metabolic status. The aim of this study was to investigate possible differences between endurance and healthy horses. For baseline biochemistry parameters of healthy horses the official reference values of the Laboratory of Internal Diseases Clinic, University of Zagreb, Faculty of Veterinary Medicine, were used. Blood samples were obtained from 65 endurance horses on the race day. The horses enrolled in the study successfully finished the race and passed all the mandatory veterinary inspections. All the horses arrived at the event venue on the same day. During transport, as well as before the sampling, the horses had unlimited access to food and water. Venipuncture was performed from the jugular vein after the first veterinary inspection, but prior to the race. Blood was collected using the vacutainer system in 5 ml gel tubes. Once centrifuged, serum was stored in a portable refrigerator and transported to the laboratory. Three muscle damage indicators were analyzed: creatinine kinase (CK), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST), as well as two kidney perfusion markers: urea and creatinine, Gamma-glutamyl transferase (GGT), glucose, triglyceride and cholesterol were analyzed as metabolic status indicators, while sodium, potassium, calcium and magnesium for electrolyte evaluation. The obtained results showed that CK [299 (237–377)] U/L and glucose [5.8 (5.3–6.5)] mmol/L values were above reference limits for healthy horses. Average CK was 130% and average glucose was 16% higher than the upper reference value.

An increase in some biochemical markers in serum has already been noted in studies on healthy and trained endurance horses. The results of the present study indicate that the specific equine sport discipline should be considered when interpreting biochemistry blood results, since all sampled animals successfully passed the veterinary inspection and therefore the obtained results were not indicators of disrupted health.

BOWEL DISLOCATION AS CAUSE OF DEATH IN HORSES AT THE FACULTY OF VETERINARY MEDICINE ZAGREB

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Bowel dislocations in equine patients are frequent emergency diseases which require immediate veterinary attention, and are often considered indications for surgical procedures. In the present study, the incidence, outcome and type of bowel dislocations in equine patients submitted for treatment and pathological analysis to the University Clinic are investigated.

Database analysis of a 7 year period with 75 horses recorded with bowel dislocations diagnosed during rectal examination, during surgery or during necropsy. The probability distribution of data was analysed using the chi-square test. There were 52 cases of large and 23 cases of small intestine displacement. Of the admitted horses, 53% did not survive, most of them (63%) were submitted for euthanasia. The part of the intestine involved in the pathology was found to significantly influence survival ($p<0.05$), where a higher mortality rate was found in horses with small intestine involvement. According to the breed, there were 16 draft horses and 59 Warmbloods. Breed had a significant influence on survival ($p<0.05$), with Warmbloods having significantly higher chances of survival. There were 45 mares and 30 male horses included in the study, although the gender had no influence on horse's survival or intestine part involved in the pathology. Diagnostic laparotomy was proposed to the owners in 48% of the cases, however, 47% refused it. Of the horses that underwent surgery, 3 were euthanized or died during surgery, and of the horses that survived the procedure 55% were discharged from the clinic. However, surgery was found to significantly ($p<0.01$) increase the chance of survival.

The surgical procedure was often refused by the owner for economic reasons and poor prognosis. Summarising the results, the Warmbloods with large intestine involvement that underwent surgery had the best chance of survival. Furthermore, this retrospective analysis also included data from a period when there were no suitable surgical facilities available, which could have influenced the results. A survey with detailed analysis of different clinical parameters is warranted for possible survival prediction.

MICROPHTHALMIA IN ''CROATIAN COLDBLOODED" FOAL – CASE REVIEW

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Microphthalmia is a sporadic congenital eyeball malformation and occurs in all horse breeds. It is either an autosomal recessive or not inherited disease, and it is considered to be caused by epigenetic factors such as various pathogens and use of drugs in the first trimester of pregnancy. Microphtalmia may be unilateral or bilateral, and of varying severity. Most reports of ''clinical anophthalmia" are actually severe cases of microphthalmia, because rudiments of pigmented uvea and neuroepithelium are found in orbital tissue. Foals with subtle microphthalmia may appear to have almost normal-size eyes, but show obvious visual deficiency, while those with bilateral microphthalmia are often blind. Severe microphthalmia is easily diagnosed because of the associated enophthalmos and the passive prolapse of the nictitating membrane.

In this report, the case of a two-month old „Croatian Coldblooded" breed foal with congenital bilateral microphthalmia is presented. Upon examination, unusually small eyeballs with partially developed light-brown coloured irises, were observed. The pupils were white with the absence of pupillary reflex, however, palpebral reflex was present in both eyes. Inbreeding was observed amongst the parents (grandparent + granddaughter). Since it could be a hereditary disease, all mares pregnant by that stallion should be examined, and similar cases of inbreeding should be forbidden. The best method for detecting the malformation would be ultrasound screening of all pregnant mares sired by the stallion. The diameter of an orbit is easily measured in a foal fetus and it serves as an indicator of its growth and development in the second and third trimesters of pregnancy.

Considering that this breed is of national value in Croatia, it is important to notice and exclude all potential malformations on time, analyse the whole gestation period, examine other mares in breeding and eliminate all potentially harmful agents, to avoid the occurrence of this pathology in the future.

ENTOMOLOGICAL SURVEY OF INSECTS OF THE GENUS *CULICOIDES* LATREILLE, 1809 (DIPTERA: CERATOPOGONIDAE) IN 2016 IN CROATIA

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Insects of the genus *Culicoides* are some of the most numerous haematophagous insects in the world. Nearly 1400 different species of *Culicoides* have been described worldwide. Insects of the genus *Culicoides* Latreille, 1809 (Diptera: Ceratopogonidae) are biological vectors of bluetongue virus, transmitting it by sucking blood from an infected ruminant host.

An entomological survey of insects of the genus *Culicoides* as bluetongue virus biological vectors was conducted in 2016, in order to determine *Culicoides* fauna in Croatia. The *Culicoides* identified morphologically were compared with the selected species COX1 gene sequences obtained.

A total of 76,977 *Culicoides* specimens were collected and morphologically identified; 200 specimens were identified by molecular methods. The presence was demonstrated of 21 known *Culicoides* species and 11 new species, showing 84% to 94% similarity to the sequences available in the NCBI GeneBase. *C. obsoletus* and *C. scoticus*, and 1 *Culicoides* showing 94% similarity to *C. obsoletus* were identified by *C. obsoletus* group vector sequencing. *C. pulicaris* group vector sequencing identified *C. pulicaris*, *C. punctatus*, *C. lupicaris* and *C. newsteadi*, and 1 specimen showing 94% similarity to *C. newsteadi*. Sequencing of other *Culicoides* species identified the following species: *C. nubeculosus*, *C. riethi, C. puncticollis*, *C. fagineus*, *C. flavipulicaris*, *C. circumscriptus, C. fascipennis, C. achrayi*, C. *parroti*, *C. salinarius*, *C. griseidorsum*, *C. simulator*, *C. picturatus*, *C. festivipennis*, *C. alazanicus,* 1 *Culicoides* with 94% similarity to *C. paradoxalis*, 1 *Culicoides* with 85% similarity to *C. oxystoma*, 1 *Culicoides* with 85% similarity to *C. circumscriptus, C. salinarius* and *C. manchuriensis* species, 2 *Culicoides* with 86% similarity to *Culicoides* sp., and 4 new *Culicoides* spp.

Data on the *Culicoides* species present in Croatia, obtained by morphological and molecular identification, are now available in Croatia for the first time. This entomological survey revealed the predominance of the vectors of *C. obsoletus* and *C. pulicaris* groups as a major risk factor for the spread of bluetongue virus, which is important for the epidemiology of bluetongue as an infectious disease. The finding of various *Culicoides* species is highly relevant for knowledge of the fauna and biodiversity of *Culicoides* insects in Croatia.

SUBCLINICAL HYPOTHYROIDISM IN GRAVID RATS CAUSES DELAYED OSTEOGENIC DIFFERENTIATION IN PUPS

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The effects of maternal hypothyroidism on the development and function of different organs of their offspring are well known. It is unclear how its subclinical form affects some of these processes, including endochondral ossification during prenatal and early postnatal development. Our previous studies have demonstrated that hypothyroid rat pups had reduced growth plate thickness.

The aim of the study was to assess bone turnover by defining the expression of tartrate-resistant acid phosphatase (TRAP), alkaline phosphatase tissue nonspecific isozyme (ALPL) and mRNA for dentin matrix acidic phosphoprotein (DMP1) in the tibia of pups from subclinical hypothyroid dams in early postnatal development. Subclinical hypothyroidism (SHT) was induced with propylthiouracil through drinking water (1.5 mg/l) in pregnant Albino Oxford dams from the first day of gravidity and during lactation. The control group was untreated. Five, seven-day-old pups from each experimental group were euthanized. All histological examinations were performed on paraffin sections of the proximal tibial growth plate. Histochemistry was used to assess the expression of TRAP, immunohistochemistry for ALPL and *in situ* hybridization for DMP1. The area of the TRAP positive signal, optical density of ALPL immunostaining, and the number of DMP1 positive cells were evaluated using the Image J. Expression of TRAP and ALPL in the proximal tibial growth plate were lower in the hypothyroid pups, and the number of cells expressing mRNA for DMP1 was decreased.

TRAP is a marker of osteoclast activity. Osteoclasts are multinucleated cells which are necessary for bone and mineralized cartilage resorption. In osteoblasts ALPL and DMP1 are expressed and they have an important role in the process of endochondral ossification. As we have shown that TRAP, ALPL and DMP1 are decreased, we may conclude that maternal SHT in rats leads to compromised cartilage to bone transition, and delayed osteogenic differentiation in the early infantile period.

THYROID HORMONES AFFECT THE PROLIFERATION AND/OR MOBILIZATION OF BULGE STEM CELL POPULATION

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Thyroid hormones (TH) play a pivotal role in the development of mammalian skin, and are necessary for both the initiation and maintenance of hair growth. Adult hypothyroid rats showed impaired epidermal proliferation, hair growth and wound healing. Stem cells (SCs) located at the bulges of the hair follicles are responsible for hair cycling, and contribute to the regeneration of the new epidermis after wounding. Therefore a reduction in the number or function of bulge stem cells could be a cause of disturbed skin reepithelization and hair follicle maintenance. We hypothesized that a decrease in TH during prenatal development would affect the bulge stem cell population.

Subclinical hypothyroidism was induced with propylthiouracil through drinking water in a dose of 1.5 mg/l in pregnant Albino Oxford rats, from the first day of gravidity and during lactation. The control group was untreated. Five, seven-day-old pups were euthanized and skin samples were taken from the dorsal part of their bodies. The number of hair follicles was estimated on an area of 1mm^2 of dermis. For the immunohistochemical study, proliferating cell nuclear antigen (PCNA) (Santa Cruz Biotechnology) and NANOG (Thermo Scientific) antibodies were used. The number of PCNA and NANOG positive cells was estimated on the area of 1mm^2 of hair follicle.

The number of hair follicles was reduced and the expression of PCNA was decreased in the cells of the inner and outer sheath of hair follicles in the hypothyroid pups compared to the controls. They also had an increased number of NANOG positive bulge cells which we presume are multipotent SCs.

Decreased expression of PCNA demonstrates low proliferation and increased expression of NANOG, which may be explained in two ways: 1) the inhibited differentiation of putative SCs and/or 2) the reduction of mobilization of these cells to the epidermis or sebaceous gland. Our results confirm that the lack of TH influences the formation of hair follicles in early infantile rat pups.

OVARIAN SURFACE EPITHELIUM IN NEWBORN RATS: GERM CELLS WAREHOUSE

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The ovarian surface epithelium (OSE) is a very dynamic part of the ovary, with an important role in ovulation during the reproductive period. It represents a source of germ cells in the fetal period but data regarding the role of the OSE in the postnatal period are contradictory. Although the OSE has been the subject of research in the past few years, its ultrastructure in newborn rats has not yet been studied. The aim of this study was to examine rat OSE ultrastructure features in the first 24h hours after birth.

Six newborn rats were euthanized at 12h±2 hours after birth. Their ovaries were fixed in 4% glutaraldehyde, post-fixed in 1% osmium tetroxide, routinely dehydrated and embedded in araldite. Semi-thin sections were stained with toluidine blue, and used to select areas of interest. Ultra-thin sections of selected areas were mounted on copper grids and examined under a Philips CM12 transmission electron microscope. Ultrastructural analysis revealed the presence of a typically single-layered OSE with sporadic pseudostratified appearance, both consisting of cuboidal and/or columnar cells. The OSE cells' nuclei had irregularly dispersed chromatin, and a central or apical location. From the surface of the OSE cells numerous microvilli and cytoplasmic projections extended towards the peritoneal cavity. Continuous *tunica albuginea* was not found, but occasionally groups of fibroblast-like cells were present, indicating the onset of its formation. Numerous oogonia and oocytes were identified between OSE cells or up to their basal part, suggesting that extrusion (the release of germ cells into the peritoneal cavity) may be an important mode of germ cell elimination.

In the early postnatal period in rat ovaries massive death takes place of oogonia and oocytes, mostly via apoptosis and autophagy. Further investigation might reveal the contribution of extrusion as another form of germ cell elimination for both, maintaining and balancing their number in the ovary, which is necessary for proper reproductive function.

INFLUENCE OF DIETARY LIPIDS ON THE HEPATIC EXPRESSION OF GENES FOR THE β-OXIDATION OF FATTY ACIDS IN THE RATS WITH STREPTOZOTOCIN INDUCED DIABETES

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Fatty acids found in the tissues originate from food, *de novo* lipogenesis and bioconversion. Fatty acids generated *de novo,* as well as fatty acids derived from food, are bioconverted by a series of desaturation, elongation and β-oxidation steps, into different saturated, monounsaturated or polyunsaturated fatty acids. The regulation of bioconversion is complex, and involves induced expression by different metabolites (glucose), hormones (insulin) and transcriptional factors. Nutrition (substrate availability) and competition for rate-limiting enzymes, as well as lipid oxidation and hormonal status, could substantially contribute or even override other regulatory mechanisms. Therefore, the aim of our study was to investigate the influence of dietary n6/n3 ratio on the expression of genes involved in the β-oxidation of fatty acids, in the streptozotocin model of diabetes mellitus type 1.

The rats were divided into 4 groups: CON (Control, n6:n3, 7:1), CON-STZ (n6:n3, 7:1 and STZ injection), N3 (n6:n3, 0.05 and STZ injection) and N6 (n6:n3, 60:1 and STZ injection). RT-qPCR was performed using a One-Step SYBR PrimeScript RT-PCR Kit II, on a Stratagene MxPro3005 thermocycler. For statistical data analysis, GraphPad Prism 7.0 and MetaboAnalyst 3.0 were used. Data were compared by analysis of variance and the Tukey post hoc test. Mitochondrial β-oxidation, analysed by carnitine palmitoyltransferase I (CPT1) gene expression, was increased in the CON-STZ and N6 groups, and decreased in the N3 group, compared to the CON. Peroxisomal β-oxidation, analysed by D-bifunctional protein (D-BP) gene expression, was decreased in the N3 group compared to the CON, while the CON-STZ and N6 groups were not different in comparison to the CON group.

The results showed that dietary lipids significantly influence mitochondrial and peroxisomal β-oxidation in streptozotocin treated rats. In diabetes mellitus type 1 significant changes are present in the fatty acid profile of different tissues. Therefore, it would be interesting to examine further how changes in gene expression caused by dietary manipulation influence the actual fatty acid composition of tissues in diabetes mellitus type 1.

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/ / / / / PHD DAY OF THE FACULTY OF VETERINARY MEDICINE UNIVERSITY OF ZAGREB / / / / /

/ / / / / INVITED LECTURES / / / / /

CENTERS OF RESEARCH EXCELLENCE

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In the world of science, Centers of Research Excellence support thematic, multidisciplinary projects that augment and strengthen national research capacity. This is usually accomplished by expanding and developing capability and enhancing research infrastructure, including the establishment of core facilities needed to carry out the objectives of a multidisciplinary, collaborative program. Center of Research Excellence is usually led by principal investigator, who is an established research scientist with expertise central to the research theme of the center, has an active research laboratory, has relevant peer-reviewed funding and has demonstrated administrative leadership and mentoring experience. Centers promote collaborative, interactive efforts among researchers with complementary backgrounds, skills and expertise. In some instances, Government support facilitates the development of new, specific research centers or augments the capability of existing centers. In Croatia, Centers of Research Excellence gathers and crosslinks the best researchers in a particular field at a national level that are focused on contemporary research topic. They also have to be internationally competitive and recognized in terms of quality and scope of scientific production, capable of effective international cooperation and have to give significant contribution to the development of science, higher education and the economy at the national level. The objective of the establishment of Centers of Research Excellence is to identify and evaluate researchers and research that are innovative, have the potential of the discovery, that are possible milestones in science and, at the same time, are internationally relevant in terms of quality and vision and in line with the strategic needs and priorities of the Republic of Croatia and objectives of the Strategy Europe 2020 and Strategy for Education, Science and Technology. Research programs within established Centers of Research Excellence provide educational and research opportunities for Graduate Students and Postdoctoral Fellows.

INCREASING THE RESEARCH CAPACITIES THROUGH ERA CHAIR PROJECT

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ERA Chair projects are one of the horizontal activities within the FP7 and HORIZON 2020, directed to overcome the differences in research and innovation area in Europe. Goal of the ERAChair initiative is to support universities and other research organizations that have the possibility to improve their research, innovative systems and policies under the guidance of esteemed scientist, in order to become the competitive participants in European scientific programs and by that to fully integrate in the research area. University of Zagreb The Faculty of Veterinary Medicine was granted ERAChair project among 111 applications. Through this project The Faculty of Veterinary Medicine gain the opportunity to hire 4 experienced researchers and one esteemed European scientist on a period of five years. Beside that, EU commission allowed the purchasing of 585 thousand Euros worth equipment, execution of working meetings, education, training and visits of scientist from different countries. The main goal of the VetMedZg project are to improve, develop and increase the research capacity and improvement of scientific excellence in the area of molecular veterinary medicine, especially postgenomic technologies (proteomics and metabolomics), comparative analysis of tumours and wildlife research. The fundamental part of our molecular research is in application of new, innovative methods and technologies, in other words proteomic technologies (omics) in veterinary medicine. The Laboratory for proteomics is equipped with 60 instruments, among which special place takes the mass spectrometer (LC-MS/MS). Experienced researchers and state-of-the-art, innovative technology enables the application of proteomics – interdisciplinary science that connects biology, chemistry and bioinformatics, in various fields of veterinary medicine, from species identification, analysis of signalling pathways, disease research to development of new vaccines and application in forensic veterinary medicine. Special meaning has clinical proteomics in discovering of new biomarkers important for diagnostic, therapy and prognosis of diseases.

THE EFFECTS OF NATRIURETIC PEPTIDES ON THE BRADYKININ SIGNALING IN PRIMARY CULTURE OF NEURONS AND ASTROCYTES

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Blockage of cerebral arteries lead to stroke and subsequent development of cerebral edema. *In vivo* experiments in a mouse model of stroke showed that i.v. application of natriuretic peptide prevents the formation of brain lesions . In this study we investigated the effect of an agonist of guanylate cyclase A, guanylate cyclase B and guanylate cyclase C on primary cultures of astrocytes and neurons , and the effect of natriuretic peptides on the bradykinin signaling system using the patch clamp technique . Binding of bradykinin to B2R receptor in neurons and astrocytes leads to depolarization of the cell membrane. Activation of Ca^{2+}-dependent Cl^- channel is responsible for the effect of bradykinin on neurons and astrocytes. GC-A agonists and GC-C agonists inhibit depolarisation effect of bradykinin in primary culture of neurons and astrocytes, while GC-B agonist, does not. The mechanism of interaction of signaling pathways of GC-A and GC-C agonists and bradykinin in neurons and astrocytes is conducted by RGS protein regulation. Natural antagonism of GC-A and GC-C agonists on B2R signaling pathway shown in cellular models of neurons and astrocytes can be used to develop new therapeutic approach to the treatment of pathophysiological conditions mediated by bradykinin, such as ischemic brain injury.

EFFECT OF DIETARY EICOSAPENTAENOIC AND DOCOSAHEXAENOIC FATTY ACID SUPPLEMENTATION DURING LAST MONTH OF GESTATION ON LIPID METABOLISM AND FASN AND ACACA GENE EXPRESSION IN CHAROLAIS COWS AND CALVES

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The significance of eicosapentaenoic (EPA) and docosahexaenoic (DHA) polyunsaturated fatty acids is especially important in the last trimester of gravidity. The aim of this research was to investigate the influence of EPA and DHA on the fatty acid metabolism of cows and their calves as well as FASN and ACACA gene expression. Based on chemical and fatty acid composition of colostrum and early milk, our aim was to determine the concentration of EPA and DHA and their influence on other milk parameters, cholesterol concentration, and oxidative stability.

In a feeding trial, a total of 20 Charolais cows were included during the last month of their gravidity. Cows were divided into 2 groups: a control group (Control) and an experimental group (DHA + EPA), each group consisting of 10 animals. All the animals were fed the basal diet, consisting of haylage and corn concentrate, and had constant access to drinking water. For the period of one month before expected calving, cows in the experimental group (DHA + EPA) were supplemented with fat supplement, consisting of 9.1g/cow/day of EPA and 7.8 g/cow/day of DHA. Milk samples were collected on the 1st day (6 hours after calving; colostrum) and on the 7th day of lactation. Blood samples were obtained from cows and their calves on the day of calving. Colostrum and milk were analyzed to determine fatty acid composition, chemical composition, cholesterol concentrations and total phenolic compounds. In serum, we conducted an analysis of blood biochemistry parameters: glucose, triglycerides, cholesterol, HDL cholesterol, LDL cholesterol. In plasma, we determined the fatty acid composition and malondialdehyde concentrations. FASN and ACACA gene expression were examined using quantitative PCR in the blood of cows and their calves.

Fatty acid composition of colostrum and milk in experimental group (DHA + EPA) was significantly altered: we found higher concentrations of fatty acids EPA, DHA, docosapentaenoic and n-3 PUFA in colostrum, and EPA, DPA and n-3 long-chain polyunsaturated fatty acids (LCPUFA) in milk on the 7th day of lactation, but summed profile of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) was not altered. The concentration of milk fat and protein in colostrum and milk did not differ between groups. In cow plasma, concentrations of PUFA, n-3 fatty acids and n-3 LC PUFA were significantly higher in experimental (DHA + EPA) group, meanwhile, plasma fatty acid composition of calves was not affected by the supplementation. Body weight of calves also showed no significant difference between groups. Supplementation of EPA and DHA altered cholesterol level in plasma in the experimental group (DHA + EPA) of cows. Oxidative stress markers in plasma, MDA concentration and in colostrum and milk, the concentration of total phenolic compounds, showed no difference between groups, both in cows and in calves. Relative mRNA abundance for genes FASN and ACACA in cow and calves blood was not affected by the treatment.

We conclude that supplementation of low dosage fatty acids EPA and DHA alters colostrum and milk fatty acids composition trough elevation of n-3 LCPUFA without affecting milk fat and protein concentrations and oxidative stability. Plasma composition in cows during supplementation was significantly altered, meanwhile, the same effect was not detected in calf plasma which implies to low a transplacental transfer of LCPUFA in ruminants. No significant change in mRNA expression for genes ACACA and FASN was detected in the blood which leads to the conclusion that there is tissue-specific mechanism regulating expression of lipogenic genes.

INFLUENCE OF DGAT1, FASN, PRL, BRCA1 AND TLR1 GENE POLYMORPHISM ON MILK PRODUCTION AND UDDER HEALTH IN COWS

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Daily milk, fat and protein yield and amount of somatic cells in cow´s milk are very important factors that influence milk performance traits. The present thesis were undertaken to study the association between polymorphism of genes *DGAT1*, *FASN* and *PRL* and milk performance traits as well as association between polymorphism of genes *BRCA1* and *TLR1* and udder health. The part of dissertation was to evaluate the molecular biology tool for detection of mastitis pathogens in milk samples in field conditions.

A total of 110 milk samples from one dairy farm were collected, from pure Holstein (HF) and Simmental (S) breed and their crossbreed (HF x S). Milk fat and DNA were isolated from milk. Milk fatty acid composition was analyzed using gas chromatography. Polymorphisms of *DGAT1*, *FASN*, *PRL*, *BRCA1* and *TLR1* genes were detected using PCR-RFLP. Detection and quantification of mastitis pathogens was done using real-time PCR.

In *DGAT1* gene, allele K, that was associated with higher fat content, had higher frequency. In *FASN* gene, the TW haplotype had very low frequency. In the HF x S crossbreed diplotype AR/AR and in HF breed diplotype TW/AR were associated with lower SFA content and higher content of C18:1 n9, MUFA and MUFA/SFA. For the *PRL* gene, the genotype AA wasn't found and the gentopye AG was found in low frequency. In the primiparous HF x S crossbreed, cows with genotype AG had significantly higher daily milk yiled ($P<0,05$). For the *BRCA1* gene the alel T was correlated with lower SCC, same as for the GG genotype of the *TLR1* SNP A1762G in the HF x S cows. From the five researched genes (6 SNPs), gene *DGAT1* was confirmed as a possible candidate gene for milk fat content. *BRCA1* gene could be a possible candidate gene for the SCS, but given the low amount of researches done, it is necessary to further define its associations.

VIRULENCE FACTORS OF ESCHERICHIA COLI AND THEIR IMPACT ON THE PATHOMORPHOLOGICAL AND HISTOPATHOLOGICAL LESIONS IN PIGLETS DIED FROM COLIBACILLOSIS

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Fimbrial and non-fimbrial adhesins, as well as heat-labile and heat-stable enterotoxins are main virulence factors in ETEC, EAEC, EPEC and STEC which affect the occurrence of intestinal colibacillosis. Pathological lesions in piglets are depended on involved pathotype of *Escherichia coli*.

The aim of this study were to demonstrate lesions caused by pathogenic *E. coli*, to isolate strains of *E. coli* and to determine the presence of genes for virulence factors, including fimbrial (F4, F5, F6, F18, F41) and non-fimbrial (INTIMIN, PAA, AIDA-I) adhesins, heat-labile (LT) and heat-stable (STa, STb, EAST1) toxins and Shiga toxins (Stx1, Stx2, Stx2e), by molecular methods. We have submitted 55 piglets for complete necropsy and further laboratory investigation.

Results showed that 84,48% of *E. coli* strains carry at least one of the virulence factors. Occurrence of six virulence genes *astA* (EAST1), *estII* (STb), *faeG* (F4), *estI* (Sta), *elt* (LT) and *paa* (PAA) is high and they are represented with, 67,24%, 63,97%, 55,18%, 50%, 48,27% and 44,82% respectively. The most common pathotype ETEC-F4 was found in 57% of cases. The most prominent lesions were found in the small intestine, and virotipe STa:Stx2:Stx2e caused the most serious overall pathological changes.

In conclusion, more extensive lesions in piglets than previously described in ETEC are the result of the expression of acquired EAST1 and PAA genes. The lesions were more pronounced in suckling piglets with diarrhea, and the degree of pathological changes in organs of piglets is correlated with the possession of genes for F4, LT, STA, STB, PAA and EAST1 ($p < 0.05$) as well as with the expression of hemolysis.

MORPHOLOGICAL AND PROTEOMIC ANALYSIS OF FLAGELLATES FROM TRYPANOSOMA GENUS IN CROATIA

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The species of *Trypanosoma* genus are spread worldwide. Some species can cause serious health problems in humans and animals, although some species are apathogenic for their host. In Croatia apathogenic species were isolated: *T. melophagium* from sheep ked, *T. theileri* from cattle, *Trypanosoma* sp. from cervids and *T. rotatorium* from frogs. For seroepizootiological studies as antigen source, *in vitro* cultured trypanosomes are used. Except by *in vitro* cultivation, antigen research can be done with proteomic analysis. The aims of this study were to describe *T. rotatorium* bloodstream forms based on morphometric parameters, *in vitro* cultivation of isolated tripanosomes in new *in vitro* culture system medium as antigen source for further seroepizootiological research and their proteomic profiling. Also, by *in vitro* cultivation, goal is to describe the vitality and morphology of all the trypanosome species present in Croatia and culture the bloodstream forms for further seroepizootiological investigations. Proteomic analysis of trypanosome isolates will elucidate their differences and similarities.

T. rotatorium morphology was described using standard morphometric parameters. A new in *vitro culture*, Biphasic Chocolate Agar for Trypanosomatids was compared with Liver Tryptose Medium by *in vitro* cultivation of isolated trypanosomes. Proteomic profile of trypanosomes was analyzed by two dimensional electrophoresis and mass spectrometry.

Morphometric analysis of *T. rotatorium* bloodstream forms showed high polymorphism. By culturing the trypanosomes in new culture system, antigen for further seroepidemiological research was produced. Proteomic analysis showed marked difference between analyzed trypanosome flagellates.

This is for the first time that *T. rotatorium* bloodstream forms were analyzed in Croatia. We showed here a new *in vitro* culture system media. For the first time proteome profile of *T. rotatorium*, *T. theileri*, *T. melophagium* and cervid trypanosome were presented.

EFFECTS OF AUTOLOGOUS INTERLEUKIN 1 RECEPTOR ANTAGONIST PROTEINE ON ACHILLES TENDON HEALING IN RABBITS

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The cytokine interleukin-1, more precisely its beta form (IL-1β), plays an important role in the initial stages of the inflammatory response in almost all tissues including tendons themselves. It is involved in the activation of inflammatory factors cascade and other molecules and also directly or indirectly increases its own local and systemic concentration by positive feedback. Recent studies are trying to work into this pro inflammatory cytokines by reducing or inhibiting completely its effects in inflammatory response. Precisely guided by the latest literature data demonstrating an important role of IL-1 in inflammation of tendons we decided to explore possibilities of preventing and improving treatment of inflammation of the Achilles tendon in rabbits by adding IL-1β (IL-1Ra) receptor antagonist protein from autologous conditioned serum. In this paper the assumption was that after longitudinal tenotomy of Achilles tendon inflammation will follow resulting in an increased concentration of IL-1β which will cause degradation of collagen fibers and extracellular matrix. Also it was expected that local administration of the therapeutic agent Orthokin (IL-1Ra, IRAP ®) in the first 48 hours after injury, will decrease the concentration of IL-1 in tendons. One of the hypothesis was that the Achilles tendon tissue of irap experimental group of rabbits, four weeks after the application of the antagonist, histologically will be more similar to normal healthy tissue without pronounced inflammatory changes and without histological signs of chronic inflammation and degeneration in relation to the tissue of the control group. Considering the stated facts aim of this study was to determine the effect of autologous interleukin-1 receptor antagonist protein (IL-1Ra, IRAP ® Orthokin) on reducing iatrogenic created inflammation of the Achilles tendon in rabbits and its effect on the overgrowth of the same tendon 4 weeks after scarification and longitudinal tenotomies using histological and immunoenzymatic indicators. Also, we wanted to determine the amount of IL-1 with ELISA kit for rabbit IL-1 in tendon tissue and serum four weeks after the administration of antagonists. The goal was to measure residual inhibitory effect of IRAP® IL-1Ra on IL-1β and to determine the residual effect of the aforementioned doses of antagonists in the prevention of chronicity of the inflammatory process. The work was carried out on 26 Californian white rabbits, aged about 1-2 years and of both sexes. Rabbits were randomly divided into two equal groups (irap and control). The Achilles tendons of rabbits were used as a model. In both groups of rabbits tendons were prepared and dissected from the surrounding tissue. After tendon sheaths were opened tenotomies and scarification were performed using surgical scalpel no.15 along the entire length of tendon in the direction of the fibers. Longitudinal tenotomies resulted in the inflammation of the tendon. After closing of tendon sheaths in irap group of animals autologous conditioned serum rich with IL-1 receptor antagonist protein (IRAP ®, Orthokin)

was injected into tendon in three points. In the control group buffered saline was administered with an equal amount, equal injection site and equal intervals as serum in the irap group (0.2 ml per injection site and 0, 24 and 48 hours after tenotomies). Four weeks after administration of the active components animals were sacrificed and the Achilles tendon were completely taken for morphological measurements, histological analysis and measurement of the amount of IL-1β by ELISA test kit for rabbit IL-1β. Worked parameters included morphological and histological differences between experimental groups (irap and control) of animals differences were compared to normal physiologically tissue of the intact tendons. Appearance, layout, the number of collagen fibers, cellularity, the amount of glycosaminoglycans (GAGs) and elastic fibers were compared. Histological parameters of healing were assessed using specific staining and Bonar gradation scale from 0-3 with a statistically significant difference in irap group. One hundred µl of supernatant per one Achilles tendon was obtained after tissue underwent grinding, lysis and centrifugation and supernatant was used for ELISA. ELISA test was read at 450nm and concentration of IL-1 was determined using standard curve. More pronounced changes were observed in the control group such as tendon degeneration of collagen, improper orientation of the fibers, fiber thinning, hyper cellularity, scattered capillaries and an increased amount of GAG between the fibers. Four weeks postoperatively there was a distinct overall tendon-like appearance with thick collagen bundles and cells oriented along the tension fibers in tendons exposed to irap treatment. Tendons were thicker, had more unique and characteristic collagen fiber arrangement and more histologic maturity of the repair tissue than in control group. There were also differences in tendon healing of different parts of tendon tissue. We found increased amount the of IL-1β by 2.5 fold in the tissue and tendons of control group with negligible increase in the blood serum of the control group after 4 weeks. Difference in tissue samples histology and IL-1 concentration between the PBS and IL-1Ra group was statistically significant at week 4. There were also some statistically important differences between groups in platelet and white blood cell count. Increase in platelet number was noted in all control group rabbits. Neutropenia was noted in irap group. Inhibitory effect of IL-1Ra on the IL-1 prevented binding of IL-1 on to target cells surface of the tendons and consequently resulted in reduction of inflammation and degradation of collagen and extracellular matrix with histologically proven accelerated tendons healing. We concluded that IL-1Ra prevented morphological deterioration of the Achilles tendon in irap group. With minimal side effects such as local irritation and neutropenia with no apparent deteriorated health of experimental animals the prevention and treatment of inflammation of the Achilles tendon in rabbits using autologous serum rich with IL-1β receptor antagonist protein showed a good alternative for commonly used NSPU drugs and corticosteroids. Our study demonstrated that treatment of Achilles tendon iatrogenic inflammation with irap has the potential to improve tendon healing and could be considered as a treatment modality for Achilles tendon inflammation.

CORRELATION BETWEEN PATHOLOGICAL CHANGES, VIRUS AND REGULATORY T LYMPHOCYTES TROPISM IN ORGANS OF COWS AND HEIFERS INFECTED WITH BOVINE LEUKEMIA VIRUS

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Enzootic bovine leukosis is a chronic viral contagious disease of cattle caused by an oncogenic RNA Deltaretrovirus of *Retroviridae* family. It has an important economic impact because it causes reduction in milk production, increased culling rate, shorter longevity and increased susceptibility to secondary diseases. Clinical signs can vary from primary infection with flu like symptoms, persistent infection and persistent lymphocytosis which can lead to dysregulation of immune system and development of different secondary diseases, to lymphoma. In this study, gross and histopathological changes in BLV-seropositive cattle have been described for the first time in Croatia. According to recent literature data, regulatory T lymphocytes play an important role in progression of viral diseases. Their role in enzootic bovine leukosis is untill today not sufficiently investigated. This study reports first findings of Treg-lymphocytes in organs of BLV-seropositive cows. Recent data suggests new approaches for faster, highly sensitive and specific detection of BLV with real-time PCR. Aim of this study is to determine whether there is a correlation between pathological changes, virus and regulatory T lymphocytes distribution in organs and to evaluate the susceptibility to secondary diseases of BLV-seropositive cattle. Stage of the disease in which BLV-seropositive cattle were at the time of slaughter was determined with hematological analysis. Serological testing and real-time PCR was performed on blood samples to verify the absence of BLV infection in control group animals. Gross and histopathological examination of carcasses and organs from BLV-seropositive and BLV-negative animals was performed in order to investigate presence of changes caused by bovine leukemia virus and/or secondary bacterial and parasitic infections. Secondary bacterial and parasitic infections were additionally evaluated with bacteriological and parasitological examinations, respectively. Modified real-time PCR protocol according to HEENEMANN et al. (2012) for the detection of proviral DNA was performed on blood and organ samples of BLV-seropositive animals.Tracheobronchial lymph node, mesenteric lymph node and spleen were immunohistochemically stained for $FOXP3^+$ Treg-lymphocytes. Hematological examination showed statistically significant ($p<0,05$) increase in total leukocyte count of BLV-seropositive animals. Neutrophil count was also elevated ($p=0,051$), while no increase in lymphocyte and eosinophil count in BLV-seropositive animals was observed. Gross examination showed changes in organs of 20% of BLV-seropositive animals, of which most common pathological change was tracheobronchial lymph node hyperplasia. Histopathological findings in both BLV-seropositive animals and control group were scored mostly mild and include interstitial pneumonia, tracheobronchial lymph node reactive hyperplasia, myocardial sarcocystosis,

lymphoplasmacytic infiltrate in myocard, liver, renal interstitium and abomasal mucosa, mixed inflammatory cell infiltrate in intestinal mucosa and submucosa and mesenteric lymph node reactive hyperplasia. However, findings in spleen of BLV-seropositive animals differ from those in control group. Statistically significant ($p<0,05$) increase of neutrophil infiltrate score was found in marginal zone of spleen in BLV-seropositive animals. The degree of reactive hyperplasia of spleen was also significantly ($p<0,05$) higher than in control group. Modified real-time PCR protocol according to HEENEMANN et al. (2012) detected proviral DNA in blood samples of 70,77% and in organs of 60,99% BLV-seropositive animals. To determine tropism of BLV for certain organs, real-time PCR results of 7 organs from 26 animals were compared to their real-time PCR results in blood. Proviral DNA was detected in tracheobronchial lymph nodes and lungs of all animals with positive blood results (100%), 94,44% in spleen, 83,33% in liver and mesenteric lymph node and 77,78% in heart and kidney. Immunohistochemical staining showed higher average value of Treg-lymphocytes in traheobronchial lymph nodes in BLV-seropositive animals (9,05) compared to control group (7,06). Beta hemolytic *Streptococcus* sp. was isolated from 2 lung samples and 2 liver samples from BLV-seropositive animals. Parasitological examination of feces showed negative results in 53,12% of BLV-seropositive animals and 70% of control group. Positive results consisted of very low number (<10 eggs/gram) of strongylid eggs, *Eimeria* sp. oocysts, *Giardia duodenalis* and *Buxtonella sulcata* cysts. No *Cryptosporidium* sp. oocysts were found.

BLV-seropositive animals were in persistent infection stage of the disease at the time of slaughter. Gross and histopathological examination, as well as bacteriological and parasitological examination showed no secondary bacterial nor parasitic infections in BLV-seropositive animals. Potential viral tropism to trachebronchial lymph nodes and lungs of BLV-seropositive animals was determined with modified real-time PCR protocol according to HEENEMANN et al. (2012). Real-time PCR results in blood and organ samples from BLV-seropositive animals indicate lower sensibility of this method compared to results from aforementioned paper. Significantly higher total leukocyte count and higher neutrophil count hematologically, as well as statistically significant increase of marginal zone neutrophil infiltrate and degree of reactive hyperplasia of spleen in BLV-seropositive animals is a new finding in cattle which suggests a B-helper role of neutrophils in spleen marginal zone. These neutrophils activate B-cells and enhance their antibody production to different antigens, in this case probably to BLV. Higher average value of Treg-lymphocytes in tracheobronchial lymph nodes of BLV-seropositive animals compared to control group indicates their potential immunosuppressive function during early stages of infection.

MOLECULAR EPIZOOTIOLOGY OF BOVINE RESPIRATORY SYNCYTIAL VIRUS

INFECTION

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Bovine respiratory syncytial virus (BRSV) infection represents one of the most important respiratory tract disease in cattle. High degree of genetic and antigenic diversity among BRSV isolates, high mutation rate and tolerance to mutation fixation result in reccurent infections, short term immunity and vaccination ineffectiveness. There is a lack of comprehensive information about the prevalence of infection in cattle herds in Croatia as well as in other countries outside the European Union. The aim of this thesis was to investigate molecular epizootiology of BRSV strains circulating in Croatia within time period December 2011 – April 2016.

Molecular epizootiology of BRSV infection in Croatian beef herds was investigated using RT PCR and semi nested PCR for the gene coding for BRSV glycoprotein G on samples of nasal swabs, lungs, lymph nodes, sera, liver and spleen. Study also included samples recived within regular respiratory disease diagnostic activities. Phylogenetic analysis was performed on the most representative sequences obtained, direct imunofluorescence test on nasal swab smears and virus isolation on continious and primary cell cultures. Seroprevalence estimation was carried out using direct and indirect enzyme-linked immunosorbent assay (ELISA).

Estimated antigen prevalence is 60% in herds included in the study. Phylogentic analysis revealed clustering of Croatian strains in genotype II and V, while nine isolates failed to cluster in any of existing six genotypes indicating existence of new BRSV genotype. These findings are strongly supported by bootstrap values (100) obtained by bootstrap method in 2000 repetition. For the first time permisivness of canine adipose derived mesenchymal stem cells (cASCs) for BRSV was demonstrated.

Findings from this study indicate a high prevalence of BRSV infections in beef cattle in Croatia, which may have a significant influence on health status and animal performance. Revealing new genotype existence is important for production of efficient vaccine. Virus affinity for the stem cells reveal the need for estimation of the role of stem cells in viral pathogenesis and makes stem cells potential new model for investigating viral peculiarities *in vitro*.

/ / / / / POSTER LIST / / / / /

THREE DIFFERENT TYPES OF HOSTS FOR FASCIOLOIDES MAGNA IN WILD ANIMALS

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Digenean trematode *Fascioloides magna* (large American liver fluke) is a parasite of primarily North American deer species. According to the genetic evidences it was introduced to Europe at least two times, due to the introduction of for Europe exotic deer species like white-tailed deer or wapiti. Finding suitable conditions for maintaining of its life cycle, it expanded its distribution through the Danube River valley and continued through all the surrounding floodplains. It has been diagnosed in Croatia over 16 years ago and has expanded from Baranja region to almost central Croatia. In wild animals we can find three different hosts depending on the pathogenesis, clinical signs and manifestation of the disease. These include definitive hosts, dead-end hosts and aberrant hosts. Definitive hosts in Croatia are red deer and fallow deer. Parasites mature in the pseudocyst in the liver parenchyma. In each pseudocyst are two or more mature flukes which are producing high number of eggs. Some of the pseudocysts have communication with the bile ducts and this is the way through which they are excreting the eggs to the gastrointestinal system. In dead end hosts (wild boar) parasite reaches the liver, but rarely matures, and if eggs are produced they are not shed to the environment. Parasite stays isolated inside the pseudocyst. The aberrant hosts, there is no pseudocyst formation and flukes constantly migrate leading to excessive tissue damage. Aberrant wildlife hosts in Croatia are roe deer, mouflon and chamois. This hosts usually succumb to the disease due to excessive bleeding and destruction of the liver tissue. Infection with *F. magna* is also possible in domestic ungulates. Physiologically, parasites are trying to live in balance with their final hosts, which enables them both to survive. This three totally different manifestations of infection by *F. magna* are interesting for future research and for monitoring of host-pathogen relationship.

Title of PhD thesis: Variability of the genes of major histocompatibility complex in red deer (*Cervus elaphus*) in relation to *Fascioloides magna* infection

Financed by: Croatian Science Foundation, grant UIP 3421 ''Molecular epidemiology of selected parasitic diseases of wildlife".

Mentor: Assist. Prof. **Dean Konjević**, Dipl. ECZM

MORPHOLOGY OF ORGAN DYSFUNCTION IN DOGS WITH LETHAL BABESIA CANIS INFECTION

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Babesiosis, caused by tick-transmitted, hemoprotozoan parasite *Babesia canis,* is a hemolytic disease which significantly affects Croatian dogs. Organ dysfunction in this systemic infection was so far accurately described through hematological and plasma proteins deviations. Pathological survey on dogs that died due to *B. canis* infection has never been systematically conducted.

To describe morphology of organ dysfunction, 17 dogs found with jaundice, anemia, hepatosplenomegaly, pigmenturia, parenchymal and serosal petechiation were dissected during a two-year period. Genotyping from affected organs confirmed *B. canis*, as a cause of infection in all dogs. Lungs, heart, liver, spleen and kidney were selected for histology.
In all cases, minimally three organs were morphologically changed. In the lungs of 16 dogs, capillary dilation and multifocal necrosis of alveolar septa followed with leakage of protein-rich fluid and erythrocytes caused hemorrhagic edema. Liver damage was evident in all dogs in form of centrolobular necroses, compressive atrophy, degeneration and dissociation of hepatocytes, mostly caused by hypoxia or severe diffuse dilation of sinusoids and spaces of Disse due to accumulation of protein-rich fluid. Massive red pulp histiocytosis with marked extravascular hemolysis was a constant finding in spleen samples, while segmental infarctions and hemorrhages were found in five dogs only. In 10 of 17 dogs, renal lesions differed from mild tubular epithelial degeneration, pigmenturia with pigment and protein tubular cast formations up to necrosis of tubular epithelial cells. Myocardial lesions were recorded in six dogs; it consisted of endomisial edema, focal necroses and hemorrhages. Presence of microthrombi within capillaries indicating disseminated intravascular coagulation were present in the liver of three dogs and in the lungs of five dogs.

B. canis causes vital organ damage by hypoxia due to hemolysis and massive capillary network dilation accompanied by increased vascular permeability. Cellular atrophy, and degeneration as well as single cell necrosis are consequence of aforementioned pathogenesis. Focal or disseminated segmental necroses and hemorrhages are direct outcome of capillary and/or venous and arterial thrombosis.

Topic of the PhD thesis: Postmortal and molecular research of bacterial and protozoal vector-borne diseases of cats and dogs

Mentors: Assoc. Prof. **Ana Beck, Relja Beck**, PhD, DVM

A SHEEP MODEL FOR AUTOLOGOUS OSTEOCHONDRAL BIOREACTOR ENGINEERED GRAFT TRANSPLANT

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A sheep model of chronic osteoarthritis was used. 24 animals were included in the study, and 48 defect sites were obtained for the analysis. A full-thickness cartilage defect placed on lateral and medial femoral condyles was used to accomplish that.

The protocol included two surgical procedures. During the first procedure two cartilage defects, 4 mm in size were created on the lateral and medial femoral condyle with a standard mosaic-plasty kit. Cartilage specimens were harvested from the created defect. In the nasal cartilage group, nasal cartilage was harvested from the nasal septum. After the first procedure the animals were transferred to the farm for a period of 6 weeks, which enabled construct production, A perfusion bioreactor was used for graft production. The articular and nasal chondrocytes were expanded and seeded into a biphasic collagen/hydroxyapatite scaffold. After 6 weeks, the second surgical procedure took place, as the sheep were divided into four treatment groups: 1) CTR: Empty defect – negative control; 2) CFS: Cell-free scaffold; 3) AC: Articular chondrocytes cartilage graft; 4) NC: Nasal chondrocytes cartilage graft. Before the implant, 4 mm chondral defects were converted to 6 mm osteochondral defects, and the bioreactor-engineered grafts were implanted (Figure 1). In the AC and NC groups, engineered autologous grafts were implanted into the defect site. In the CFS group, the defect sites were treated with cell free scaffolds. CTR sheep underwent a defect conversion surgery, but without implantation. Two animals from each group were euthanized after 6 weeks, 3 months and 12 months, respectively, and the specimens harvested for analysis. The specimens were analyzed by gross examination, histology, and μCT. Each specimen was divided into three levels – PLATE, DEFECT and SUBCHONDRAL (Figure 2). Before specimen excision, defects were photo documented and evaluated with semi quantitative macroscopic scoring system developed by ICRS. The medial and lateral condyles containing the cartilage defects were removed and divided into two halves. Undamaged medial or lateral condyle of the contralateral knee joint were taken for control.

The purpose of this study was to test the feasibility and safety of using bioreactor-based tissue engineered cartilage grafts for the regeneration of articular cartilage in the knee. The purpose of this thesis is to analyze subchondral bone microarchitecture of the explanted osteochondral units using μCT.

Mentor: Prof. **Dražen Matičić**

THE INFLUENCE OF DAIRY COWS AGE AND CALVES GENDER ON THE STRESS LEVEL IN EARLY WEANING

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In the first 24 hours after calving, a strong bond between dam and calf is created. In natural conditions, this bond would last for a period of six to nine months before the natural weaning occure. Under intensive breeding conditions, calves are often forcedly separated from their mothers immediately or several hours after birth. This procedure is called early weaning and does not imply only the denial of suckling, but also includes the physical separation of the calf from the dam, and presents an extreme stress in terms of adaptation to many physical and social factors of the environment. The stress response in the early weaning is very complex and most often accompanied by an increase of cortisol concentrations in serum, saliva, milk and other secretions, and in significant changes of animal behaviour. These behavioural changes can be identified through the assessment of the emotional state of the animal, ie the recognition of several specific behavioural patterns such as frequent movement (reciprocally search) and frequent vocalisation which sometimes is followed by highlighting the sclera. Above mentioned patterns of behaviour are often interrupted by the periods in which animal express a certain form of depression – periods of "protest" or "despair". Early weaning presents stress both for calves and mothers, and it is justifiably considered to be one of the leading problems of animal welfare in intensive dairy production.

The study will investigate the influence of early weaning on the occurrence of specific behaviours in both categories of cattle (cows and calves), and cortisol concentrations in cow's milk and calves saliva. With further distribution of cows to age groups and calves according to gender, study will show whether there are differences in their response to early weaning. Outcomes of the study will be used for improving the breeding conditions of calves and dairy cows, respectively reducing the level of stress at early weaning.

Mentor: Prof. **Željko Pavičić**

GENDER INFLUENCE ON THE EXPRESSION AND DISTRIBUTION OF NA/K-ATPase IN PORCINE SMALL INTESTINE

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Na/K-ATPase is an enzyme found in animal and human cell membranes. It actively transports sodium and potassium out and into the cells, respectively. By doing so it regulates resting potential, transport and cellular volume. In the intestines it is localized to basolateral membrane enterocytes where it facilitates maintenance of the sodium ion gradient required for transepithelial transport of nutrients with sodium dependent cotransporters located on the brush border membrane of enterocytes.

The aim of this study was to determine the influence of gender on the Na/K-ATPase expression and distribution along the small intestine of pigs. The study was conducted on 20 pigs Swedish Landrace breed divided into 4 groups (males, females, castrated males, castrated females). Tissue samples were taken from duodenum, jejunum and ileum and were analyzed by immunostaining of tissue cryosections and Western blotting.

Preliminary results indicate the existence of gender dependent differences in the Na/K-ATPase expression in pig's small intestine. The expression of Na/K-ATPase shows significant differences between genders in the proximal part of the small intestine, whereas the differences in expression between genders decrease in the distal part of the small intestine. Differences in the expression in pigs of the same gender but different groups (male/castrated male and female/castrated female) suggest that sex hormones may affect the Na/K-ATPase expression in the small intestine. The distribution of Na/K-ATPase along the small intestine shows a significant increase of expression towards the distal part of the small intestine which could indicate that the distal parts of small intestine are more active in transepithelial transport of nutrients with sodium dependent cotransporters.

Topic of the PhD thesis: Influence of gender and castration on the expression and distribution of transporters involved in transepithelial glucose transport in porcine small intestine

Mentors: Assist. Prof. Hrvoje Brzica, Prof. Marcela Šperanda

MOLECULAR DETECTION OF CHLAMYDIACEAE IN FREE-RANGE CHICKEN FLOCKS

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Recently, three chlamydial agents (*Chlamydia psittaci*, *C. gallinacea* and *C. avium*) were associated with avian, with *C. psittaci* and *C. gallinacea* being detected in chicken flocks. *C. psittaci* infection may or may not result in clinical manifestations but is considered a highly zoonotic agent. The occurrence of *C. gallinacea* results in poor production performance; however, its zoonotic potential remains to be investigated. With free-range poultry production becoming popular, increasing its production performance while keeping the flock healthy is among the priorities. The investigation on the presence of *Chlamydia* species in free-range poultry flocks will contribute to the understanding of the impact of these bacteria on poultry production and health performance.

This study detects *Chlamydia* spp. in free-range poultry flocks in Croatia and in the province of Bohol, Philippines. Triple swabs (conjunctival, pharyngeal and cloacal), litter-fecal and/or water samples were collected from 70 free-range chicken flocks in Croatia (n=54) and in the province of Bohol, Philippines (n=16). Detection of *Chlamydiaceae* was done by real-time qPCR assay, targeting the *Ch23S rRNA* gene. Samples with Cq value of $\leq$40 were considered positive. A flock with at least one positive sample is considered *Chlamydiaceae*-positive.

qPCR assay detected *Chlamydiaceae* in 62.86% (44/70) of flocks examined. Thirty out of 54 (55.56%) and 14 out of 16 (87.50%) flocks from Croatia and the Philippines, respectively, were *Chlamydiaceae*-positive. These *Chlamydiaceae*-positive samples will be subjected to specific assay for *C. psittaci*, *C. gallinacea* and *C. avium*. Furthermore, samples which are positive for species-specific qPCR assay will be sequenced for phylogenetic analysis.

The results showed that *Chlamydiaceae* is prevalent in free-range chicken flocks. Furthermore, this is the first report of *Chlamydiaceae* detection in free-range chicken flocks in Bohol province, Philippines.

Topic of the PhD thesis: Prevalence and molecular characterization of *Chlamydia* spp. in free-range poultry flocks in Croatia and in the Philippines

Mentor: Prof. Estella Prukner-Radovčić, Dipl. ECPVS

BIOCHEMICAL AND MOLECULAR ANALYSIS OF ESCHERICHIA COLI ISOLATED FROM FOOD OF DIFFERENT ANIMAL ORIGIN AND CARCASS SWABS

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Escherichia coli (*E. coli*) is a Gram negative, aerobic and facultatively anaerobic, non-sporogenic, rodshaped bacterium, member of the *Enterobacteriaceae* family. It is commonly found in the intestine, but some serovars can cause intestinal and other infections. Pathogenicity is linked to the presence of virulence genes, like verotoxin coding genes (*vtx*) and intimin (*eae*), as well as phylo-group determination and biochemical properties. Most *E. coli* that cause intestinal infections belong to phylo-group A and B1, while virulent ekstraintestinal strains mostly belong to phylo-group B2. Biochemical properties, virulent profile and phylo-group of the strains will be determined. A link between biochemical proprerties, presence of virulence genes and phylo-group will be researched in strains isolated from food of different animal origin with the purpose of evaluating *E. coli* zoonotical potential in Republic of Croatia.

Topic of the PhD thesis: Biokemijska i molekularna analiza izolata bakterije Escherichia coli izdvojenih iz hrane životinjskog podrijetla i obrisaka klaoničkih trupova

Mentors: Assist. Prof. Andrea Humski, Assoc. Prof. Vesna Dobranić

///// ONE HEALTH /////

ONE HEALTH APPROACH IN HUMAN AND ANIMAL INFECTIOUS DISEASES CONTROL

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One Health approach is a worldwide strategy for widening interdisciplinary collaborations in all aspects of health care for humans, animals and the environment. Recognizing that human health, animal health, and ecosystem health are inseparably linked will accelerate biomedical research and expand the scientific knowledge base which will result in enhancing public health efficacy and improvement of the medical education and clinical care. Collaborative effort of multiple disciplines has already shown its value in the field of the infectious diseases surveillance and control. It is estimated that six out of ten human infection agents has been transmitted from animals showing clear connection between animal and human health. Emergence of many infectious diseases that have recently appeared within a population or those whose incidence or geographic range is rapidly increasing can be linked to increasing contact between humans and animals, intensification of food production, expansion of international travel and the climate changes.

In Croatia the first larger scale outbreak caused by emergent animal pathogen was at the beginning of the 21st century. In 2001 blue tongue disease was recorded for the first time and has become endemic ever since. There is clear evidence that blue tongue spreading and endemisation is the result of climate changes as well as new vectors involved in disease transmission. Like in the case of blue tongue, over the last few decades viral infectious diseases appear to be emerging and spreading faster than ever with vector borne viruses are the most commonly identified in Croatia and world-wide. In 2010 the first autochthonous case of Dengue fever was detected in humans in Croatia. At the same time viral activity of the West Nile virus was identified in sentinel horses with first neuroinvasive cases in humans in 2012 and ever since. In 2013 it was confirmed that the Usutu virus was identified as the new cause of human neuroinvasive disease in Croatia only two years after the confirmation of the Usutu virus's circulation in horses. More recently, during 2016, Lumpy Skin Disease had also started spreading in the southeast Europe and had reached the Croatian border. During 2016, imported human clinical cases of Chikungunya and ZIKA virus infections were reported. Competent vectors for Chikungunya and ZIKA virus are already present with in Croatia which means we can have an outbreak at any time.

The appearance of new emergent infectious diseases in Croatia encouraged an institutional collaboration which established surveillance of emerging infectious diseases. Close collaboration between veterinary service and human medicine is necessary so we can be prepared for any outbreak that would put human and animal health at risk.

ONE HEALTH CONCEPT: IS THERE A WALL OR A BRIDGE BETWEEN HUMANS AND ANIMAL PARASITES?

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Parasites constitute a diverse group of organisms that take benefit from another (a „host"), usually causing some damage to it. They may affect a wide range of animal and plant hosts. Zoonotic are those parasites that can be transmitted from animals to humans. Parasitic infections can be spread in a number of ways through contaminated water, food, waste, soil, and blood. Furthermore, parasitic arthropods, besides being annoying (e.g. ticks, fleas, flies, mosquitoes, phlebotomine sandflies etc.), are vectors of a large number of bacteria, viruses, and parasites, affecting animals and humans worldwide. Vector-borne diseases are generally spread during feeding (blood sucking), they impact human and animal health and cause millions of dollars in losses annually. Control of these diseases is complex and expensive.

If the zoonotic potential is considered as a „bridge" through which an animal parasite is transmitted to humans, logically someone may assume the existence of a „wall", preventing infection with the non-zoonotic parasites. Indeed, parasites normally do not infect different species of animals indiscriminately under natural conditions; they show varying degrees of preference for hosts and for habitats within them. On the other side, incidental (syn. accidental) parasites are those which occasionally appear in unusual hosts under natural conditions. Incidental parasites usually do not survive, or at least do not reproduce in the „faulty" host, but in some cases, they can be extremely pathogenic because of a lack of the evolutionary adaptation. Zoonotic parasitic infections represent a major public health issue not only in developing countries but also in developed nations, such as in the USA and European countries.

The One Health concept is a global strategy for widening of interdisciplinary collaborations in all aspects of humans, animals and the environment health care. Besides adequate education and experts training, animal owners, (in fact, all potentially exposed individuals) should be properly instructed about the diseases, and this kind of education plays an important role in the prevention (or even eradication) of certain zoonotic parasitic diseases. People should be educated how to avoid infection with zoonotic parasites – this implies keeping up on routine veterinary care for animals, but also hand washing, attempting to drink only clean (tap) water and thoroughly washing or cooking foods. Parasites also can be the cause of some food-borne diseases.

Both protozoan and helminth parasites can be found in muscle tissue. *Toxoplasma* and *Sarcocystis* are examples of zoonotic protozoans infective to humans and not being searched during meat inspection. Instead, consumers at risk (and animal owners) should be advised not to eat, but also, not to offer raw meat to animals. Helminths are larger in size, and some of the species can be seen by naked eye, but a few can't. Of helminths, *Taenia* and *Trichinella* species can be transmitted to humans after consumption of the raw meat or meat products, and official meat inspection is regulated by the law. Problems appear after „backyard slaughter" along with meat inspection avoidance before consumption (human, but also animal). Indeed, to prevent meat-borne parasitic infections, it is the best to advise thoroughly meat cooking, although this is not always the most desirable procedure from a culinary standpoint.

ONE HEALTH CONCEPT: THE IMPORTANCE OF RATIONAL ANTIMICROBIAL DRUGS USE

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Antimicrobials are used widely in veterinary medicine. This includes therapeutic use to treat clinically sick animals. It also includes non-therapeutic use such as for growth promotion and/or as prophylaxis to try to prevent infections developing in food animals. The advantages generated by the use of antimicrobials for food animals transcends more than just the well-being of the animals, as it has also brought about economic benefits for the food animal producers and a more secured and safer health for the general public. Nevertheless, there are conflicting opinions regarding the proper role of antimicrobials in the production of livestock and poultry.

As there are only a limited number of antimicrobial classes available and as a range of antibiotics is required to treat the many different species of animals that face particular disease threats, some classes of antibiotics are used in both people and animals. Certain antibiotic classes are categorised by the World Health Organization (WHO) as *"critically important antibiotics (CIAs)"* for human use, of which several are designated as "highest priority critically important antibiotics (HP-CIAs)". In December 2014, the European Medicines Agency (EMA) published scientific advice on the risk to humans from antibiotic resistance caused by the use of HP-CIAs in animals. Based on the current scientific evidence supporting a reduction of antibiotic usage practices because they may pose a serious risk to both animal and human health through ever-increasing rates of antimicrobial resistance, the recommendations provided by the EMA gives veterinary surgeons an important piece of scientific evidence to factor into their clinical treatment decision making and to responsibly prescribes antibiotics.

In conclusion, it is important to stress that the unnecessary or wasteful use of antibiotics should be reduced when their use for a particular disease condition is not efficacious or when non-antibiotic solutions are readily available. It is upon this common ground that the human and veterinary medical communities call for the proper and prudent use of antibiotics.

CANINE AND FELINE OBESITY - A MODERN LIFE DISEASE

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Basic postulate of One Health is that the health of people is connected to the health of animals and the environment andas a consequence animals also share our susceptibility to some diseases. There is a tendency for increase in obesity among people and companion animals in developed countries. Obesity is the most common form of malnutrition in small animal practice and 25% to 40% of cats and dogs are overweight or obese. The levels of human obesity vary from 5% in some Asian countries to over 30% for adults in the USA.

Obesity is a clinical syndrome that involves the excess accumulation of body fat and can have effects on health. Obesity has been associated with an increased incidence of many diseases in humans and companion animals, e.g. *diabetes mellitus*, arthritis, hepatic lipidosis, urine incontinence, infertility, reduced life expectancy, urinary tract diseases, constipation, cardiovascular issues, respiratory issues, dermatitis, and increased anesthetic and surgical risk. In human medicine obesity is usually diagnosed by Body Mass Index (BMI) and by measuring body fat (bioimpedance and dual energy X-ray absorbtiometry), and in veterinary medicine morphometric methods are commonly used for assessing the body condition of dogs and cats (Body Condition Scores - BCSs).

In both humans and companion animals obesity develops when energy intake exceeds daily energy consumption, and many environmental and social factors contribute to the development of obesity. The most common factors are similar for animals and humans: decreased daily exercise and overfeeding. Sometimes food is used as a palliative agent for stress situations or it is a sort of compensation for lack of time spent in quality physical activity. We humans also tend to eat the same volume of food or feed our animals with the same volume of food despite changes in energy requirements and despite the facts that daily energy requirements vary according to the environmental temperature, growth, pregnancy, lactation, age, the activity level etc. Several studies showed a relationship between obesity in dogs and their owners: for an owner that is obese it is more likely that their dog will be obese.Also, an important factor for obesity in dogs is a tendency of owners to humanise the animals and communicate through food; the owners of the obese dogs care less about their own health and are of lower social status.

For adequate understanding, prevention and treatment of animal obesity it is important to investigate human obesity. Along with special diets, a healthy lifestyle with enough exercise is likely to have a positive effect on the prevention of a number of human and animal lifestyle-related diseases.

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