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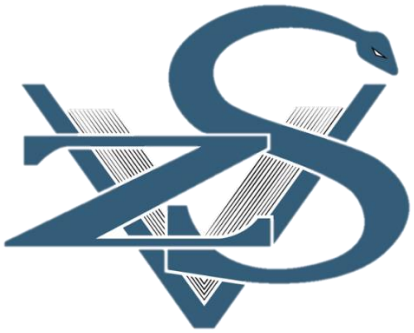
BOOK OF ABSTRACTS

FACULTY OF VETERINARY MEDICINE

UNIVERSITY OF ZAGREB

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9TH

INTERNATIONAL CONGRESS

“VETERINARY SCIENCE AND PROFESSION”

/ / / / OCTOBER 9TH 2021 / / / /

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Dear Colleagues,

It is with great pleasure that I welcome you to the 9th International Congress "Veterinary Science and Profession" on behalf of the Organising Committee.

This year we meet in an online format with the programme divided into six sessions – Veterinary Public Health and Emerging Diseases, Farm Animals, Horses, Exotic and Wild Animals, Small Animals and Free Communications.

We are pleased to welcome a panel of distinguished invited speakers from multiple countries, presenting us with the latest trends in veterinary science and profession.
COVID-19 marked our lives during the past 2 years and this hot topic will be discussed with focus on our companion animals – dogs and cats. We also have an impressive number of abstracts from a wide variety of topics, that are exhibited as e-posters on the Congress web page.

We would like to thank all participants, sponsors and invited speakers who have made the Congress possible in this online format.

We hope you discover new ideas and techniques during the Congress.

Wishing you all the best!

Assoc. Prof. Zoran Vrbanac, DVM, PHD, DECVSMR, DACVSMR

President of the Organising Committee

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/ / / / / PROGRAMME / / / / /

OCTOBER 9TH 2021

09:00–09:10 Welcome

HOT TOPIC

09:10–09:30 **Vladimir Stevanović** (CRO)

- ***Is COVID-19 a new disease of dogs and cats?***

VETERINARY PUBLIC HEALTH AND EMERGING DISEASES SECTION

Chairpersons: Nevijo Zdolec, Vladimir Stevanović

09:30–09:55 **Dragan Antic** (UK)

- ***Abattoir interventions in a risk-based meat safety assurance system***

09:55–10:20 **Tamaš Petrović** (SRB)

- ***African Swine Fever in Serbia: challenges of controlling the spread of infection***

HORSES SECTION

Chairpersons: Nika Brkljača Bottegaro, Jelena Gotić

10:20–10:45 **David Sutton** (UK)

- ***A guide to investigating dysphagia and abnormal feeding activity in the horse***

10:45–11:10 **Marco Marcatili** (UK)

- ***Wounds with synovial structure involvement: diagnosis and management***

11:10–11:20 Break

EXOTIC AND WILD ANIMALS SECTION

Chairpersons: Maja Lukač, Magda Sindičić

11:20–11:45 **Alessandro Massolo** (ITA)

- ***The Old world keeps invading the New one: a lecture from the invasion of the European strain of Echinococcus multilocularis in North America***

11:45–12:10 **Endre Sós** (HUN)

- ***The principles of wild bird medicine – biodiversity in your practice***

SMALL ANIMALS SECTION

Chairpersons: Zoran Vrbanac, Marin Torti

12:10–12:35 **Roswitha Dorsch** (GER)

- ***Ureteral obstruction in cats***

12:35–13:00 **Cristian Falzone** (ITA)

- ***Ischemic myelopathy and most common differential diagnosis in dogs and cats***

13:00–13:10 Break

FARM ANIMALS SECTION

Chairpersons: Nino Maćešić, Sven Menčik

13:10–13:35 **Vlado Vuković** (MKD)

- ***Pig breeding challenges in the next decades***

13:35–14:00 **Zhihua Jiang** (USA)

- ***Advancing Genome to Phenome Research in Swine: Progress and Perspectives***

14:00–14:10 Conclusions and closing words

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/ / / / / INVITED LECTURES

IS COVID-19 A NEW DISEASE OF DOGS AND CATS?

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COVID-19 pandemic started in late December of 2019 in China and has since spread around the globe resulting in millions of human infections and deaths. Causative agent SARS-CoV-2 is a betacoronavirus closely related to human SARS-CoV and some bat coronaviruses. Early epidemiological data pointed to wet markets as the most probable place where SARS-CoV-2 had gained access to the human population. In Asian wet markets, various domestic and wild animals are sold together. It is a unique place for the spillover of zoonotic pathogens to the human population, as had been the case in 2002. Like in 2019, in 2002, another animal coronavirus (SARS-CoV) spread from China around the world, but the outbreak was contained after approximately nine months following strict infection control measures.

First cases of natural infections of dogs and cats only a few months into the pandemic sounded an alarm among pet owners and veterinary practitioners. Susceptibility of cats and dogs was confirmed in experimental conditions, with cats much more permissive to infection and shedding virus in higher titre. Additionally, experimentally infected cats and dogs did not show any signs of clinical disease.

At the time of writing, several epidemiological and clinical studies of SARS-CoV-2 infections in dogs and cats have been concluded in Croatia. Probably the most remarkable is the portion of animals with evidence of SARS-CoV-2 infection. In the households with confirmed human coronavirus disease 2019 (COVID-19) cases, 43.9% of dogs and 20.83% of cats tested enzyme-linked immunoassay (ELISA) positive. A high level of animal exposure was also evident in the general population at the end of the second wave of pandemics. In December 2020, 18.56% of dogs and 13.79% of cats in the general population tested serologically positive. When compared to the results of serosurvey in the human population, there was no statistically significant difference in seropositivity rate between dogs, cats and humans.

As SARS-CoV-2 is a newly emerged pathogen, the consequences of infection to animal health are still not fully understood. In the beginning, SARS-CoV-2 was considered a respiratory pathogen of humans, often prograding to pneumonia. The same was true for the first cases of natural infection in cats and dogs. Some owners of infected animals observed mild respiratory or gastrointestinal disease. In the study of cats and dogs from

COVID-19 positive households in Croatia, mild respiratory and gastrointestinal disease signs were often noticed in pets during owner self-isolation. Still, cats and dogs showing clinical signs did not test more positive to SARS-CoV-2 antibodies.

Today, COVID-19 is defined as a multisystemic human disease with long-term sequels in some cases. The clinical study in over a thousand dogs admitted to the Veterinary Teaching Hospital at the Faculty of Veterinary Medicine in Zagreb has shown some possible long-lasting effects of SARS-CoV-2. Dogs with clinical signs of the acute central nervous system (CNS) disorder were 1.97 times more likely to test positive for SARS-CoV-2 IgG antibodies. Antibodies of IgG class are produced later on in the course of infection, so observed CNS disorders cannot be attributed to the acute infection but rather as a subacute or chronic clinical manifestation.

At the moment, there is no indication that dogs and cats have any role as a source of human infections and the virus is spread by human-to-human transmission. Our studies gave strong evidence that the infection incidence among pet animals and humans is not very different. It seems possible that dogs and cats could have a more significant role in the epidemiology of SARS-CoV-2 infections in the future. Recent evidence of infections in pet animals with new SARS-CoV-2 variants raises the possibility that a new virus variant could also emerge in animals.

Finally, there is a strong possibility that pets are not just being infected, but infection affects their health. It is essential to determine the extent of this effect and risk factors and behaviour to protect animal health without affecting other welfare aspects.

ABATTOIR INTERVENTIONS IN A RISK-BASED MEAT SAFETY ASSURANCE SYSTEM

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Implementation of successful interventions against relevant microbial hazards in the meat chain up to and including the chilled carcass stage is now recognised as an essential component of a risk-based meat safety assurance system (RB-MSAS). In such a system, high-risk animal batches are subjected to additional slaughter hygiene control measures complemented with (hide and meat) interventions (Blagojevic et al., 2021). Therefore, the use of interventions is intended to accomplish the meat safety targets on chilled carcasses, particularly in situations when an abattoir is unable to sufficiently reduce risks arising from specific farms/animal batches by using process hygiene alone. Furthermore, interventions are considered whenever food safety authorities identify meat production processes associated with high risks for consumers (Antic et al., 2021). This paper overviews the role of abattoir interventions in a risk-based, meat safety assurance system.

The most relevant meat-borne biological hazards categorised by the European Food Safety Authority (EFSA) as of high- or medium-priority for control in the beef chain are *Salmonella* and verocytotoxin-producing *Escherichia coli* (VTEC), and in the pork chain *Salmonella* and *Yersinia enterocolitica*. In many cases under commercial conditions, reliance on Good Manufacturing Practice/Good Hygienic Practice (GMP/GHP) and Hazard Analysis and Critical Control Point (HACCP) based procedures is not sufficient to control microbial contamination. Therefore, it must be accompanied by implementation of appropriate additional interventions. In some countries, e.g. the USA, decontamination treatments of hides and carcasses are regularly used and integrated within HACCP system; such interventions have not been commonly used under commercial conditions in Europe. The EU Regulation 853/2004 allows the use of decontamination treatments during slaughter, following appropriate consideration and a risk assessment by EFSA and approval of such treatments by the regulatory authorities.

In the RB-MSAS, the regulatory authority will set meat safety targets for chilled carcasses in abattoirs. The targets (Performance Objectives; POs) need to be clear and measurable and need to contribute to the Food Safety Objective and Appropriate Level of Protection. The system is supposed to be coordinated by a risk manager who will be responsible for adjusting control options in the farm-to-abattoir meat chain, ultimately ensuring that the hazard-based targets for chilled carcasses are achieved. This can lead to different decisions, e.g. whether high or low risk animals will be sent to high or low risk abattoirs; and to use additional abattoir interventions when these are the only available solution to meet the microbiological targets in carcasses (Blagojevic et al., 2021).

Interventions are usually GHP- and hazard-based measures. GHP-based measures are founded on empirical knowledge and experience (e.g. hide removal methods,

rodding, bunging, knife-trimming, chilling, equipment sanitation). Such measures serve as pre-requisites to, and complement, the hazard-based interventions that are evidence-based, i.e., developed from scientific research to control certain hazard(s). The examples include a range of skin and carcass interventions mostly aiming at microbial removal, immobilisation or elimination, and they provide demonstrable and quantifiable reductions in hazard loads.

Categorisation of cattle based on their cleanliness significantly reduces (by about 1 log) the microbial contamination, including faecal microbiota, of resulting beef carcasses. In contrast, hide water washing of live cattle in lairage with ambient temperature water and hide clipping are both largely ineffective. Chemical decontamination or hide clipping of live cattle are not recommended due to animal welfare concerns and/or practical considerations. Cattle hide interventions, i.e., chemical hide washes and microbial immobilisation treatment with shellac, significantly reduce transfer of aerobic bacteria and *Enterobacteriaceae* to beef carcasses by 1–1.5 logs. Therefore, they are recommended to be applied post-exsanguination and before dehiding, as proactive interventions, to reduce microbial contamination of resulting beef carcasses. Beef carcass hazard-based interventions to control microbial contamination after dehiding and pre-chilling can also be recommended. Carcass pasteurisation treatments with hot water and/or steam are efficacious against microorganisms when temperatures of carcass surfaces achieve more than 70°C. These treatments reduce indicator bacteria by 1–2.5 logs, with an additional reduction of 0.5–1 logs if organic acids are used sequentially. The time-temperature combinations required to achieve significant reductions are specific to an individual commercial abattoir and subject to validation. Chemical washes, particularly with lactic, acetic or citric acids, are efficacious, reducing all indicator bacteria by 1–1.5 logs. Knife trimming and steam vacuuming are also highly efficacious, reducing all indicator bacteria by 1–2 logs. However, the reductions achieved highly depend on the skill and diligence of the operator to spot visible contamination and efficiently remove it, and these interventions' parameters are difficult to optimise to achieve a consistent effect. Carcass water washing to remove microorganisms is largely ineffective, with reductions up to 0.5 log achieved. Use of multiple, sequential carcass interventions has the biggest impact on microbial reduction on beef carcasses; reductions are up to 3 logs, greater than any of these interventions applied alone (Antic et al., 2021).

GHP-based measures for pig carcasses, such as scalding and singeing, have a prime purpose to remove pigs' hair, but they do contribute in reducing microbiological contamination. Scalding under commercial conditions can reduce aerobic bacteria by 3 logs, and singeing by additional 2 logs. Other GHP-based measures, dehairing and polishing, increase levels and/or prevalence of aerobic colony counts and *Enterobacteriaceae*. Rectum sealing is effective in reducing the prevalence of *Y. enterocolitica* on pig carcasses indicating that this procedure should always be used. Hot water washing of pig carcasses significantly reduced the prevalence of generic *E. coli* and numbers by 1.2 logs and reduced aerobic bacteria by 1.3 logs. In Denmark, hot water wash is used on pig carcasses from batches coming from *Salmonella* positive pig herds and it was found to be more cost effective than steam vacuum and lactic acid wash. Blast

chilling combined with conventional dry chilling was found to be effective in reducing *Enterobacteriaceae* prevalence on pig carcasses under commercial abattoir conditions. Combined effects of multiple interventions showed strong evidence that they were effective in reducing aerobic bacteria by 3 logs. Overall, the results suggest that scalding, singeing, washing with hot water and dry chilling are effective in reducing indicator bacteria on pig carcasses and can be recommended for use in pig abattoirs (Zdolec et al., 2021).

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AFRICAN SWINE FEVER IN SERBIA: CHALLENGES OF CONTROLLING THE SPREAD OF INFECTION

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African swine fever (ASF) is a viral disease of domestic pigs and wild boars and currently represents a major threat to the pig farming sector and swine industry worldwide. In Serbia, the first official measures started from 2013, with raising the laboratories capacity and testing of shot wild boars on presence of ASF virus-specific antibodies. During 2017, the National Expert's team, National Crisis Centre and the Decision team were established. In order to increase awareness, the lectures for veterinarians, farmers and hunters were organised, and appropriate written material was printed (leaflets, brochures) and distributed all over the country. At the country borders informative posters were set up. Sharing the information in the media was continuously conducted. From 2017/2018, due to the situation in the neighbouring countries – confirmed presence of ASF in domestic pigs in Romania (2017), in the wild boars in Romania, Hungary and Bulgaria (2018) and domestic pigs in Bulgaria (2018), both active and passive surveillance of wild boars in buffer area (about 50 km) from the mentioned countries' borders was introduced. In addition, in cooperation with farmers, hunters and representatives in enclosed hunting grounds intensive passive surveillance has been carried out. At that time, it was suspected that the virus will be introduced by wild boar movement and human activities in border areas, as well as near the main roads (highways). Unexpectedly, the first ASF case in Serbia was detected in a backyard population of domestic pigs in the central region of Serbia (Mladenovac municipality), on 30th July 2019. Human activities (people movement around country borders, and possible illegal trade of pork or home-made traditional pork products) were found to be the most possible route of infection. Soon after the first case, a new ASF outbreak in domestic pigs was detected further north, near the border with Romania on 12th September 2019. From September until the end of 2019, an intensive active surveillance in domestic pigs has been carried out throughout the country. Each pig holding, regardless of the size, has been clinically, and if needed, laboratory monitored and tested in weekly or biweekly intervals. An active surveillance in domestic pigs was/is also operational during 2020 and 2021. However, in the beginning of 2020, ASF has been detected in wild boars in the east of the country, close to the country's borders with Bulgaria and Romania. Since then, numerous outbreaks in domestic pigs and wild boars have been reported in south-east, but also central part of the Serbia. In April 2021, a large commercial farrow-to-finish pig farm, located close to the border with Bulgaria, was affected with the ASF virus, and the virus was detected in the area neighbouring with Romania, both in domestic pigs and in the wild boar population located in an enclosed hunting ground (Vršačke planine).

Currently, the recognised challenge in ASF control is certainly the structure of pig production. Like in Romania, in Serbia the ASF epidemiology seems to follow a dominantly

domestic cycle: the ASF virus circulates among smallholdings, affecting also commercial holdings and the wild boar population. Serbia has the highest pig density among the Western Balkan countries (2.7 million pigs). Besides industrial pig farms, backyard farming and smallholdings remain the basic traditional system of keeping pigs, with low or without any implemented biosecurity measures. In the ongoing ASF situation, human activity was identified as the main risk factor for possible further spreading of the virus. It became obvious that the domestic pig cycle, human activities involving pigs or pig-derived meat products are the dominant driver of the virus transmission. Even more, directly or indirectly, human activities are frequently a connection (link) between domestic pigs and wild boars. Certainly, all previously mentioned factors create a challenging situation for farm biosecurity – the more people are involved, the more challenging it becomes. In conclusion, in the absence of an effective vaccine, in wild boar habitat and domestic pig epidemiological cycle the implementation of strict biosecurity measures by all involved actors is the key factor for disease control. We found the social aspect to be a very important obstacle, especially in low-income countries like Serbia. Existing pig production systems urgently need to shift to a pig sector that is more commercialised, professional and better integrated by farmer education.

A GUIDE TO INVESTIGATING DYSPHAGIA AND ABNORMAL FEEDING ACTIVITY IN THE HORSE

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Horses may develop difficulty in swallowing or altered feeding activity for many reasons, and a detailed history is required, with systematic approach to investigation. In mild chronic cases of dysphagia, the first clinical sign noted by the owner may be progressive loss of weight. In more severely affected horses, clinical signs are likely to include reduced speed of ingestion, which might be caused by prolonged chewing, quidding of feed or dropping of feed after mastication. Coughing may accompany feeding if there is inhalation of ingesta, and there may be appearance of feed or water at the nostrils. Inhalation of feed may lead to the development of aspiration pneumonia in the cranioventral lung lobes, with malodorous breath, tachypnoea and pyrexia. Gagging and stretching of the head and neck after feeding often indicates disordered oesophageal transit of the feed bolus. Gastric pain may also result in horses dropping feed after chewing, or reversing from the feed manger due to pain in the cranial abdomen. Sham drinking, ptyalism or 'lip smacking' behaviour may also be manifestations of pain relating to the orad gastrointestinal tract.

All of the above will ultimately result in a reduction in forage intake with progressive weight loss. Clinical signs are therefore varied and careful observation is required to determine the exact point in the cycle of prehension, mastication, feed manipulation, swallowing and oesophageal transit at which the problem is occurring. Some of the more common disorders affecting these different functions of the orad gastrointestinal tract are discussed in this lecture, and case examples are discussed.

Abnormal Prehension

Effective prehension requires sensory innervation of the muzzle (CNV), functional motor control of the rostral facial muscles (CN7) and efficient grasping by the incisor teeth in additional to central control of these processes. Conditions such as facial nerve paralysis, or central disorders affecting motor coordination or balance will impair the ability of the horse to prehend accurately. Pain resulting from soft tissue trauma or incisors (e.g. equine odontoclastic tooth resorption and hypercementosis (EOTRH)) should also be ruled out as part of an investigation.

Problems of Mastication

Atrophy of the masseters or temporal muscles may be primary or secondary to other conditions affecting mastication. Nutritional myodegeneration is a primary cause of masseter muscle atrophy. Disuse atrophy may occur secondary to temporomandibular joint disorders or temporohyoid osteoarthropathy. Guttural pouch endoscopy and TMJ radiographs will be required for evaluation.

Dysphagia

Thorough evaluation of the tongue should be undertaken to rule out impaired feed manipulation caused by conditions such as foreign bodies, abscessation, CNXII dysfunction or neoplasia of the base of the tongue. Tongue base masses can be surprisingly extensive and further diagnostic imaging and histopathology are likely to be needed to decide the best approach to treatment. Dysphagia also may arise from mechanical disorders such as persistent entrapment of the epiglottis or any pharyngeal mass. Cleft palates (palatoschisis) may result in the regurgitation of liquid or feed and may go undetected until adulthood. An effective endoscopic examination should include evaluation of the swallowing mechanism, by repeated stimulation of the roof of the pharynx with water, to rule out subepiglottic cysts and evaluate neurological function. Loss of pharyngeal sensation may result from guttural pouch mycosis or neoplasia, and careful evaluation of the guttural pouches is required. CNIX neuropathy may impair both oropharyngeal sensation and pharyngeal motor function, and most commonly results from guttural pouch mycosis.

Oesophageal dysmotility

The commonest anatomical locations for oesophageal obstruction are the orad oesophagus, the thoracic inlet, and the cardia. Recurrent episodes of choke may arise due to oesophageal stricture formation resulting from progressive fibrosis; this is usually the result of circumferential damage following severe obstruction, or external fibrosis from traumatic injury to the neck. Maximal fibrosis is present approximately 60 days after the original insult and often results in increasingly frequent episodes of choke and dysphagia. Bougienage or oesophagomyotomy may be required to improve intraluminal pressures. Pulsion or traction diverticuli often progress with age, becoming less elastic and more prone to obstruction. Endoscopic examination of the dilated oesophagus is required to detect strictures or diverticuli and contrast radiography may help to determine the anatomical details of the abnormality, facilitating treatment planning.

Equine dysautonomia causes ptyalism, dysphagia and retrogradal oesophageal peristalsis which results in characteristic spontaneous appearance of large quantities of saliva at the mouth. Linear oesophageal erosions are also present in the aborad oesophagus as a feature of dysautonomia. Other clinical features such as persistent tachycardia, ptosis or delated intestinal transit are usually supportive diagnostic signs for this condition.

Connective tissue disorders also have been linked to oesophageal diverticuli and megaoesophagus, and these have been extensively investigated in the Friesian breed.

Gastric abnormalities

Although gastric abnormalities are not often associated with dysphagia, severe gastric pain will cause anterior abdominal pain shortly after swallowing. This results in pauses in mastication, backing away from feed or dropping of mouthfuls of feed prior to swallowing. Severe gastric glandular disease, pyloric outflow restrictions or gastric impactions are most likely to be associated with these clinical signs. Chronic gastric

impactions may develop and progress in size over several weeks, resulting in a gradual reduction in food intake, and even a pendulous abdominal profile.

Gastric ultrasound evaluation should be part of any diagnostic investigation of dysphagia, chronic colic or weight loss. Extension of the gastric silhouette over more than four intercostal spaces could be indicative of a gastric impaction. Treatment of chronic impactions is difficult, requiring a combination of repeated gastric lavage and constant rate infusion of gastric fluids via a fluid delivery system. After emptying is complete, a thorough gastroscopic examination should be undertaken, to rule out conditions such as pyloric polyps or stenosis, or squamous cell carcinoma. Long-term feeding management is required to prevent recurrence of this condition in animals affected by gastroparesis, with a pelleted hay replacement ration or grass being the best forage sources.

Discrete polyps in the gastric outflow may be removed by transendoscopic loop cautery if pedunculated. Concurrent anti-ulcer medication is often required. The success of management strategies for gastric dilatation/gastroparesis can be assessed by periodic follow-up ultrasound evaluation of the gastric silhouette. The aetiopathogenesis of this condition is still not fully understood.

Take home messages: In addition to dental pain, functional disorders of the tongue, pharynx, oesophagus and stomach must be considered in horses with dysphagia or abnormal feeding behaviour. Early recognition and treatment are vital in improving the prognosis for affected horses.

WOUNDS WITH SYNOVIAL STRUCTURE INVOLVEMENT: DIAGNOSIS AND MANAGEMENT

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Synovial sepsis in the adult horse develops most commonly as a result of a penetrating injury (wound). In rare cases, synovial sepsis can also develop without the presence of a wound. Initial assessment of horses presenting with wounds located near synovial structures is vital. During the initial assessment, one should take into account: location of the wound, duration of the condition (i.e. when the wound/penetration first occur), degree of lameness, amount of discharge and potential soft tissue structures that may be involved (i.e. tendons or ligaments).

Synovial fluid collection and synovial structure distension are the two most useful tests available to rule in or out the presence of synovial sepsis. The Total Nucleated Cells Count (TNCC) is the single most important parameter that allows diagnosis of synovial sepsis. This can be combined with the Total Protein level in the synovial fluid.

If treatment is instigated in the first 24–36 hours the prognosis is good. However, in those cases where a wound is not present and the duration of the sepsis exceeds 36 h, the prognosis is significantly reduced.

Figure 1. Laceration of the distal palmar metacarpal region, tendon tissue of the superficial digital flexor tendon protruding through the wound margins.

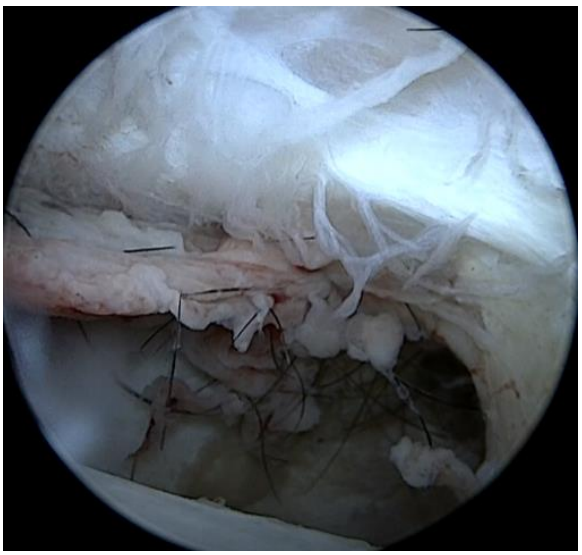

Figure 2. Tenoscopy of the case in Figure 1. Severe synovial contamination of the digital tendon sheath.

THE OLD WORLD KEEP INVADING THE NEW ONE: A LESSON FROM THE INVASION OF THE EUROPEAN STRAIN OF *ECHINOCOCCUS MULTILOCULARIS* IN NORTH AMERICA

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Parasitic invasions are global and mediated by several epidemiological, ecological, social or cultural processes. The outcomes of such invasions, when it comes to zoonotic infectious agents, can be dramatic and with significant public and animal health consequences. In some cases, the invasion process may not involve different parasite species, but different variants within the same species. These variants may have very diverse pathogenicity and transmission rates in host species that are competent and susceptible to the local variant, but had developed adaptations to fight its infections, but are naïve to the new variant, thus allowing for its establishment and spread. In case of parasites with complex life cycles as those whose transmission is mediated by a predator-prey relationship, the invasion of a new variant may have very complex effects on these parasites transmission, as their cycle involves multiple hosts, and it is difficult to predict how they may react to new parasitic strains.

Among these, *Echinococcus multilocularis* (*Em*) is the aetiological agent of Alveolar Echinococcosis (AE), a severe zoonosis emerging in Europe and, more recently, in North America. *Echinococcus multilocularis* circulates in wild and domestic canids (fox, coyotes, jackals, wolves, dogs) as definitive hosts, and mostly small rodents as intermediate hosts. *Echinococcus multilocularis* exists in several strains across the northern hemisphere with three main clades identified in Asia, Europe and North America, each one with sub-strains. These classifications are based on mtDNA characterization, and confirmed by means of EmsB, a highly variable tandem-repeated multiloci microsatellite, that allows for more detailed discrimination at local scale. More relevantly, these genetic variants are deemed to have differential infectivity and pathogenicity.

In North-America *Em* was present in two main strains, N1 typical of the northern latitudes, mostly Arctic, and N2 that was described in the central continental regions. In the last decade, European-like *Em* genotypes (similar to the EU strains, but with some endemic SNPs) were detected in several wild and domestic definitive hosts (foxes, coyotes and dogs), and, more relevantly, in human AE patients from an unprecedented outbreak in continental North America, in Alberta, Canada. The EU-like strains found in these patients presented the same mtDNA SNPs found in the most common strain in wild definitive hosts in North America, but not in Europe. Interestingly, in the very same region the N2 strain was not detected in sylvatic and domestic hosts, if not occasionally. It has been then hypothesised that the EU-like strains found in North America are the result of invasion events, more likely through pet-dog movements, or red fox translocations for

fur-trade purposes. Nonetheless, it cannot be excluded that the EU-like strain is an undetected strain recently spread in Western Canada.

To test this hypothesis, *Em* samples have been collected from across Western Canada, with a focus on Alberta, where the human cases outbreak occurred. Parasite adults were searched in the two main hosts of *Em* in North America: coyotes and red foxes. Specimens were genetically characterised via sequencing of mtDNA target genes (*cox1*, *nad2* and *cob*) and using fragment analysis of the EmsB nuclear marker. The analysis of these mtDNA sequences and EmsB profiles showed that the genetic diversity observed was lower than expected for an undetected strain, supporting the alternative hypothesis of a recent invasion. More interestingly, the distribution of the different variants, using both mitochondrial and nuclear markers, suggested multiple invasion events rather than a single one.

The overall situation of *Echinococcus multilocularis* in North America mandates new measures to mitigate and track the occurrence of invasion events that may lead to the establishment not only of new parasites, but also of more pathogenic genetic variants. At this stage, it may be necessary to reassess the current deworming policies that are implemented when translocating pets or other potential hosts between countries where the parasite is endemic, but with different variants.

THE PRINCIPLES OF WILD BIRD MEDICINE – BIODIVERSITY IN YOUR PRACTICE

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Birding and caring for birds attract the attention of many people, therefore bird rescue is something where the general public often act to help animals in need. Veterinarians frequently serve in the forefront and even if one is not an avian specialist, a lot can be done before more specialised diagnostic or treatment effort are initiated. In order to implement this task, it is worth knowing some basic rules.

We were taught, "a good history is a half diagnosis". When rescued birds are brought to our practice there are no classical owners, but the circumstances (how, when and where the animal was found) can help greatly to narrow down the possible causes. Last, but not least, the exact identification of the species and age determination is crucial, as many conditions, even viral diseases (such as West Nile Virus) are strongly linked to certain taxa or genuses and young birds often do not require medical treatment, but proper care and husbandry (which is more of a task of a specialised rescue centre). But do not worry! You do not have to be both a veterinarian and an avid ornithologist. Birds are probably the best studied animal group by the general public, therefore there are ample sources and identification groups on the internet.

After the aforementioned steps, we have to make a quick decision and decide whether to continue with diagnostics or to provide emergency medical care to a given individual. Most of the birds admitted to rescue centres are at least mildly dehydrated. Many of them are also in a negative energy balance. We have to provide fluid therapy (IV/IO/SQ) and offer proper food/force feed, depending on the given condition and situation.

If the bird is not in a critical state, the next steps are purely diagnostic. The easiest and fastest way to gain further information is through radiology, which will help us in many cases and our diagnostic work is not possible without it. Other tools include bloodwork (CBC and biochemistry), microbiology, toxicological investigations, endoscopy or even CT/MRI.

The need, the knowledge, the experience and the technical conditions will determine until what stage we diagnose/treat our patients and when we ask the help of a specialist.

MANAGEMENT OF URETERAL OBSTRUCTIONS IN CATS

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Over the last 15 years, veterinarians are increasingly faced with the problem of ureteral obstruction in cats. Osborne et al. 2009 described a tenfold increase in upper urinary tract stones over the last 20 years (1). The higher incidence of upper urinary tract stones is complicated by the fact that most these stones are calcium oxalate stones for which medical dissolution is not an option. If they do not pass spontaneously and instead cause obstruction, diversion of urine is warranted. Stones are the most common cause of ureteral obstruction (65–80%) followed by ureteral strictures (ca 20%) (2). Other less common reasons are dried solidified blood calculi that can also not be managed by medically dissolution, bladder neoplasia that obstruct urine outflow, accidental ligation of the ureter and in rare cases obstruction of the ureter with inflammatory products in cases of pyelonephritis.

Ureteral obstruction can be partial or complete, unilateral or bilateral. Studies in dogs have shown that complete ureteral obstruction decreases the renal blood flow to 40% within the first 24 hours and to 20% within two weeks (3). The GFR declined permanently to 65%, 56% and 0% after 7, 14 and 40 days, respectively. If both ureters are completely obstructed, this will be fatal within 48 to 72 hours (4). In another experimental study investigating partial ureteral obstruction in dogs, relief of obstruction after 7 days, 28 days and 60 days, resulted in recovery of GFR to 100%, 35% and 8%, respectively (5). These are experimental studies in dogs with no pre-existing azotaemia. Many cats that are presented with ureteral obstruction due to ureteroliths already have compromised renal function due to CKD. Treatment should therefore be implemented as soon as possible to avoid further damage to the obstructed kidney. Unilateral obstruction leads to compensatory hypertrophy of the contralateral kidney. Such a compensatory response is however not possible in cats with pre-existing CKD or in cats with bilateral ureteral obstruction.

Clinical signs in cats with ureteral obstruction are anorexia, lethargy and vomiting because of acute uraemia. Other clinical signs are weight loss, polydipsia/polyuria, signs of abdominal pain, vocalization, haematuria and pollakiuria. On physical examination, cats show signs of abdominal pain, and the kidneys are typically asymmetrical with one kidney enlarged and painful and the other kidney being small and irregular in shape ("big kidney – little kidney" presentation). Bilateral renomegaly is less common and can be identified in cases of bilateral ureteral obstruction. Cats with unilateral partial obstruction and good functioning of the second kidney can be asymptomatic (2, 6, 7).

Most cats with ureteral obstruction are azotemic even when unilateral obstruction is present (2, 6, 8). This is certainly also because more than 50% to 70% of cats have pre-existing chronic kidney disease. In addition to azotaemia, metabolic acidosis, hyperphospataemia and varying degrees of hyperkalaemia can also be seen. A complete

blood count shows anaemia in two thirds of affected cats (8). A urinalysis including a urine culture should be performed in all cats. If the bladder is empty due to bilateral ureteral obstruction, a urinalysis and culture can be performed using urine obtained from the renal pelvis during surgical intervention.

The diagnosis of ureteral obstruction is based on physical examination findings and confirmed by diagnostic imaging of the abdomen. Abdominal ultrasound is the primary imaging method and should be performed in all cats with suspected ureteral obstruction. Ultrasound also allows to evaluate the intrarenal architecture, geometry and to assess whether chronic alterations are present. In addition, ultrasound can be used to monitor the treatment response by evaluating the renal pelvis and ureter size after medical and surgical intervention. Plain abdominal radiographs should be performed in all cats to look for radiodense stones in the kidney and ureter and for renal asymmetry. In 20–30% of cats no discrete calcified material is identified with ultrasound or plain abdominal radiographs. In these cases, antegrade pyelography can be performed to evaluate the patency of the ureter and to identify non radiodense material in the ureter and ureteric strictures. In the author´s facility this is usually performed immediately before an interventional therapy.

Treatment options include medical treatment, traditional surgery, ureteral stent implantation and subcutaneous ureteral bypass. Medical treatment includes fluid therapy, diuresis with mannitol, analgesia, symptomatic treatment of uremic signs and amitriptyline or α-sympatholytic drugs (e.g. tamsulosin or prazosin). Medical treatment has been shown to be effective in only around 10% of cats. If medical treatment is not successful after 24 to 48 hours, or if the patient is unstable (hyperkalaemic, oliguric, overhydrated) immediate intervention is indicated to decrease the intrarenal pressure and to re-establish urine flow. During medical management, the renal pelvis should be checked for further enlargement. Traditional surgery on feline ureters which have a diameter of only 0.4 mm is technically demanding. Perioperative mortality rates of 21% and perioperative complication rates of 30% have been reported (9, 10). The most common complications of ureteral surgery are perioperative leakage, stricture formation and recurrent obstruction, which are observed in around 40% of cats with ureteral stones. Interventional techniques with surgical assistance for renal decompression and reestablishment of urine flow such as ureteral stents and the subcutaneous ureteral bypass device (SUB) have been developed in the last decade and have improved the prognosis for cats with ureteral obstructions. With the placement of feline double pigtail ureteral stents urine is diverted from the renal pelvis to the urinary bladder. The reported success rate of stent placement is up to 96% (2) with perioperative mortality rates of 8 to 15% (2, 11). Apart from procedural difficulties, complications include dysuria (seen in up to 35% of cats with ureteral stents), stent migration, stent mineralization or fracture.

The SUB system includes a nephrostomy tube and a cystotomy tube, which are placed permanently and are connected via a subcutaneously placed shunting tube. With this system, urine can flow through the attached catheters from the renal pelvis to the bladder. Implantation of a SUB device was originally developed as a salvage procedure for cats in which a stent could not be placed due to a very narrow lumen of the ureter or

excessive ureteral stones. In recent years, it has been shown that the SUB device is advantageous over stents for all causes of ureteral obstruction regarding mortality and long-term complications. In a retrospective study of 137 cats with ureteral obstruction due to ureterolithiasis (67%), stricture (13%), stricture and stones (20%) or pyonephrosis (0.5%) placement of the SUB device was successful in all cases. Perioperative complications were device leakage (3.4%), kinking (5%), occlusion with blood clots (7.5%) and catheter mineralization (25%). Of the 137 cats, 94% survived to discharge and the median survival time was 827 days (11). Dysuria which is a common complication in cats with ureteral stents (> 30%), is an uncommon complication in cats with SUB devices (< 5%). A smaller study that investigated initial outcomes and complications of the SUB procedure at two university hospitals reported a need for blood transfusion in the peri- or post-operative period in 8/19 cats, pleural effusion in 2/19 cats, and a 21% rate of bacterial urinary tract infections within 10 days of surgery (12). None of these cats had a positive culture or an active urine sediment before surgery. Two patients had recurrent multidrug-resistant UTI infections. Recurrent and resistant infections and the potential for biofilms are therefore a significant concern in SUB patients. Two more recent studies report higher complication rates of 67% and 80%, respectively (14, 15). In these studies, the most common complications were SUB obstruction, lower urinary tract infection and pyelonephritis. The median overall survival time in these studies was 274 days (1–311 days) and 820 days (1–1915). The use of fluoroscopic guidance is advantageous to ensure correct placement and coiling of the nephrostomy catheter in the renal pelvis.

Ureteral obstructions in cats are increasing in frequency. Adequate diagnostics should be performed as soon as possible in order to identify this condition and to improve the chance for recovery of renal function with earlier intervention. Good collaboration between primary care veterinarians, medicine and surgery specialists is necessary for successful management of affected cats. Despite the promising results with interventional procedures, owners also need to understand possible short and long-term complications of these interventions.

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ISCHEMIC MYELOPATHY AND MOST COMMON DIFFERENTIAL DIAGNOSIS IN DOGS AND CATS

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Vascular myelopathies with particular reference to the ischemic variant are relatively frequent in dogs and to a lesser extent in cats. In fact, vascular myelopathies can be divided into two large groups, the haemorrhagic and the ischemic type. The latter are undoubtedly the most frequent. They have been described in numerous species besides dogs and cats, including humans, pigs and horses. From an etiopathogenetic point of view, the occlusion of a blood vessel by fibrocartilaginous material, histologically similar to the nucleus pulposus of the degenerated intervertebral disc, was most frequently identified in the vast majority of the cases reported in the literature; for this reason, these vascular disorders of the spinal cord are often identified with the name of fibrocartilaginous embolisms (FCE). Several theories have been advanced on how this disc material can gain vascular access, but to date the real pathogenesis is not yet clear.

Typically, the neurological symptoms have an acute/hyperacute onset which is often accompanied by a yelp that the dog emits during intense physical activity; no progression of neurological symptoms is usually observed beyond 24 hours. These reflect the involvement of a specific area of the spinal cord and are typically characterised by severe paresis or plegia with an asymmetrical fashion: therefore, hemiparesis or back monoparesis are frequently observed, respectively with ischemic cervical or thoracolumbar ischaemic lesions. The decrease or absence of spinal reflexes affecting the anterior or posterior limbs are due to the involvement of the cervical or lumbosacral intumescence, respectively. There is usually no spinal pain present at the time of the neurological examination.

The clinical suspicion of ischemic myelopathy must be confirmed by magnetic resonance imaging (MRI). This can be completely normal in mild cases, if carried out in the initial stages from the onset of symptoms (first 48 hours). More commonly, a hyperintense intramedullary area is observed in T2 images, isointense in T1 images, with variable contrast enhancement after intravenous injection of paramagnetic medium (gadolinium) and usually more evident towards the end of the first week (Fig. 1). The hyperintense area strictly affects the grey matter of one side only, but cases with bilateral, asymmetrical and sometimes asymmetrical involvement have been reported; the lesion can also be focal but also spread to a relatively large area of the spinal cord. Usually, no significant increase in the transverse diameters of the spinal cord or evident alterations of the epidural space are observed, unlike what can occur in the so-called "high speed and low volume" disc extrusions, which represent the pathological entity that more closely mimics FCE clinically and from an imaging point of view.

As for therapy, there are no drugs that are effective in reducing ischemic cord damage, especially after the first hours from the onset of symptoms, and therefore physiotherapy is of particular importance.

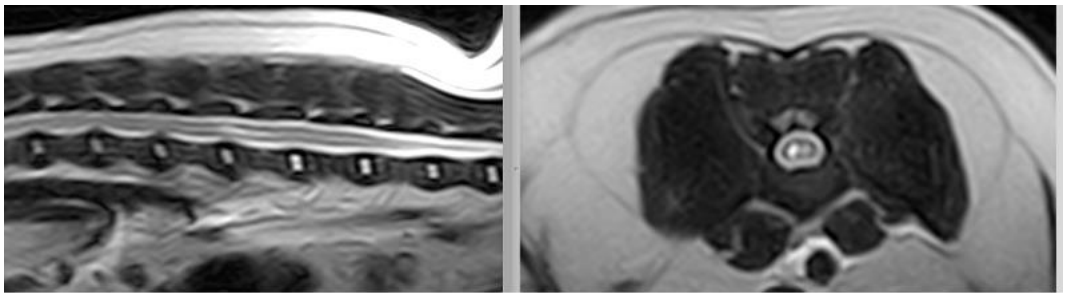

Figure 1. Sagittal and transverse T2-weighted MR images: note the typical hyperintense lesion affecting the grey matter, especially on the dog's right side.

Prognosis depends on the severity of the clinical symptoms at the time of presentation; factors such as the loss of deep sensation, the involvement of intumescence and the symmetry of neurological deficits have been indicated as negative prognostic signs. In addition, with the increasing use of magnetic resonance it has been shown that patients with presumed FCE in the absence of MRI alterations have a favourable prognosis and how the extension of the lesion is directly related to the possibility of recovery of the subject. Specifically, hyperintense areas with a ratio between lesion length and vertebral (C6 for the cervical and L2 for the lumbar) greater than 2 or with a percentage of the affected area of the spinal cord in cross section greater than 67%, are associated with a negative prognosis in 100% of cases.

Vascular myelopathies of a haemorrhagic nature are very rare in our patients and are mostly associated with spinal trauma (endogenous or exogenous), with systemic coagulation disorders (primary or secondary, for example with poisoning with anti vit K rodenticides), with congenital vascular malformations or spinal neoplasms. Among all, epidural haemorrhage secondary to acute disc extrusion is of particular importance in terms of frequency. In this circumstance the herniated disc material violently hits the venous sinuses, breaking them with consequent epidural haemorrhage. This frequently provokes cranial and caudal diffusion of the disc material with secondary compression of the spinal cord for a relatively long area and therefore requires extensive decompression surgery (Fig. 2).

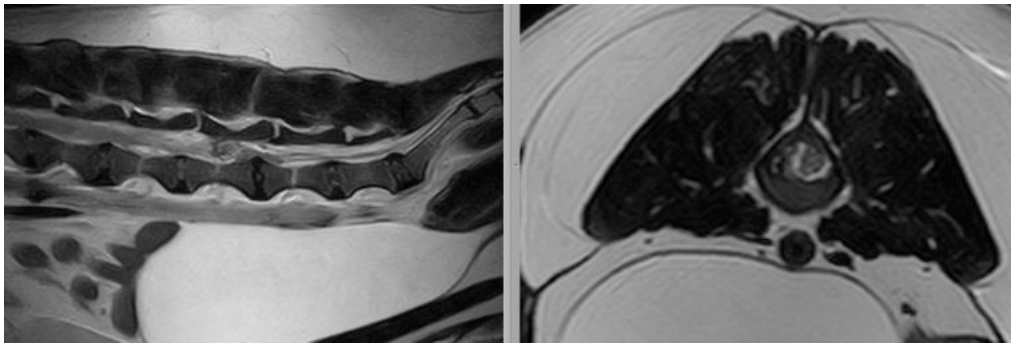

Figure 2. Sagittal and transverse T2 weighted MRI images of disc extrusion associated with epidural haemorrhage.

Disc herniation are undoubtedly the most common differential diagnosis of ischemic myelopathies. Disc herniation consists of a partial or complete dislocation of the disc in the vertebral canal, with compression and/or damage of the spinal cord. In the great majority of cases, at the base of the disc herniation there are degenerative phenomena affecting the disc itself; in exceptional cases, the aetiology can be traumatic. Disc herniation can manifest itself in two main forms: extrusion of the nucleus pulposus or protrusion of the annulus fibrosus.

Extrusions, also known as Hansen's type I, are typically associated with chondroid degeneration and consists in the herniation of the nucleus pulposus through defects in the annulus: the extruded material causes then a ventral, ventrolateral or circumferential compression of the spinal cord with usually acute or hyperacute onset of neurological symptoms. Hansen type I disc herniations mainly affect chondrodystrophic dogs (Dachshund, Lhasa Apso, Pekingese, etc.) with a peak incidence around 4–6 years of age. Disc protrusions, also known as Hansen type II, occur predominantly in older (5–12 years of age), large, non-chondrodystrophic dogs (German Shepherd, Dalmatian, Doberman, etc.). German Shepherd Dogs are frequently affected. The cause of protrusion is the fibroid degeneration of the disc and the weakening of the dorsal portion of the annulus fibrosus. This portion progressively protrudes into the vertebral canal until it reaches dimensions such as to compress the spinal cord ventrally or ventrolaterally; nerve roots may also sometimes be affected (Fig. 3). The onset of neurological symptoms is usually subtle and the course is progressive. The symptoms are related to the affected medullary area; therefore, patients with thoracolumbar pathologies will have ataxia and/or paresis affecting the hind limbs and those with cervical protrusions will manifest sings affecting all four limbs. Spinal pain, when present, is often "dull" and relatively focal. Disc herniations therapy can be conservative or surgical. Conservative therapy consists of rest/reduction of physical exercise and, when indicated, the administration of anti-inflammatory drugs (steroidal or non-steroidal) and analgesics. As for surgical therapy, various techniques have been described and all of them have the ultimate goal of decompressing the spinal cord. Prognosis is generally good for patients with intact deep pain sensation.

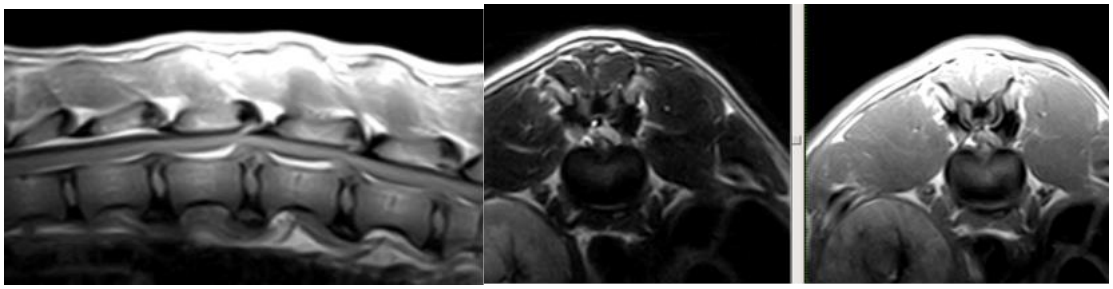

Figure 3. From left to right, T1 sagittal and transverse T2 and T1 weighted images of a subject with multiple thoracolumbar disc protrusions.

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PIG BREEDING CHALLENGES IN THE NEXT DECADES

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Food production, in the new millennium, is dealing with sizeable and quite controversial challenges. From one hand, humanity's need for food is ever-increasing, while on the other, the intensification of the agricultural production leads us to the endangering of the sustainable development, all while ignoring the negative social and ecological implications. Pig husbandry, as a significant source of meat and meat products, is in a constant process of modernizing its technology, tailored specifically to the needs of the market. The genetic improvement of pig population is a continuous and a long-term process. The carefully picked and designed selection goals, the improved accuracy of the selection through large data bases, the usage of genomic selection and the breeding value estimation models, all help us to succeed in improving of the pig's economically important traits.

Modern pig husbandry implies a responsible relationship with society, especially when it comes to the well-being of animals, food safety, climate issues, ecological needs, care for the consumer as well as the market's needs. All this tells us that pig selection in the coming years will have to be focused on changing or modifying selection goals, the way of controlling of the traits and the registration of a considerable amount of data for breeding value estimation. Breeders will have to be creative and define new traits which will, directly or indirectly, help in the genetic improvement of robust and long-lived sows with high mothering abilities, vital piglets with a high rate of survival, adapt for life in big groups in the conditions of future pig farms. The traits such as resiliency to specific illnesses and technological diseases in large farms will help in stopping the (un)controlled mass usage of antibiotics in pig farms. Genetic improvement for feed efficiency will lead to a positive economic and environmental outcome.

The traditional breeders' question is to which extent will genomic selection be used and will it perhaps lead us to the use of gene editing in the future years? External factors such as the instability of the world's markets and the price of pigs and pig meat, as well as the constant spread of diseases like the African swine fever, are making it difficult to create a productive atmosphere, conducive for making new strides in the field of pig husbandry. The coming period will be a challenge which will require innovation when dealing with the subject of genetic improvement of pigs, as well as changing of the breeding philosophy.

ADVANCING GENOME TO PHENOME RESEARCH IN SWINE: PROGRESS AND PERSPECTIVES

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Pigs not only provide high quality food for human consumption, but they also serve as an important biomedical model to understand health and diseases in people. Thanks to revolutionary techniques and data management, porcine genome mapping, sequencing and annotation have advanced dramatically during the last 30 years. Such unique resources have allowed the community to efficiently profile more than 70 million DNA variants harbored in the swine genome. In terms of phenomes, long-term selection has dramatically improved swine performance, such as backfat, days to 250 pounds, pounds of lean, number born alive, number weaned, and litter weight as single traits or terminal sire index, sow productivity index and maternal line index as composite phenotypes. Using both genotypes and phenotypes collected from Duroc (29 year selection) and Yorkshire (37 year selection), we were able to characterize the phenome, genome and pathway responses to long-term selection. No doubt, such as analysis also revealed challenges we are facing to understand the relationships between genome and phenome. Although phenome responses to the long-term selection appear similar between Duroc and Yorkshire, the selected genome regions underlying the same phenotypes are rarely shared between them, for example. Somehow, long-term selection has narrowed the genetic diversity in Duroc, but broadened it in Yorkshire, making it hard to identify the mechanisms controlling the same phenotypes. In Duroc, genes responsible for genetic improvement are enriched for immune systems and stress regulation. In Yorkshire, the enriched pathways are, however, skewed to neurological function and maternal placenta development. To date, the "phenotype (P) = genotype (G) + environment (E) + genotype x environment (G x E)" principle has guided the community to detect DNA variants contributing to phenotypic variation important to biomedicine and agriculture. On the other hand, the central dogma of molecular biology clearly states that DNA makes RNA makes protein. With these advancements, I would propose to form a central dogma of phenomics to advance our understanding of the complex genome-phenome relationships.

/ / / / / VETERINARY PUBLIC HEALTH AND EMERGING DISEASES

ANTIMICROBIAL RESISTANCE OF *CAMPYLOBACTER JEJUNI* AND *CAMPYLOBACTER UPSALIENSIS* ISOLATED FROM DOG FAECES

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Increasing antimicrobial resistance in both human and veterinary medicine is recognised as a major public health problem. Since 2005, campylobacteriosis has been the most commonly reported zoonotic disease in humans (European Union). Dogs have been identified as carriers of *Campylobacter* species, and their role as a source of infection for humans has been established.

Dog faeces were plated on mCCDA agar. *Campylobacter* species were identified by polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP) and matrix-assisted laser desorption/ionization time of flight (MALDI TOF). Antimicrobial susceptibility testing was performed by disk diffusion method according to EUCAST recommendations using ciprofloxacin (5 µg), erythromycin (15 µg), tetracycline (10 µg) and azithromycin (15 µg).

Forty-two isolates of *Campylobacter* spp. were included in the study, 25 of *Campylobacter jejuni* and 17 of *Campylobacter upsaliensis*. Resistance to ciprofloxacin was found in 64.3% of the isolates, to tetracycline in 14.3% of the isolates, to erythromycin in 4.8% of the isolates and to azithromycin in 2.4% of the isolates. In this study, *Campylobacter* spp. were found to be resistant to one (47.6%) or two (14.3%) classes of antimicrobial agents. The highest resistance values were observed for ciprofloxacin and the lowest for macrolides.

The ciprofloxacin resistance observed in this study is among the highest resistance levels reported in human and veterinary isolates. As dogs live in very close contact with humans, this finding may pose a major public health challenge.

HIGH CARRIAGE RATE OF METHICILLIN-RESISTANT STAPHYLOCOCCI IN SMALL ANIMAL VETERINARY CLINICIANS – INDIRECT EVIDENCE OF ZOONOTIC TRANSMISSION

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The increase in bacterial resistance to antimicrobial agents that has been observed worldwide in recent decades is one of the most serious public health problems of today. One way to combat this problem is to adhere to biosafety measures in clinical work to prevent the spread of infection, overuse of antibiotics, and selection of resistant bacteria. The use and effectiveness of biosecurity measures in veterinary clinics is often evaluated by monitoring mucosal colonization of clinicians with methicillin-resistant staphylococci (MRS). Studies on the incidence of MRS in different populations have in many cases shown a higher incidence of infection and mucosal colonization by MRS in occupationally exposed individuals, such as veterinarians.

This study involved 79 clinicians, whose nasal and buccal mucosal swabs were self-collected. Methicillin-resistant staphylococci were isolated by pre-enrichment and selective isolation. Methicillin resistance was detected by disk diffusion phenotype assay and confirmed by detection of mecA gene by polymerase chain reaction. Methicillin-resistant staphylococci were isolated in 93.7% of individuals. More than one MRS isolate was isolated from 84.3% of the swabs. In 73.0% of individuals, MRS was isolated from both nasal and buccal mucosa. Of the 144 MRS isolates, 4 (2.8%) were coagulase-positive staphylococci (CoPS) of the Staphylococcus pseudintermedius (MRSP) species. The majority of isolates (97.2%) were coagulase-negative methicillin-resistant staphylococci (MRCoNS).

The high incidence of methicillin-resistant staphylococci is of concern because of the potential for opportunistic and nosocomial infections, as well as transmission of genes responsible for resistance to coagulase-positive staphylococci. The results of this study may contribute to an even better implementation of biosafety measures in clinical veterinary practice.

SEROLOGICAL SCREENING FOR TULARAEMIA IN HUNTING DOGS IN AUSTRIA USING QUICK SLIDE AGGLUTINATION AND TUBE AGGLUTINATION

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Tularaemia is a zoonotic disease caused by the bacterium *Francisella tularensis*. The European Union reports approx. 1200 human cases yearly. In Austria, *Francisella tularensis* supsp. *holarctica* is endemic in the eastern part of the country (Lower Austria and Burgenland). In later years, Salzburg, Upper Austria, and Tyrol report several human and animal cases. The health ministry reported seven human cases in the first quarter of 2021 (1 Lower Austria, 5 Upper Austria, 1 Salzburg). As mild symptoms can be confused with, e.g. the flu, the disease is most likely underreported. Norway is the only European country so far reporting zoonotic transmission from pet species, and reported the first clinically ill dogs in 2014 showing e.g. lethargy and fever. It has long been hypothesised that prevalence will increase with climate change. After screening hunting dogs in the known endemic areas in 2017 (6.25% tested positive) a second serological screening started in 2020 in Lower Austria, to evaluate the situation.

The study included 40 actively used hunting dogs of mixed breeds with an age-range 1 to 11 years (13 male, 27 female). Serum samples were taken before and after the annual hunting season; a third sample of positive dogs three months after the second sample. Analyses used were quick slide agglutination (Bioveta Inc., Ivanocie na Hané, Czech Republic), followed by tube agglutination (Bioveta Inc., Ivanocie na Hané, Czech Republic).

All 40 dogs tested negative in the first sample. Two dogs tested positive after the hunting season (5%); one of them tested positive in the third sample. The owners reported that the dogs did not show any symptoms.

Compared to 2017, this is an (unsuspected) slight decline. However, one must stress that large hunts were forbidden due to the pandemic, and hunting was mostly done individually at a decreased rate. Parts of the study are continued in 2021, and as an increase of tularaemia cases is noted in wildlife this year, the authors suspect an increase in positive dogs. Analyses of the immune response are ongoing and will hopefully shed some light on this entity in dogs and a possible risk of transmission.

MEAT JUICE SEROLOGY AS A POTENTIAL TOOL IN FARM RISK CATEGORIZATION – SURVEY ON *TOXOPLASMA GONDII* ANTIBODIES IN DOMESTIC PIGS AND WILD BOARS IN KARLOVAC COUNTY

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Toxoplasmosis is a zoonosis caused by the protozoan *Toxoplasma gondii* and is one of the most common meat-borne parasitic diseases. Since pork is one of the major sources of *T. gondii* infection in humans, seropositivity may be a good indicator of the presence of parasitic cysts in meat. Although most serological tests are mainly performed with blood serum, antibody screening of meat juice has been proposed as an alternative method. The aim of this study was to determine the prevalence of *T. gondii* antibodies in wild boar and domestic pig populations in relation to their origin in order to estimate the risk of toxoplasmosis in humans.

In 2020, frozen diaphragms of 92 domestic pigs and 92 wild boars were collected from *Trichinella*-negative samples at the Karlovac Veterinary Station. Pigs were grouped based on the origin data, farm size and biosecurity level obtained from a competent authority. Samples were thawed and obtained meat juice samples were tested for the presence of *T. gondii* antibodies using a commercially available ELISA test.

T. gondii antibodies were detected in 35 meat juice samples (38.04%, n = 92) from domestic pigs and in 51 meat juice samples (55.43%, n = 92) from wild boars. As expected, the largest proportion of positive samples in the domestic pig population (71.43%, n = 25) were from small farms with up to five owned pigs. Moreover, 88.57% of them (n = 31) belonged to farms with low biosecurity level (category "1" or "2").

The results suggest a high prevalence of *T. gondii* antibodies in pigs raised at family farms and especially in wild boars from Karlovac County. Meat juice serology can be proposed for continuous risk categorization of pig farms and hunting areas in the framework of the new Meat Safety Assurance System. The safety of meat from high-risk farms/hunting areas should be improved by interventions such as biosecurity measures and thermal treatment of meat during processing.

INHIBITION OF FOODBORNE RESISTANT AND PATHOGENIC BACTERIA BY DALMATIAN AROMATIC HERBS EXTRACTS

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Domestic aromatic herbs and their extracts have high potential as natural additives in local food production. The CEKOM 3LJ project aims to investigate the impact of Dalmatian aromatic herbs in cheese production in terms of taste, quality and health properties of new products. The aim of our study was to test the antimicrobial activity of alcoholic extracts of seasonal aromatic herbs from Dalmatia – basil (*Ocimum basilicum* L.), sage (*Salvia officinalis*), lavender (*Lavandula officinalis*), rosemary (*Rosemarinus officinalis* L.) and immortelle (*Helichrysum italicum*).

Antimicrobial potential was evaluated using a disk diffusion test against pathogenic and resistant bacterial strains of food and public health importance: four strains of *Salmonella* Infantis, two strains of *Listeria innocua* and *Listeria monocytogenes,* ermA+ *Staphylococcus haemolyticus*, ermC+ *Staphylococcus haemolyticus*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Listeria welshimeri* and *Listeria ivanovii.*

Alcoholic extracts were effective on nine of the 14 strains tested. Staphylococci and *Y. enterocolitica* strains were most sensitive and were inhibited by all herbal extracts. Rosemary extract inhibited the broadest spectrum, i.e. seven indicator bacteria, while lavender inhibited only three. Immortelle extract showed the strongest inhibitory activity in terms of the broadest zones of inhibition against ermA+ *Staphylococcus haemolyticus*, ermC+ *Staphylococcus haemolyticus* and *Staphylococcus aureus*. After three months of storage, the antimicrobial activity of sage, rosemary and immortelle extracts was maintained, albeit in attenuated form.

The results show a high antimicrobial potential of herbal extracts against pathogens that may compromise the microbiological safety of cheese. Their inhibitory activity will be further investigated against cheese starter cultures and indigenous dairy microbiota to gain a complete insight into the technological applicability of the extracts in cheese production.

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POSSIBILITIES OF USING EFFECTIVE MICROORGANISMS IN ENVIRONMENTAL PROTECTION

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Effective microorganisms (commercially available as EM-1® and its secondary products, EMRO, Japan) are a mixed culture of microorganisms with a beneficial effect on nature, including humans, animals, plants and other microbial species. It is known that EM contains more than 80 species of anaerobic and aerobic microorganisms such as photosynthetic and lactic acid bacteria, yeasts, actinomycetes, etc. They have been used in agriculture, forestry, animal production, environmental protection, and medicine.

Effective microorganism technology is used in more than 130 countries worldwide, being included in the state programme for organic farming and healthy food production in 12 countries. The technology is natural and organic involving growing, applying, managing and activation of native microorganisms in the environment. It has been shown that EM application promotes positive changes in the water ecosystem by increasing decomposition of organic matter and diversity of phytoplankton, and reducing total nitrogen, phosphate, pesticide and heavy metal concentrations. Also, EM has been demonstrated to inhibit the growth of pathogenic bacteria in water and soil, and to reduce the concentrations of harmful gases in air. Moreover, the number of insect pollinators is falling constantly due to the use of pesticides and climatic changes, and EM raises honeybee immunity and protects their health.

The ever-greater economic and industrial growth is leading to the environmental pollution, including water, soil and air. These problems require seeking for solutions other than the known physical and chemical technologies. Therefore, an environmentally friendly, safe, and relatively inexpensive way of protecting the environment is the use of biologically active substances such as EM to achieve positive synergistic effects with beneficial microorganisms already existing in nature.

DIFFERENTIAL DIAGNOSIS OF INTESTINAL EMPHYSEMA AND CYSTICERCOSIS (*CYSTICERCUS TENUICOLLIS*) IN PIGS

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Intestinal emphysema (IE) of pigs is a benign condition involving mainly the serosal surface and small intestinal wall. *Cysticercus tenuicollis* is a bubble-like metacestode stage of the canine tapeworm *Taenia hydatigena* that infects the abdominal cavity and liver of diverse intermediate hosts, including pigs.

In 2020, 2 samples were delivered in 4% of neutral buffered formalin, from a slaughterhouse to the Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Zagreb, and submitted for histopathological examination. Both were collected form domestic pigs. The first sample was a part of the small intestine, and the second was a bubble-like structure collected from mesenterium attached to the serosa of the small intestine. Samples were embedded in paraffin and stained with HE stain.

On the serosal surface of small intestine numerous thin-walled cystic structures were visible. Histopathological examination revealed IE; cystic structures located in the subserosa with a mild mixed cellular inflammatory reaction in the cystic walls. Gross examination of structure removed from mesenterium corresponded to *C. tenuicollis* with invaginated protoscolex. Histologically, tangential section through cysticercus demonstrated a thick, smooth tegument, subjacent layer of somatic cells, a spongy parenchymatous body cavity with numerous calcareous corpuscles.

Emphysematous lesions are the consequences of gas-dilated intestinal lymphatic vessels. The cause is unknown and not associated with clinical disease or negative pig farming economics.

Cysticercosis caused by *C. tenuicollis* is an economic and epidemiological issue. Infected organs should be safely discarded, while infection indicates the presence of raw meat-fed dogs and environmental contamination with canine faeces. The incidence of IE is markedly decreasing and is just a morphological aberration. Because of the reasons mentioned above, it is necessary to distinguish porcine IE from cysticercosis.

APPLICATION OF HISTOLOGICAL AND HISTOCHEMICAL METHODS IN KULEN COMPOSITION ANALYSIS

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Histological analysis of meat and meat products allows the evaluation of the content of animal and plant tissues, and could provide information on the quality of the raw material used and the quality of the processing steps, as well as possibly identify different tissues prohibited by regulations.

To evaluate the advantages of histological methods for meat composition analysis, six samples of dry-fermented *kulen* sausage were studied, one of which was produced in domestic conditions, while the other samples were commercial products sold in local markets. The samples were subjected to classical histological preparation. The slides obtained were stained with haematoxylin-eosin, Masson-Goldner, toluidine blue and periodic acid-Schiff/Alcian blue. The content of muscle, fat and connective tissue was determined by histomorphometric analysis.

Histological analysis of the composition of the dry-fermented *kulen* sausage revealed the following results: the presence of muscle, fat and dense connective tissue was confirmed; blood vessels, glandular epithelium, peripheral nerves, cartilage and plant tissue were identified. Histomorphometric analysis revealed that the products contained an average of 54.45 ± 2.52% muscle tissue, 7.27 ± 1.38% connective tissue and 19.82 ± 3.24% adipose tissue.

These results suggest that by using histological methods, it is possible to identify various permitted and prohibited animal tissues in dry-fermented *kulen* sausage, so that they can be used as a complementary method to standard chemical analyses in compositional analysis. It is also possible to confirm the presence of various plant tissues, although additional histological methods must be used for their accurate identification.

SARS-COV-2 IN CROATIA – GENOMIC ANALYSIS OF THE THIRD AND FOURTH EPIDEMIC WAVE

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SARS-CoV-2 as a large genome ssRNA virus (29.9 kb) is subject to frequent mutations during replication. These mutations can be silent, deleterious to viral propagation in population or can be beneficial for the virus and create variants with the selective advantage. Variants with significant impact on transmissibility spread easier and faster than other variants. This leads to more COVID-19 cases and another epidemic wave. Variants that became dominant on specific geographic areas are named Variants of Concern (VOC). European Centre for Disease Prevention and Control (ECDC) currently monitors Alpha- B.1.1.7, Beta- B.1.351, Gamma-P.1 and Delta- B.1.617.2 VOC.

To monitor epidemiologic situation and to capture circulating variants in accordance with the ECDC recommendations, whole genome sequencing (WGS) of SARS-CoV-2 positive samples in Croatia is conducted weekly from February 2021. Sequences are uploaded in GISAID database that categorise isolates in phylogenetic clades, depending on single nucleotide polymorphism (SNP) present in the genome.

By the end of August 2021, we processed 8460 samples and 7075 samples were successfully sequenced. During the first and second wave of the epidemic, virus lineages form clade G, GR and GV were dominant. Marked weekly increase of the Alpha variant from GRY clade indicated the beginning of the third epidemic wave in Croatia. The Alpha variant was dominant (more than 50% of positive samples) from the beginning of March 2021. In the first week of June 2021, we detected first Delta variants that rapidly replaced the Alpha variant and by the beginning of August 2021 reached more than 90% of all sequenced samples. Beta and Gamma variants detected in a minor number of samples were closely related to traveling abroad and did not transmit locally.

Rapid propagation of Alpha and Delta variants in Europe and Croatia led to successive epidemic waves. The course of the fourth wave will depend on the Delta variant's potential to spread and current immunity status of the population.

THE EFFECT OF FERMENTED FEED AND HUMIC SUBSTANCES ON LIPID OXIDATION OF BROILERS BREAST AND THIGH MEAT

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Fermented feed products prepared by solid-state fermentation (SSF) has been employed to enhance nutrient bioavailability and resulting in improved nutrient digestibility.

This experiment was designed to examine the effect of supplementing broiler diet with 10% fermented product (wheat bran after SSF by filamentous fungi *Mortierella alpina* CCF2861 with production of arachidonic and eicosapentaenoic acids), 0.7% of humic substances, and the effect of their combination on lipid oxidation of produced broilers breast and thigh meat. To determine the lipid oxidation changes of breast and thigh muscles, the 2-thiobarbituric acid spectrophotometric method was used.

Various feed supplements affected lipid oxidation of breast and thigh meat only on the first day of meat storage ($p < 0.01$). On the first day of storage period, which lasted 7 days, a statistically significant effect of broiler feed supplementation on lipid oxidation of breast and thigh meat was observed, $p < 0.001$ and $p = 0.004$, respectively. In contrast, malondialdehyde (MDA) content of the control group of thigh meat increased by an average of 119%. No difference in MDA content was observed amongst all the experimental groups, neither in breast nor in thigh meat samples, after seven days of meat storage ($p > 0.05$).

The results of this experiment indicate a significant effect of the enrichment of broiler feed with humic substances in combination with fermented feed, whereby effects were observed on the lipid oxidation of breast and thigh muscle of broiler chickens.

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INCIDENCE OF INFECTIONS CAUSED BY *ENTEROCOCCUS* SPP. IN ANIMALS

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Bacteria of the genus *Enterococcus* are physiological intestinal inhabitants of many animals, but as opportunistic pathogens they can cause a variety of infections. Enterococci are often involved in mixed infections, so their pathogenic role is difficult to interpret and also tends to be neglected. They have innate resistance to some commonly used antimicrobial drugs and a great ability to acquire new resistance mechanisms, which can lead to multidrug resistance. In animals, the most common enterococcal infections are urinary tract infections, followed by intra-abdominal and wound infections.

This study investigated the incidence of infections caused by *Enterococcus* spp. over a two-year period (January 2019–December 2020). Clinical samples from animals were submitted for microbiological examination to Bacteriology Laboratory of the Faculty of Veterinary Medicine, University of Zagreb. Bacterial species were identified according to standard laboratory procedures. Gram-positive and catalase-negative cocci with visible growth on kanamycin aesculin agar (black colonies) were characterised as *Enterococcus* spp. and included in this study.

Of 1817 different clinical specimens, 806 (44%) were positive for bacterial growth. *Enterococcus* spp. was isolated from 49 (6%) specimens. Among the various clinical specimens, urine culture yielded the highest number of enterococci isolates (38%), followed by wound swabs (18%) and vaginal swabs (14%). In 53% of the samples, *Enterococcus* spp. was the only bacterial species found.

In recent decades, enterococci have emerged as an important human pathogen, particularly in nosocomial infections caused by multidrug resistant isolates. It is important to raise awareness of enterococci as the primary causative agent of animal infections, especially because of the close contact between animals and humans. Appropriate surveillance and control measures are crucial to prevent occurrence and transmission of resistant enterococci from animals to humans.

REPRESENTATION OF BRUCELLOSIS IN THE DAIRY CATTLE POPULATION IN THE FEDERATION OF BOSNIA AND HERZEGOVINA

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Brucellosis in Bosnia and Herzegovina has been a significant public health and economic problem for the past 20 years. Although sporadic cases were recorded in the 1970s and 1980s, they were successfully resolved by applying strict measures. The recurrence of sporadic cases was recorded at the beginning of this century, and in the period 2004–2008, there was a significant increase in the number of seropositive reactors.

In the period 2018–2020, a total of 221,220 blood sera of dairy cattle from the territory of the Federation of Bosnia and Herzegovina were searched. The Rose Bengal test as a "screening" method was used to detect positive and suspicious reactors, while the complement fixation reaction was used as a confirmatory method. The presence of specific antibodies against brucellosis has been demonstrated in 191 bovine blood sera. The largest number of positive reactors came from the three cantons of the Federation of BiH, Unsko-Sanski, Zeničko-Dobojski, and Srednjebosanski.

Due to the common keeping of small ruminants and cattle on the same pastures and in the same dwellings, the number of detected cases of positive reactors is correlated with the occurrence of positive reactors in sheep and goats.

Although the programme of laboratory testing of all-female cattle older than 12 months for brucellosis has been carried out continuously since 2009, and positive individuals are harmlessly removed, the number of new cases of brucellosis in the cattle population is recorded. In the first eight months of 2021 alone, 70 cases were identified, which indicates the need for regular controls not only of cattle but also of small ruminants.

CHARACTERIZATION AND SELECTION OF *LACTOCOCCUS LACTIS* STRAINS FROM EWE'S MILK AS POTENTIAL CHEESE STARTER CULTURES

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Raw milk and traditional dairy products are a source of indigenous strains that have great potential for developing and protecting the recognizable sensory quality of local products. Therefore, the aim of this study was to select lactic acid bacteria strains from Pag raw sheep milk and test their technological and biochemical properties under controlled laboratory conditions.

The lactic acid bacteria were isolated from sheep milk using MRS and M17 agar and identified by MALDI-TOF mass spectrometry. Out of the 18 identified strains of *Lactococcus lactis* subsp. *lactis*, five (M1–M5) were selected for further characterization. Strains were tested for growth in M17 broth at 10°C, 30°C, and 45°C, at pH values of 6.5, 5.5, 4.5, 3.5, and 2.5, and at salt concentrations of 4.5%, 5.5%, and 6.5%. Cell growth was measured by plating on M17 agar and absorbance (OD_{600}). The acidification capacity of the strains was measured in MRS broth using the titration method. In addition, the lipolytic, proteolytic and antimicrobial activities of the isolates were tested, as well as their ability to produce gasses. Data analysis was performed using Statistica 13.5 software. The significance of differences between groups was evaluated using the analysis of variance at the 0.05 level.

The results show that none of the isolates produced gas by glucose degradation, all of them show the same proteolytic, lipolytic and antimicrobial activity and have a high level of acidifying ability. At different temperature conditions, pH values and salt concentrations, all isolates grew but with significant differences ($p < 0.05$), with *Lc. lactis* subsp. *lactis* M1 showing the best technological performance.

It can be concluded that all the selected strains of *Lc. lactis* subsp. *lactis* can be used as potential cheese starter cultures. Considering the above differences, the final selection of strains should be based on the technological process and organoleptic characteristics of the desired cheese variety.

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/ / / / / HORSES

ESTIMATION OF CALPROTECTIN VALUES IN SERUM OF ATHLETIC HORSES

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Calprotectin is a promising biomarker for the evaluation of neutrophil-related inflammation. It is suggested that calprotectin modulates inflammatory reactions and has a central role in neutrophil defence against microbes. Previous studies showed that faecal calprotectin can be a useful nonspecific marker of inflammatory bowel disease in horses. However, there is no data available for values of serum calprotectin in healthy horses. The aim of this study was to evaluate serum calprotectin in serum of healthy athletic horses.

The study included 23 randomly chosen horses competing at an endurance competition. The inclusion criterion was a successfully passing of clinical examination prior to the race. Blood samples were collected by jugular venipuncture 1 h before the start. Serum calprotectin concentrations were measured using Horse Calprotectin ELISA Kit (MyBioSource, San Diego, CA, USA).

There were 12 female and 11 male horses, with an average age of 11 (± 3.7) years. The study included 10 Arabian and 13 horses of other warmblood breeds. The lowest measured concentration of calprotectin was 15.7 ng/mL, while the highest was 46.6 ng/mL with an average of 30.1 (± 7) ng/mL. There was no difference in values of calprotectin according to age, breed and gender. Significantly lower values of calprotectin were recorded in horses participating in higher race categories.

Serum calprotectin has been successfully determined in all the enrolled horses. Lower values noted in horses undergoing more demanding training programmes could indicate a different effect of levels of exercise on subclinical systemic inflammation. The results of this pilot study emphasise the role of serum calprotectin as a potential biomarker of different training adaptations.

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OSTEOSARCOMA OF THE MANDIBLE IN A GERIATRIC HORSE – A CASE REPORT

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Osteosarcoma (OS), a malignant neoplasm of bone is a rare tumour in horses. Most cases have been attributed to young horses. This report presents a case of OS of the mandible in a horse.

A 21-year-old Warmblood gelding was presented with a history of weight loss, painful swelling of the left lower jaw, halitosis, quidding and reduced appetite lasting for 8 months. On presentation the horse was depressed, with BCS 3/9, bilateral temporal muscle atrophy and swelling of the left body of mandible. Intraoral examination revealed hard swelling of the left ramus mandible from 306 to 311.

Radiography showed a huge expansile, aggressive bone lesion involving most of the left mandible. Osteolysis was predominant with sun-burst periosteal proliferation. Periapical area of 306 to 311 was radiolucent with total alveolar bone destruction. Mandibulectomy was rejected by the owners and the horse was euthanised. Post-mortem analysis involved the head and included computed tomography (CT) scan, histopathology (HP) and immunohistochemistry (IHC).

CT scan revealed a tumour mass with bone destruction and lytic lesions. The lesion extended from 306 to the caudal border of the ramus mandible. The ventral border was destroyed and replaced by connective tissue stroma. HP showed densely to loosely arranged spindle to polygonal cells that form intertwined bundles. Cells had scant to moderate eosinophilic cytoplasm and roundish or irregularly shaped nucleus. Anisokaryosis and karyomegaly were present, likewise a moderate number of giant multinuclear cells. Mitoses were 0–3/HPF. Multifocally there were islets of osteoid, bone, necrosis and purulent inflammation with bacterial colonies. IHC showed positivity for vimentin, small number of cells for cytokeratin. The diagnosis of osteoblastic and fibroblastic OS (mixed type) with epithelioid differentiation was made.

This report emphasises the importance of considering OS as a differential diagnosis in cases of mandibular mass in old horses.

EVALUATION OF CLINICAL AND LABORATORY VARIABLES AS A PROGNOSTIC INDICATORS IN HOSPITALISED COLIC HORSES

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There are several reports on predictors for the outcome of equine colic. Identification of clinical and laboratory variables associated with prognosis can help the clinician in the process of decision making. The aim of this study was to determine whether there was an association between outcome and data of clinical examination and blood analyses to provide a better guideline for determining prognosis in horses with colic.

The present study included hospitalised colic horses during 5 years. Clinical and laboratory data were obtained at the time of the admission. The outcome was survival/non-survival/euthanised. Horses were divided into groups: heart rate beat/minute (HR≤60/HR>60), presence of reflux (yes/no), gut sounds (decreased/normal/increased), haematocrit percentage (<32/32–48/>48), lactate concentration mmol/L (<4/>4), performed treatment (medical/surgical), location of colic (small intestine/large intestine/other). Data were analysed by Chi-squared test.

There were 87 horses enrolled, with the average age of 11.3 years (SD ± 5.7). Eight horses died, 43 survived treatment and 28 horses were euthanised. The following variables proved to be significantly associated with survival: HR ≤ 60 (p = 0.01), no reflux (p = 0.03), normal gut sounds (p = 0.007), haematocrit percentage between 32–48 (p = 0.007), lactate concentration lower than 4 mmol/L (p = 0.002), and problem located in large intestine (p = 0.01). There was no association between performed treatment and survival.

This study demonstrated that different clinical and laboratory variables could be used as prognostic indicators in colic horses. Lactate concentration at admission of higher than 4 mmol/L could be a useful indicator of disease severity and indicate a negative outcome. Lack of connection between medical and surgical treatment could indicate that the selected horse were good candidates for surgery what emphasise the importance of appropriate evaluation of prognostic indicators.

DIAGNOSING AND TREATING LIVER DISEASE IN HORSES – A CASE REPORT

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Liver disease is a relatively common problem in horses and is easily diagnosed with blood tests. Due to non-specific symptoms, the severity of the disease cannot be easily determined so advanced diagnostic methods, such as a liver biopsy, are needed. This report describes a case of liver disease in a 14-year-old Connemara gelding.

The horse was presented in emergency for an acute episode of laminitis, dullness and recent weight loss. Laboratory tests showed elevation of hepatic enzymes, including gamma glutamyl transferase (GGT), glutamate dehydrogenase (GLDH), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST). Other horses on the same yard showed no symptoms, and their laboratory test results were within normal range. Initial treatment included antibiotic and anti-inflammatory drugs with milk thistle supplement (silymarin) and B vitamins. Blood samples were retested after four weeks, and showed only mild elevation of GLDH, so treatment with only milk thistle and B vitamins was continued. Eight weeks from the initial examination, the horse again presented the same clinical signs. Initial treatment caused improvement but not long-lasting since biochemistry showed elevation of GGT and LDH, so a liver biopsy was performed. Main histopathologic diagnosis was moderate-marked periportal fibrosis. Colchicine and vitamin E were introduced alongside the previous treatment for the duration of two months. Blood samples were retested in two-month intervals on three occasions, and all hepatic enzymes remained within normal reference range.

Diagnosing and treating liver disease in horses can be a challenge because the cause is often unknown, and the treatment lasts long. Advanced diagnostic techniques are useful tools which provide more accurate diagnosis, specific treatment plan and prognostic information.

MANAGING A DEEP DIGITAL FLEXOR TENDON INJURY – A CASE REPORT

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The majority of forelimb lameness in the horse originate from the foot, with an injury to the deep digital flexor tendon (DDFT) being the most frequent soft tissue injury. Despite different treatment options, the prognosis is usually guarded with a high risk of reinjury. This case report describes a DDFT injury in a 12-year-old Thoroughbred mare.

The mare presented a 3/5 grade lameness of the right forelimb (RF) which was localised to the foot after a positive response to a palmar digital nerve block. The taken radiographs did not detect any anomalies which would explain the level of presented lameness. Rest and anti-inflammatory treatment with phenylbutazone were prescribed, which resulted in improvement but intermittent low-grade (1/5) RF lameness was still present. With the increase of exercise, the original level of RF lameness recurred so the RF coffin joint was medicated, and corrective shoeing was applied. Post medication lameness was still present so the mare was referred for an MRI scan. The MRI showed a tear in the medial and the lateral lobe of the DDFT which were associated with two synovial masses in the navicular bursa. Following the diagnosis of the MRI scan, the mare was referred for a navicular bursoscopy which was followed by a controlled exercise programme. Despite correctly diagnosing the problem and providing the best treatment option, the mare never fully recovered.

A correct diagnosis and treatment followed by a long rehabilitation process do not always guarantee a full recovery. Any soft tissue injury in horses is serious and may easily recur so it is important to manage the owners' expectations while keeping in mind the horses' well-being.

/ / / / / EXOTIC AND WILD ANIMALS

WILDLIFE REHABILITATION TRIAGE

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When wild animals are found sick or injured veterinarians should be aware that the clinical examination and triage differ from those in domesticated animals. In wildlife medicine, animals with the highest chances of survival are treated first. This is because the purpose of wildlife triage is not only to increase the probability of survival but to decide which animal can be released back to nature, which is the final goal of wildlife rehabilitation. For this reason, it is sometimes referred to as "reverse triage" because the least ill patient should be treated first.

The first step is taking patient history and it is followed by "in-the-box" hands-off exam to check for any visible abnormalities. The animal can then be removed from the box to assess its movement as it can often reveal injuries that are not apparent when the animal is in the box. The next step is hands-on exam which should last no more than 15 seconds. Since major threats for wildlife survival are stress and pain, clinical examination should be done in a dark and quiet room with minimal staff present and the pain should be relieved as soon as possible. After the hands-on exam, the animal should be situated in the appropriate housing before the development of correct treatment plan.

When assessing animal's condition, species characteristics, natural behaviour, age, sex, treatment options, probability of release, rehabilitation time, available resources and legislative requirements should be considered. Injuries that warrant euthanasia are, with some exceptions, amputations, traumatic joint injuries, loss of vision and spinal cord injuries with the loss of deep pain. In case of injuries that cause permanent disability, imminently fatal injuries as well as those causing heavy pain and suffering, euthanasia is justified. Although some injuries are treatable, it is important to balance the suffering with the probability of release.

The purpose of triage is to decide whether to treat or euthanise, based on the animal's chance of surviving in nature after rehabilitation.

SKIN MICROBIOLOGY OF VIETNAMESE MOSSY FROGS (*Theloderma corticale*)

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Amphibians are one of the most endangered vertebrates in the world due to their habitat loss, environmental pollution, climate changes and emerging diseases. By secretion of peptides from skin glands and by composition of skin microbiota, their skin plays an important role in the protection from various diseases.

To get more insight into the skin microbiota, skin swabs of eight Vietnamese mossy frogs (*Theloderma corticale*) situated backstage of the Zagreb Zoo were taken. Samples were taken with sterile swabs without transport media and immediately transported to the laboratory of the Department of Poultry Diseases with Clinic. Swabs were plated on non-selective culture media for detection of aerobic bacteria and incubated 24 hours at room temperature. Isolated bacteria were further identified microscopically, by catalase and oxidase reactions, and by Gram staining. Further identification to genus level was done using MALDI-TOF MS. For fungal detection, swabs were plated on Sabouraud dextrose agar and incubated for five days at room temperature. The identification was based on morphological appearance and microscopic characteristics using lactophenol staining, and by MALDI-TOF MS. Skin swabs were also tested for the presence of *Batrachochytrium dendrobatidis*, *B. salamandrivorans, Ranavirus* and *Chlamydia* spp. by qPCR after DNA extraction using Qiagen Dneasy Blood and Tissue Kit.

In total, 13 bacterial and 4 fungal species were isolated, with predominance of *Bacillus cereus* and *Candida catenuata*. Although *B. cereus* is a common isolate of amphibian skin, there is no data about its pathogenic or protective role in amphibians. Detection of *C. catenuata* could indicate immunodeficiency, which should be corrected by proper husbandry and water quality in captive enclosures. Chytrid fungi, *Ranavirus* and *Chlamydia* spp. were not detected.

Microbiological screening of amphibian microflora seems to be useful tool for distinguishing between protective and pathogenic microbiota in amphibian conservation efforts.

ANATOMICAL STUDY OF SAFE CORRIDORS FOR EXTERNAL SKELETAL PIN INSERTION IN THE RABBIT BRACHIUM

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The successful external skeletal pin insertion in different animals depends on the knowledge of topographic and cross–sectional anatomy and their species-specific anatomical characteristics. The use of inappropriate corridors can cause iatrogenic injuries. The aim of this study is to describe the topographic and cross-sectional anatomy of the rabbit brachium and to define safe corridors for pin insertion.

Fourteen forelimbs of seven rabbit cadavers were used for this study. Slaughtered rabbits were bought from the rabbit farm for meat production for human consumption. On seven forelimbs the anatomical dissection was performed and seven forelimbs were deep frozen at –14°C. The frozen forelimbs were transversally cut and anatomical structures were defined. Safe corridors for external skeletal fixation were described.

Safe corridors for external skeletal pin insertion on the rabbit brachium were defined on the craniolateral side of the brachium and at the level of the humeral epicondyles.

Caudal side of the brachium is not convenient for pin insertion because of the presence of the big triceps brachii muscle.

It is not possible to use the medial side of the brachium due to the location of important nerves of the brachial plexus.

Treatment of humeral fractures in rabbits with external skeletal fixation can be used as a primary method. An external skeletal fixator pin should not damage any big muscle, vessel or nerve during surgery. For this reason, the exact knowledge of the topographic anatomy and safe areas for pin insertion is needed.

VARIATION IN COMPLETE BLOOD COUNT AND BIOCHEMICAL PARAMETERS IN GRIFFON VULTURE UPON ADMISSION TO THE RESCUE CENTRE FOR GRIFFON VULTURE ON THE ISLAND CRES IN CROATIA

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Griffon vultures (GF) play important roles in food chain in the maintaining of the whole ecosystem. However, their population is endangered being on the threshold of extinction. Laboratory and field investigations are crucial for their rehabilitation and preservation. This study aimed to investigate variation in complete blood count (CBC) and biochemical parameters in GF upon admission to the Rescue Centre for Griffon Vulture on the island Cres in Croatia.

Blood samples were obtained from 36 GF (12 female, 16 males, 8 not determined; one nestling, 25 immature, 1 adult and 9 not determined) upon the admission to the Rescue Centre. In collected samples, CBC and biochemical parameters were analysed. Statistical analysis was performed using statistical software IBM SPSS Statistics 21® (IBM Corp., Armonk, New York, USA).

Significant differences in the means of some haematological parameters were found compared to the literature data, i.e. PCV, haemoglobin, MCV, MCH, MCHC, total WBC and a differential blood count. Particularly, decreased lymphocyte count could be a consequence of endogenous glucocorticoid release due to stressful conditions upon arrival to the centre. Monocytosis in our birds might suggest inflammatory response. Higher relative eosinophil and basophil counts in our population could point out to immune response. Additionally, there was an increase in polychromatophils in two immature vultures indicating regenerative anaemia. Significant differences in the means of glucose, total proteins, albumin, triglyceride, calcium, urea, creatinine, ALT and AST were observed as well. Increased AST and ALT in almost all birds may be due to muscle injury during falling from cliffs or manipulation.

Obtained results showed variation in some laboratory parameters suggesting alteration in physiological condition and health status. Our results will benefit understanding of pathophysiological variations of blood parameters, facilitating better preservation of this species.

ANAESTHETIC MANAGEMENT OF A FEMUR FRACTURE REPAIR IN A YOUNG RED FOX

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The red fox (*Vulpes vulpes*) is one of the most widely distributed members of the order Carnivora, being present across the entire Northern Hemisphere. It is a small mammal, with a sharp-pointed face and ears, and a large tail. Because of their ability to adapt to different habitations, it is not strange to find them in villages and cities in search of food.

A 4-month-old, female red fox from the Dumovec shelter was presented with no possibility to stand on hind legs. It was sedated intramuscular with 0.004 mg/kg dexmedetomidine, 4 mg/kg ketamine and 0.2 mg/kg midazolam. Orthopaedic examination and radiology revealed a comminuted diaphyseal fracture of the right femur. An intravenous catheter was placed in the cephalic vein, and methadone (0.1 mg/kg) was applied. General anaesthesia was induced intravenously with propofol 6 mg/kg. After the loss of palpebral reflex and jaw tone, the red fox was endotracheal intubated with a tube size 5 mm in diameter. To maintain general anaesthesia, 1.5% isoflurane was used. For multimodal analgesia, epidural anaesthesia of 2% lidocaine and 0.5% bupivacaine (0.4 mg/kg) was added. Intravenous fluids were given throughout the surgical procedure (0.9% NaCl, 5 ml/kg/h). During the whole procedure ECG, capnography, pulse oximetry, end-tidal CO_2 and non-invasive blood pressure was continuously monitored. The heart rate, respiratory rate and blood pressure were stable during the surgery and, except for the mild hypothermia at the end of the procedure, the recovery from the anaesthesia went uneventful. Postoperative analgesia was maintained with meloxicam (0.1 mg/kg) for 5 days.

Due to stabile anaesthetic parameters during the surgery, it can be concluded that multimodal analgesia approach for orthopaedic surgery is the optimal choice in a red fox. Using methadone as an opioid agent for analgesia is the first report to the author´s knowledge.

SURGICAL TREATMENT OF CUTANEOUS LOW MALIGNANCY SARCOMA IN A DEGU (*OCTODON DEGUS*) – A CASE REPORT

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Degus (*Octodon degus*) are a species of rodents originating from Chile. Originally used as laboratory animals, they are becoming more popular as pets in recent years.

A 5.5-year-old intact female degu was admitted to the clinic for a suspected mass on the left thoracic wall. Upon clinical presentation a round, well-circumscribed, incapsulated cutaneous mass 8x5 mm in diameter on the left costal region was visible. Fine needle biopsy was performed and the cytological examination suggested a malignant peripheral nerve sheath tumour. The degu was premedicated intramuscularly with combination of midazolam and ketamine and anaesthesia was maintained using isoflurane. During the whole procedure heart rate, respiratory rate and SpO_2 (pulse oximetry) were monitored. The surgical area was clipped and prepared for surgery. An elliptical incision on the skin was made. A surgical margin of 7 mm was applied and the tissue was excised down to intercostal muscles and the surgical wound was closed in layers. Pathohistological examination of the mass revealed a low-grade sarcoma and indicated a complete surgical excision. The patient was discharged the same day following the procedure. On check-ups no recurrence was reported and radiologically no metastases were found. The degu survived 512 days following surgery but was euthanised due to severe dental disease.

Margins used for complete excision of cutaneous malignant neoplasia in companion animals are not applicable in small mammals due to their size. Comparing the average size of both the degu and guinea pig, with the former being smaller and lighter than the latter, a 0.5–1 mm margin as used in guinea pigs for removal of malignant cutaneous neoplasia still allows for a complete excision and successful wound closure.

This case report suggests that the same margin as the one used in guinea pigs should be sufficient for complete removal of cutaneous masses in degus.

PRELIMINARY STUDY OF FATTY ACID COMPOSITION OF PERIRENAL ADIPOSE TISSUE OF FREE-RANGING BROWN BEARS FROM CROATIA

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There are structural and metabolic differences between adipose depots and it is generally assumed that the fatty acid (FA) composition of all adipose tissue within an individual is the same. Some studies did not detect any site-specific differences, but some consistent differences have been reported. To our knowledge, FA composition of brown bear perirenal adipose tissue (PAT) has not been investigated, so the objective of this study was to determine the FA composition of this tissue.

The study was conducted on 18 free-ranging brown bears from Croatia (8 females, F; 10 males, M) sampled during 2015 hunting season. Tissue samples were homogenised, total lipids extracted than composition of FA was determined by gas chromatography.

PAT was dominated by monounsaturated FA (F, 45.5 ± 2.6% vs. M, 43.3 ± 1.7%, $p < 0.05$). Second in the representation were saturated FA (SFA, 42.5 ± 4.8% vs. 44.7 ± 4.5%, $p > 0.05$), while least represented were polyunsaturated FA (PUFA, 3.2 ± 1.9% vs. 2.6 ± 1.2%, $p > 0.05$). Females had significantly lower percentage of C16:2 (1.0 ± 0.3% vs. 3.8 ± 1.2%) compared to males. When compared to our previously published results (F, N = 19; M, N = 57;) regarding FA composition of subcutaneous adipose tissue (SAT), PAT of females had 1.7 times higher representation of SFA and 2.1 times lower representation of PUFA. Furthermore, PAT of males had 1.7 times lower representation of PUFA than SAT of males. Essential C18:2n-6 showed difference in representation in females comparing the two adipose tissues (PAT, 2.4 times lower than SAT).

Physiological significance of differences in the FA composition of two adipose tissue locations in the body is possibly dependent on factors such as dietary fat composition, energy balance, and metabolic activity as well as some factors that have to be determined. Regarding this, larger numbers of brown bear PAT samples in different seasons, emphasizing the influence of autumn and spring, have to be investigated.

CASE REPORT: CARBOFURAN POISONING IN WILD ANIMALS

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The deliberate poisoning of wild animals is a problem worldwide and a direct danger to the environment and humans. Poisons from the carbamate group are frequently used, although prohibited in the European Union due to their toxicity for mammals, birds, fish, and wild animals. Carbofuran belongs to the carbamate group and acts on the principle of reversible inhibition of acetylcholinesterase which causes hyperstimulation of cholinergic receptors, followed by muscarinic, nicotinic and central nervous signs. The death is usually a result of heart and respiratory failure.

In the area of Mazin (Municipality of Gračac, Zadar County), 2 calves, wolf, fox, cow and golden eagle carcasses were found within a range of one hundred meters. Necropsy (N) and histopathological examination (HE) were performed; likewise, toxicological analysis was done (TA) using the gas chromatography-mass spectrometry (GC-MS) method.

Unknown foreign substance was found in the wolf's stomach, the trachea, and the fox's stomach. In fox, wolf, and golden eagle, N and HE revealed extensive multi-organ haemorrhages and congestion of parenchymal organs. In calves and the cow, multiple post-mortem injuries of the skin, subcutaneous tissue, and skeletal muscles were found, with the same content within the lesions. The unknown foreign substance in all carcasses was detected as carbofuran. The calves died without a suspicion of violent death.

On the basis of the findings from the scene of the incident and N, the suspicion of poisoning had been assumed, which was confirmed by TA. The carcasses of the calves and cow were used as the source of the poison bait, which is still a usual method of deliberate wildlife poisoning of animals. The pathological findings in the fox, wolf, and golden eagle were non-specific but corresponded to the descriptions of carbofuran-induced pathological changes from the literature.

COMMON CALCANEAL TENDON REPAIR IN A BLACK-AND-WHITE RUFFED LEMUR (*VARECIA VARIEGATA*) USING A CALCANEUS TUNNEL AND MODIFIED KRACKOW SUTURE PATTERN

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An 8-month-old, female, intact, healthy black-and-white ruffed lemur from the Zoo was presented with peracute lower leg swelling and non-weight-bearing hindlimb. Clinical evidence of complete rupture of the common calcanean tendon was present. An X-ray study noted signs of soft tissue oedema in the calcaneal region. General anaesthesia was induced with propofol 2 mg/kg IV. Endotracheal intubation and 2% isoflurane were used to maintain anaesthesia. Hydration was maintained with 0.9% NaCl 10 mL/kg/h IV. Epidural analgesia was provided with 2% lidocaine 0.15 mg/kg.

A caudal approach to the common calcanean tendon was used. Reposition and reconstruction was achieved using a calcaneal tunnel and Modified Krackow (MK) suture pattern. The suture configuration of the MK suture pattern was completed with the OrthoFiber 5® pre-sterilised suture. The wound was reconstructed with a Maxon ® 2-0 suture in two layers. The skin was reconstructed intradermally with Maxon ® 2-0 to avoid suture removal procedures and associated handling with possible sedation risks. Analgesia was provided with meloxicam (0.1 mg/kg IV) and maintained perorally for the next ten days.

Recovery from the surgery was uneventful, and the lemur was reintroduced into the group upon recovering from anaesthesia. Initial weight-bearing started within 9 days after the surgery, with progressive limb loading in the upcoming weeks. 43 days post-surgery normal activity with full weight-bearing was observed.

ANALYSIS OF TEMPORAL OVERLAP BETWEEN WOLF AND ITS PREY

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Animal activity patterns are driven mainly by physiological constraints, but also resources availability, weather and interaction with sympatric species. Research of temporal distribution of activity in animals living in sympatry helps understanding factors shaping interspecific interactions. This is especially interesting in predator – prey systems, so we analysed the temporal overlap between the wolf (*Canis lupus*) and coexisting ungulates – roe deer (*Capreolus capreolus)*, red deer (*Cervus elaphus*) and wild boar (*Sus scrofa*).

Our study was conducted in Lika, Gorski kotar and northern Dalmatia in Croatia, with the total surface of the study area of around 6000 km^2. During the period from May 2018 to November 2020 automatic camera traps with infra-red sensors were set at 158 locations. Camera traps were checked at least every two months to collect data and replace batteries. Collected images were processed in program Camelot and for each event, the species, number of animals and age category (juvenile or adult) were defined. An event is defined as one recording of an animal lasting 10 minutes during which several photos and videos could be taken. Patterns of interspecific temporal overlap were estimated using the software R, package Overlap. The coefficient of overlapping (Δ_4) was calculated, and it can range between 0 (no overlap) and 1 (total overlap). A total of 7497 photos of target species were collected and all revealed largely nocturnal activity.

Our analysis showed that in the study area the temporal overlap amongst the wolf and observed ungulates was substantial. The highest coefficient of temporal overlap ($\Delta_4 = 0.87$) was reported between wolf and red deer, while it was the same for the wild boar and roe deer ($\Delta_4 = 0.70$). This suggests that in our study system, prey plays an important role in influencing wolf activity, however, further research is needed to discern the behavioural responses of observed species.

SEQUENCE ANALYSIS OF COX-1 GENE OF THE GIANT AMERICAN LIVER FLUKE (*FASCIOLOIDES MAGNA*) FROM CROATIA AND SERBIA

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Fascioloides magna is a non-native parasite in Europe, originally parasitizing in deer species of North America. After arriving to Europe during the 19th century, the fluke formed new host-parasite associations, and established three permanent natural foci: in Italy, Czech Republic and Danube floodplains. Favourable environmental conditions and a variety of available intermediate and final hosts have resulted in relatively rapid spread of *F. magna* to new geographic areas and until now, invasion of wild and domestic ruminants was confirmed in Italy, Czech Republic, Slovakia, Hungary, Germany, Poland, Austria, Croatia and Serbia.

The goal of this research was to analyse cox-1 gene and to collect data on fluke diversity in Croatia and Serbia, presenting first molecular data from Serbia and first molecular analysis of samples covering almost the entire distribution area in Croatia. After DNA isolation, PCR and sequencing we analysed 355 base pairs of cox-1 genes of 58 *F. magna* samples collected from red deer (*Cervus elaphus*) in Croatia and 55 in Serbia. In Croatia, the presence of two previously known haplotypes of the Danube focus was confirmed and the most common haplotype (Ha3) was also present in Serbian samples. Another haplotype, previously not described in the literature, was shared among samples from Croatia and Serbia. The finding of new haplotypes highlights the need for further research.

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SEROLOGICAL SURVEY OF *LEPTOSPIRA* SPP. IN WILD BOARS FROM MEDVEDNICA NATURE PARK

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During the last few decades, a growing wild boar (*Sus scrofa*) population and their colonization of suburban and urban areas has been noted across Europe. As wild boars are known potential hosts of several zoonotic pathogens, more frequent interactions with humans and animals may pose a public health concern. The aim of our study was to assess their potential role as reservoirs for leptospirosis in areas with pronounced human activity.

A total of 96 wild boar serum samples were collected within health monitoring project in the Medvednica Nature Park over the period of four years (2014–2016; 2018). For antibody detection, microscopic agglutination test was performed using an antigen panel of 12 different serovars; cut-off titre of 1:100 was considered positive.

An overall prevalence of 21.88% was determined with variation that was related to year and season of collection. Higher prevalence of leptospirosis was observed in 2014 (30.77%) and 2015 (31.58%) as opposed to 2016 (6.25%) and 2018 (9.09%). Odds ratio indicate 4.44 times higher possibility of infection in 2014 compared to 2018 (CI 95% .893-22.118), 6.66 compared to 2016 (.787 – 56.405) and 4.6 in 2015 compared to 2018 (.805-26.454). Seasonally, 20% of seropositive boars were hunted during spring, 13.79% during summer, 42.11% during autumn and 17.86% during the winter season. Higher prevalence ($p < 0.05$) was found in adults (> 2 y). As opposed to most of the previous studies, we found higher overall seropositivity in females ($p < 0.05$). However, these variations were not temporally consistent. The distribution of most prevalent serogroups also varied over time with overall domination of serogroup Pomona (38.1%), followed by Javanica, Grippotyphosa (23.81% each) and Australis (9.5%).

Our results confirmed that wild boars are a potential source of pathogenic *Leptospira* spp. Further monitoring of the prevalence of leptospirosis in wild boars, as well as confirmation and clarification of associated risk factors, are needed.

THE PECULIARITIES OF THE COMPLETE BLOOD COUNT OF THE UTILA IGUANA (*CTENOSAURA BAKERI*)

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This research dealt with the physiological blood parameters of the highly protected and rare iguana species, Utila iguana which naturally resides in only one habitat.

Samples were extracted from ten adult animals residing in the Zagreb City Zoo, out of which there were 2 females and 8 males. The samples were then used to make double blood smears for each animal, while the rest of the blood was put into the eppendorf test tubes containing heparin. Determined blood parameters were: PCV, total red blood cell count, leukocyte count, haemoglobin concentration, red blood cell indices and differential blood cell count. The results were statistically compared to the haematological parameters of the green iguana.

Physiological parameters were obtained for 8 males since one female had inflammatory leukogram due to the bite wound, while the other had anaemia. Considering the pathological conditions, those data were not taken into consideration. By comparing the red blood cell count, Utila iguanas had significantly lower number of erythrocytes but higher concentrations of haemoglobin than green iguanas. Furthermore, in the differential blood cell count data between Utila and green iguanas, the only difference was significantly higher absolute number of basophils in Utila iguanas.

Differences in some blood parameters between two species of iguanas could be due to the external factors. The data obtained in this study determine the blood parameters of clinically healthy Utila iguanas, which contributes to a better understanding of physiological blood picture of these animals, and thus allows more efficient detection of pathological conditions and faster medical treatment which is an important factor in preserving this rare species.

PARASPIDODERA UNCINATA IN GUINEA PIG

Nika Konstantinović, Lea Lovrić

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Guinea pigs (*Cavia porcellus*), also known as cavies, are popular laboratory and pet animals. Even though their popularity as pets is on the rise, there is still little data on several endoparasites they can carry. However, a parasitological coprological examination is seldom recommended by veterinary practitioners. *Paraspidodera uncinata* is one of their most common intestinal parasites. Infection is usually harmless, but clinical signs such as diarrhoea, weight loss, and weakness may occur.

Small and large intestines of the pet guinea pig were delivered from the Department of Veterinary Pathology to the Department of Parasitology and Parasitic Diseases with the clinic for parasitological examination. Coprological parasitological analysis was performed using a flotation technique with $ZnSo_4$ solution (s.g. 1.35) and sedimentation technique.

Two adult nematodes that were recovered from the cecum of the animal were identified as an adult male and female of *P. uncinata* based on morphological characteristics according to other authors. Using coprological analysis, thick-shelled eggs, ellipsoid to oval in shape, were found. The length varied from 54 to 69 µm, while the width ranged between 41 and 47 µm.

The eggs are described as thick-shelled and ellipsoid in appearance, length varying from 43 to 73 µm, and width from 31 to 53 µm, according to different authors. The life cycle of this parasite is direct, and it is transmitted through feed and water contaminated with infective eggs. Therefore, adequate sanitation is essential to prevent infection in both laboratory and pet cavies. Since *P. uncinata* can occasionally cause severe infections, coprological examination of a guinea pig should more often be recommended by veterinary practitioners.

SUMMER BROOD INTERRUPTION AS EFFECTIVE AND SUSTAINABLE VARROOSIS CONTROL

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Varroosis is caused by parasitic mite *Varroa destructor*. It causes major economic losses worldwide to the beekeeping sector, so it is necessary to regularly monitor and control mite infestation levels in honeybee colonies. The *V. destructor* mite contributes to weakening and immunosuppression of colonies what can increase the susceptibility to other diseases. Despite the existence of many control measures, there is a need for implementation of new management practices that would be based on adopting specific biotechnical methods in combination with soft acaricides treatment.

One health approach requires achieving a reduction in the *V. destructor* mites number, and at the same time ensure safe production of honey and other honeybee products which are food for humans.

Brood interruption consists of various methods that artificially stop the queen egg laying and consequently induce the absence of brood during summer months. This is increasing the anti-varroa treatments efficiency. The experience acquired from seasonal brood interruption studies showed that queen caging with combined oxalic acid treatment presents an effective treatment. As efficiency of the same treatment varies in different geographical areas and pastures, for the first time it is implemented during chestnut pasture, in the continental part of Croatia.

This way of integrated pest management can be successfully implemented in the studied area, but it is necessary to pay attention to satisfactory natural food sources in order to enable the development of a strong bee population that will survive the winter.

PRELIMINARY STUDY – STRATIFICATION AND INTER-SPECIFIC DIFFERENCES IN FATTY ACID COMPOSITION OF DOLPHIN'S BLUBBER

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Cetaceans have been shown to exhibit three layers within the blubber with each layer performing a different function; for example, stable outer layer used for structural support, more variable inner layer for energy storage. The aim was to determine fatty acid (FA) composition of blubber in two dolphin species found in the Adriatic Sea.

The study was conducted on 8 animals, bottlenose dolphin (*Tursiops truncatus*; 2 females, F and 4 males, M) and striped dolphin (*Stenella coeruleoalba*; 1 F, 1 M) sampled during 2018. Blubber was divided horizontally into outer (OL), middle (ML) and inner layer (IL). Tissue samples were homogenised, total lipids extracted than composition of FA was determined by gas chromatography.

Blubber OL and ML of striped dolphin was dominated by monounsaturated FA (MUFA, 55.2 ± 8.2%; 49.1 ± 2.8%) while in IL by polyunsaturated FA (PUFA, 41.7 ± 2.6%). In contrast, all three blubber layers of bottlenose dolphin were dominated by MUFA (OL, 63.1 ± 6.6%; ML, 55.1 ± 6.8%; IL, 49.0 ± 5.7%). Regarding blubber FA composition in striped dolphin most differences were between ML vs. IL (very long n-3 PUFA, 14.1 ± 4.8% vs. 36.8 ± 3.4%; n-3, 15.2 ± 4.2% vs. 37.5 ± 3.6%, all at $p < 0.05$). In bottlenose dolphin most differences were between OL vs. IL (C18:0, 1.5 ± 0.2% vs. 3.8 ± 1.5%; MUFA, 63.1 ± 6.6% vs. 49.0 ± 5.7%; $p < 0.05$).

When testing differences in blubber FA composition for striped vs. bottlenose dolphin, most differences were in ML (C18:2n-6, 5.3 ± 3.1% vs. 1.9 ± 0.5%; C20:4n-3, 0.1 ± 0.1% vs. 0.6 ± 0.2%; n-6, 6.6 ± 3.2% vs. 3.4 ± 0.8%; $p < 0.05$) and in IL (C22:6n-3, 27.2 ± 13.8% vs. 10.5 ± 2.3%; PUFA, 41.7 ± 2.6% vs. 22.1 ± 2.9%; n-3, 37.5 ± 3.6% vs. 18.2 ± 2.6%; very long n-3 PUFA, 36.8 ± 3.7% vs. 17.0 ± 2.3%; $p < 0.05$).

Although more samples are required, this stratification can be attributed to different levels of metabolic activity within layers. Differences in FA composition within and between species could be due to diet, since many long-chain PUFAs can be traced back to a specific organism and geographic location in the food chain.

/ / / / / SMALL ANIMALS

RETROSPECTIVE STUDY OF TOXICITIES ASSOCIATED WITH CHEMOTHERAPY IN DOGS WITH MULTICENTRIC LYMPHOMA TREATED AT THE CLINIC FOR INTERNAL DISEASES, FACULTY OF VETERINARY MEDICINE, UNIVERSITY OF ZAGREB (2005–2021)

Tea Dodig, Mirna Brkljačić, Martina Crnogaj, Ivana Filipčić, Jelena Gotić, Ines Jović, Iva Šmit, Marin Torti, Nada Kučer

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Systemic chemotherapy is the therapy of choice for canine multicentric lymphoma. While implementing a chemotherapeutic protocol, it is important to inform the owners about the possible side effects which could interfere with the patient's life quality. Chemotherapeutics can be associated with numerous toxicities. In most cases, toxicities are acute, but chronic or delayed effects do occur. The most common toxicities include bone marrow suppression, gastrointestinal injury, alopecia and other drug-specific toxicities (e.g. cardiotoxicity from doxorubicin). All of the documented toxicities are associated with dosage, concurrent diseases/organ dysfunction, specific breeds, idiosyncratic reactions and/or drug specifics.

The aim of this study was to investigate the most common toxicities and their prevalence in the population of dogs with multicentric lymphoma treated at the Clinic for Internal Diseases, Faculty of Veterinary Medicine, University of Zagreb, from 2005–2021.

Thirty-two dogs (15 females, 17 males) were diagnosed with multicentric lymphoma on the basis of cytology (66%) or immunocytochemistry (34%) of fine needle lymph node aspirates. Different drugs according to multiple protocols were used: vincristine (100%), doxorubicin (88%), cytarabine (66%), cyclophosphamide (50%), lomustine (13%), and methotrexate (3%). All of the dogs received prednisolone. Most common toxicities were neutropenia (28%) and gastrointestinal side effects (diarrhoea 50%, nausea and vomiting 47%, inappetence 28%). Other included lethargy (13%), haemorrhagic cystitis (9%), local reaction after chemotherapeutic application (6%), skin erythema (6%) and fever (3%). Toxicities were most frequently seen with the use of vincristine (34%), followed by doxorubicin (28%) and cyclophosphamide (22%).

In conclusion, noted toxicities were mild and self-limiting with neutropenia and gastrointestinal side effects being the most common. The majority of toxicities was attributed to vincristine and doxorubicin.

LONG TERM ENTERAL NUTRITION IN A KITTEN – A CASE REPORT

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Motility disorders in the pregastric region can disable feed passage requiring enteral nutrition support for adequate energy and nutrients supply. Nevertheless, long-term enteral nutrition is challenging especially in growing animals due to tube-related complications, limitations posed by gastric volume, and extensive follow-up. Additionally, there is limited data on enteral nutritional support of growing animals. Therefore, the aim of this case report is to showcase a detailed enteral nutrition approach during growth in a kitten.

A European shorthair kitten, female, 4 m old, was presented at Ghent University Teaching Hospital with a complaint of coughing up phlegm, regurgitating, and breathing with an open mouth. Weight loss of 20% was noted over the past 7 days. Upon conducting a contrast radiograph a diagnosis of focal dilatation of the oesophagus due to congenital diverticulum or congenital or acquired focal stenosis and aspirational pneumonia was made. A gastric tube was placed the following day.

Combining principles of enteral nutrition and growth, a nutritional protocol was prepared. Energy requirements were calculated to support growth using the formula 1.75-2 x 418 kJ/kg$^{0.67}$. Energy was incrementally increased to reach daily energy requirements over 6 days. A polymeric liquid diet appropriate for growth was fed. The frequency of feeding was adjusted not to exceed an estimated gastric volume of 4 ml/100 g BW/meal. The nutritional protocol was adapted weekly according to the growth curve to ensure approximate growth of 10 g/day. Tube-related complications included wound dehiscence and tube disintegration. In spite of incomplete resolution of focal dilatation, the diet was successfully introduced *per os*, gradually, over the period of 3 weeks.

Enteral nutrition during growth poses a challenging task, nevertheless, an appropriate and detailed nutritional plan and follow-up can ensure prevention of malnutrition and optimal growth and musculoskeletal development.

CO-INFECTION WITH *DIROFILARIA REPENS* AND *DIROFILARIA IMMITIS* IN A DOG – A CASE REPORT

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Canine dirofilariases are mosquito-borne zoonoses with a continuous expansion in Europe. While *Dirofilaria immitis* poses a great risk to animal health causing heartworm disease with a potentially fatal outcome, *Dirofilaria repens* usually causes non-pathogenic subcutaneous dirofilariasis. Over the past decade, an increasing number of cases of *D. immitis* and *D. repens* infections in dogs and humans have been reported. The following case describes accidental finding of *D. immitis* and *D. repens* co-infection in a dog.

In 2021, a 9-year-old male dog from the city of Sisak was autopsied at the Faculty of Veterinary Medicine University of Zagreb. Three adult nematodes were found in the right ventricle of the heart. One nematode and blood clot from the right heart were collected for further examination. Modified Knott's test was performed from mechanically lysed blood clot and revealed 11,050 microfilariae/mL. The same sample was screened for *D. immitis* circulating antigen using FASTest® HW Antigen (MEGACOR) and yielded negative. After DNA extraction from nematode, blood clot and lysed blood clot using NucleoSpin Tissue (Macherey-Nagel, Düren, Germany), PCR amplification of a cytochrome oxidase subunit 1 (COI) gene fragment of *D. immitis* and *D. repens* showed positivity for both species in blood clot samples, while nematode was positive for *D. immitis*.

Previous studies suggest that *D. immitis* and *D. repens* has expanded in Europe in the last decade due to increasing movement of dogs and global climate changes. In Croatia, *D. repens* was found to be widespread throughout country, while *D. immitis* was found mainly in the coastal area of Croatia, but also in the continental parts including the city of Sisak. Knott's test is considered the gold standard for detection of microfilariae, but detecting mixed infections can be challenging because of the morphological similarities between microfilariae of both nematodes. Therefore, molecular diagnostic tests are recommended to use.

DOES STORAGE OF BLOOD IN EDTA TUBE AFFECT HAEMATOLOGIC PARAMETERS OR NOT?

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The complete blood count (CBC) provides crucial data for clinicians. The storage conditions after blood collection are an important step in the preanalytical phase and errors can lead to false results. The purpose of this study was to document which parameters of CBC are most affected by storage.

Thirteen dogs in random order were included in the study. Blood samples were collected from the cephalic vein into EDTA tubes. Haematological analysis was performed using the Horiba Scil Vet ABC Plus analyser (Scil, Germany) within one hour of collection. After the initial analysis, 1 mL of the blood was aliquoted and stored at 4°C, while the remaining blood was stored at room temperature (RT). The CBC analysis was repeated after 24, 48, 72 and 96 hours. Blood stored at 4°C was mixed for 20 minutes on room temperature before analysis.

Leukocytes, haemoglobin (HGB), erythrocytes and haematocrit (HCT) did not change ($p > 0.05$) from the initial analysis and differed less than 5% during the studied period at both temperatures. MCV was higher at 96 hours post blood collection in samples stored at 4°C and at 72 and 96 hours stored at RT compared to the initial values ($p < 0.05$). Red blood cell distribution width was increased at 48, 72 and 96 hours in samples stored at 4°C and at 72 and 96 hours in samples stored at RT ($p < 0.05$). MCHC was decreased at 96 hours post-extraction in samples stored at RT ($p < 0.05$), but remained stable in samples stored at 4°C during the studied period. Platelet counts decreased during the study from 5% to 45% compared to the initial measurement ($p > 0.5$) with more pronounced decrease in refrigerated samples. Based on our results, leukocytes, erythrocytes, HGB, and HCT could be determined in whole blood stored up to 4 days after collection.

When stored in EDTA, erythrocytes swell, which may result in a falsely high MCV and consequently low MCHC. A decreased platelet count during storage could be explained by platelet aggregation at low temperatures.

IS IT POSSIBLE TO USE EDTA PLASMA AS A SAMPLE FOR BIOCHEMICAL ANALYSES IN VETERINARY LABORATORY DIAGNOSTICS?

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The choice of anticoagulant is an important preanalytical factor in laboratory diagnosis. Errors in anticoagulant selection can be avoided or reduced if it is found to affect certain biochemical analytes. The aim of this study was to compare values of some biochemical parameters in dog EDTA plasma and serum samples.

Paired plasma (EDTA anticoagulant) and serum samples were collected from cephalic vein of 15 dogs. Serum and EDTA plasma samples were obtained after centrifugation at 1600 g for 10 min. Concentrations of urea, creatinine, total proteins, albumin, glucose, bilirubin, and C-reactive protein (CRP), and activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), and creatine kinase (CK) were measured in the paired plasma and serum samples with Abbott reagents on the Abbott Architect Plus c4000 biochemical analyser.

The differences in concentrations of urea, creatinine, total proteins and albumin, and in activities of AST, ALT and GGT between serum and EDTA plasma were less than 5%. Glucose concentrations were up to 40% lower in EDTA plasma than in serum. Bilirubin concentrations were lower in EDTA plasma than in serum (16–30%). Alkaline phosphatase activity was significantly lower in EDTA plasma (78–95%) than in serum. Activity of CK ranged from –18% to +68% in EDTA plasma compared with serum. Concentrations of CRP in EDTA plasma samples were higher than in serum (38–80%) in samples where the CRP was less than 10 mg/L. When the CRP concentration was above 23 mg/L, the CRP differences in EDTA plasma vs. serum were up to 11%.

In conclusion, urea, creatinine, total proteins and albumin, and in AST, ALT and GGT can be reliably assayed from either serum or plasma samples in dog samples. Glucose, bilirubin AP, CK should always be assayed in serum. The determination of CRP in EDTA plasma can provide an approximate result, but the control samples must be repeated in EDTA plasma.

WHAT HAEMATOLOGICAL PARAMETERS IN DOGS ARE AFFECTED WHEN THE BLOOD IS LIPEMIC?

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Interferences are an important source of results variability in laboratory diagnostics. Lipemia is considered second most often interference in haematology in veterinary laboratory medicine, after haemolysis. Aim of this study is to determine what complete blood count (CBC) parameters in dogs are affected by lipemia.

The study was conducted on 10 dogs. Blood samples were collected from cephalic vein into EDTA tubes. Blood samples were neither lipemic nor haemolytic. The complete blood count was performed on Horiba Scil vet ABC Plus+ haematological analyser (Scil, Germany). SMOFLipid 20% solution was used for sample preparation of lipemia interference. Diluted lipid solutions were prepared according to lipemic index (Abbott Architect biochemical analyser: 0, 1+, 2+, 3+, 4+) and final lipid concentrations were 0.3, 0.9, 1.4, 2.0 and 5.0 g/L. Lipid solution was added to whole blood sample aliquot after performing initial haematological analysis. Measurements were done in duplicates.

Results indicate that lipemia statistically significantly increased haemoglobin (HB), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) in dog samples ($p < 0.05$). Red blood cell count (RBC), haematocrit (HTC), mean corpuscular volume (MCV) and red cell distribution width (RDW) were not changed significantly with increase of lipemia and differences were less than 5%. White blood cell count (WBC) and platelet count (PLT) increased with lipemia, with differences from 11% to 30% and 8% to 24% respectively ($p > 0.05$ both). Mean platelet volume (MPV) decreased from 2% to 8% with increasing of lipid concentration.

In conclusion, RBC, HTC and MCV can be measured reliably in lipemic samples. Due to turbidity of a sample HB, MCH and MCHC should not be assayed in lipemic samples. The determination of WBC, PLT and MPV in lipemic samples results in approximate values.

VARIATIONS OF STEMNESS GENES EXPRESSION OF CANINE MESENCHYMAL STEM CELLS DURING *IN VITRO* CULTIVATION

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The ultimate goal of successful expansion *in vitro* is to maintain the stemness of adipose-derived mesenchymal stem cells (AD-MSCs), reflected in their ability to maintain self-renewal, i.e., undergo extensive proliferation while maintaining their multipotent differentiation potential. Since stem cell therapy involves cellular manipulation and in vitro cultivation, it is necessary to reveal its impact on the expression of stemness genes during cultivation and consequently stem cell desirable properties.

We analysed changes in expression of several genes (FGF, INS, LIF, OCT4, SOX2 and WNT3A) responsible for stemness in eight healthy young female canine donors of different breeds (poodle, Rhodesian ridgeback, dachshund, beagle, Jack Russell terrier, Belgian shepherd, and two mix breed) referred to elective surgical procedure as it is the opportunity to collect the sample and bank the cells for use in adult age.

Changes in the expression of stemness genes were studied, using validated RT2 Profiler PCR Array Format R with SYBR Green-optimised primer assays, in cells obtained by collagenase digestion of adipose tissue samples cultivated and analysed in two-time points; passage (P) P3 and P6.

Variations of expression occurred between early (P3) and late (P6) passages, along with increased FGF and LIF expression, stable INS, SOX2 and OCT4, and decreased expression of WNT3A. The decreased expression of WNT3A, despite the stable expression of other stemness markers, explains the proliferation arrest and occurrence of senescence. It was already shown that blocking WNT signalling arrests growth, and a decline in WNT3A signalling is necessary for mesenchymal stem cells to proceed with replicative senescence. Though, stable expression of other stemness markers in cells cultivated in standard condition between P3 and P6 indicate their preserved stemness properties prior and optimal timeframe for transplantation. These results will be further validated on protein level and more canine donors' cells.

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CAN SERUM CALPROTECTIN BE A RELIABLE BIOMARKER IN DOGS WITH GASTROINTESTINAL SYMPTOMS?

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Calprotectin is a protein involved in the inclusion of inflammatory cells in the body's immune response by interacting with endothelial cells. Measured in serum it is used as a prognostic factor for dogs with sepsis and systemic inflammatory response syndrome (SIRS). Many studies showed promising results using faecal calprotectin in diagnosing inflammatory bowel disease. The aim of this study was to determine if serum calprotectin can be a reliable biomarker in dogs with gastrointestinal symptoms (GS).

The dogs enrolled in this study were patients admitted at the Veterinary Hospital. Inclusion criteria for healthy dogs (control group, n = 5) were: absence of signs of inflammation or infection seen in clinical examination, complete blood count and biochemistry. Animals assigned to the gastrointestinal group (GI group, n = 8) had GS (vomiting and diarrhoea). Serum samples were taken from all the dogs. Serum concentrations of canine calprotectin (cCP) were determined with sandwich enzyme immunoassay for in vitro quantitative measurement of canine calprotectin concentration (AbClonal technology, Woburn, Massachusetts, United States).

All samples were measured in duplicates and their mean value was taken for statistical analysis. The highest cCP concentration was 328.2 µg/L, and the lowest 18.1 µg/L. According to study done by Heilmann (2012) the reference range of calprotectin in serum is 76.4 to 563.6 µg/L. The median of serum cCP in the control group was 105.4 µg/L and in GI group 115.7 µg/L, both values were within reference range.

There were no differences in calprotectin concentrations between the control and GI groups. Based on this preliminary study it can be concluded that serum calprotectin can't be used as a reliable biomarker in dogs with gastrointestinal symptoms.

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AETIOLOGY OF VIRAL GASTROENTERITIS IN DOGS – PREVALENCE AND RISK FACTORS

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Viral gastroenteritis is a common disease in dogs. Causative agents of canine viral gastroenteritis include canine parvovirus type 2 (CPV-2), canine distemper virus (CDV), canine coronavirus (CCoV), canine adenovirus (CAV) and canine bocavirus (CBoV).

A total of 97 dog rectal swabs, admitted for molecular diagnostics at the Virology Unit of the Faculty of Veterinary Medicine, the University of Zagreb, from January 2017 to August 2021. These samples were tested for the presence of five viral pathogens (CPV-2, CDV, CCoV, CAV and CBoV) by conventional polymerase chain reaction (PCR) and reverse transcription-polymerase chain reaction (RT-PCR). For statistical analysis, available signalment and medical history data were used. Data were analysed using the two-tailed Chi-square test or Fishers' exact test, and $p < 0.05$ were considered statistically significant.

Out of 97 samples screened, 48 samples were found positive for at least one of the viral pathogens. Prevalence of CPV-2, CCoV, CBoV and CDV were 26.80%, 17.53%, 15.12% and 3.09%, respectively. None of the submitted samples was positive for CAV. Co-infections were found in 13 samples. Five rectal swabs were positive for CPV-2 and CCoV, five for CPV-2 and CBoV, two for CPV-2 and CDV and one for CCoV and CBoV. There were no specific clinical signs that could be used to distinguish between causative viral pathogens. Dogs younger than two years were more likely to be positive to CPV-2 ($p = 0.027$), as well as dogs younger than one year to CCoV ($p = 0.007$).

In this study, the presence of four viral pathogens that cause canine gastroenteritis was confirmed. Due to nonspecific clinical signs caused by these viruses, objective diagnosis is needed. High exposure to the virus and developed immunity seems to be the most probable explanation for a sharp decrease of CCoV prevalence in dogs over one year. A higher incidence of CPV-2 in dogs younger than two years is probably a consequence of inadequate initial puppy vaccination and the absence of booster dose.

RARE CASE OF PERIOCULAR SWELLING IN A DOG

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Periorbital fat protrusion is a rare benign condition that is caused by degenerative changes or trauma of the periorbital septum, muscular fascia, or Tenon's capsule. The described condition can appear in several species and in some cases, surgical treatment is required.

A 13-year-old male, mixed-breed dog, was presented with a history of periocular swelling that appeared 18 months ago and showed signs of progression over the last 6 months. Ophthalmic examination showed facial asymmetry caused by the mass on the lateral canthus of the left eye leading to medial strabismus. The mass was the size of a small walnut, brown pigmented and with an irregular surface. STT1 on the right eye was 15 mm/min while on the left eye it was 25 mm/min with visible epiphora. Other ophthalmological findings were within normal limits. Furthermore, eye ultrasound, radiology and cytology were used and were unremarkable so an excisional biopsy was recommended. Mass resection was performed under general anaesthesia through the dorsolateral conjunctival sac. Access to the left lateral orbit was achieved through a combination of blunt dissection and undermining of connective tissue. Reconstruction was done in two layers with resorptive sutures Maxon 5-0 and Polysorb 6-0. Postoperative check-up showed that the sutures were intact and the eye in position with mild hyperaemia of the mucosa. Histopathological findings revealed that the mass was fibroadyposal tissue consisting mainly of fat cells and less connective tissue with normal morphology.

The most common causes of retrobulbar formations include neoplasia, inflammatory and vascular changes such as trauma-induced hematomas, orbital cysts such as sialocele, immunologically mediated conditions, and emphysema. In our case, it was suspected that the finding was a protrusion of the retroorbital fat pad caused by an old trauma that could have led to the rupture of the periocular diaphragm.

LOCKDOWN CLEAR-OUT? – INCIDENCE OF EUTHANASIA AT CLINIC FOR INTERNAL DISEASES AT FACULTY OF VETERINARY MEDICINE, UNIVERSITY OF ZAGREB, CROATIA DURING THE LOCKDOWN IN 2020

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The "Christmas clear-out" is well known term in veterinary medicine which describes an increased number of cases of euthanasia during the Christmas period. The similar situation may have happened during the lockdown due to pandemic of coronavirus when there was significant decline in majority of veterinary procedures.

In order to investigate incidence of euthanasia during the lockdown, first we have compared data on all dogs treated at Clinic for Internal Diseases, Faculty of Veterinary Medicine during 2020 and 2019 (considering it as any other "normal" year) analysing number of admitted dogs, number and percentage of euthanasia in relation to admissions. Furthermore, in order to minimise errors, we have compared month by month separately between two years. Finally, we have extracted data from first, most strict lockdown (between 21st March and 15th May 2020) and compared them with data from the same period in 2019. We have analysed all the data using chi-squared test with $p < 0.01$ considered as statistically significant.

There was no significant difference in the number of admissions as well as in the number of cases of euthanasia in 2020 (2584; 168) compared to 2019 (2930; 236), but the percentage of euthanasia was significantly lower in 2020 (6.5%) compared to 2019 (8.1%). Monthly analysis showed the significantly higher percentage of euthanasia in April (11.4:5.4) and significantly lower percentage in March (6.2:10.0), May (4.0:10.9), June (4.9:10.5) and October (3.3:7.5). During the lockdown there was significantly lower number of admissions (206:436) and sadly, our hypothesis that there might be a higher number of cases of euthanasia during lockdown was confirmed regarding the number and percentage of cases of euthanasia (21; 10.2%) compared to same period in 2019 (40; 9.2%).

In conclusion, our results showed that there was a "lockdown clear-out" which could be attributed to COVID-related reasons (stress, anxiety, health, finances, transport, strict clinic admission policy) potentially exacerbated with simultaneous earthquake.

MOLECULAR IDENTIFICATION OF ANIMAL-DERIVED *CANDIDA* SPECIES AND THEIR SUSCEPTIBILITY TO MICONAZOLE DETERMINED BY THE BROTH MICRODILUTION METHOD

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Miconazole (MCZ) is an antifungal drug very often used in topical treatment of local yeast infections in animals. Azole resistance in *Candida* isolates in human medicine is a well-known fact for decades. Although *Candida* infections are not so frequently reported in animals, there is growing evidence of resistance to both *C. albicans* and some non-albicans *Candida* species such as *C. tropicalis*. In this study, we identified thirteen *Candida* species isolated from healthy and diseased animals to the species level and evaluated their susceptibility to miconazole using the broth microdilution method.

Species identification was performed using phenotypic and molecular methods. Polymerase chain reaction was performed with ITS-1 and ITS-4 primers for the amplification of ITS1, ITS2 and 5.8S rDNA region followed with phylogenetic analysis. Susceptibility testing against MCZ was determined according to the European Committee on Antimicrobial Susceptibility Testing guidelines for yeasts. The range of tested MCZ concentrations were from 0.0625 to 32 μg/mL. Testing was performed in triplicate on three separate microplates for each sample.

The most commonly isolated species was *C. albicans* with six isolates, followed with two isolates of *C. tropicalis* and *C. palmioleophila*, and one isolate of *C. krusei*, *C. lusitaniae* and *C. zeylanoides*. Minimum inhibitory concentrations (MIC) values were in the range from < 0.06 to 2 μg/mL. The activity of *C. krusei* was the highest (2 μg/mL) followed with *C. zeylanoides* (1 μg/mL) in comparison with the rest of the isolates. In conclusion, *C. albicans* was dominant species isolated from animal samples. The obtained range of MIC values indicate various susceptibility of tested *Candida* species.

Further investigation of a number of *Candida* isolates from animal species is needed to identify susceptibility patterns of this yeast in veterinary settings.

UNILATERAL SEGMENTAL APLASIA OF THE UTERINE HORN WITH PYOMETRA

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A 15-year-old intact female Pekingese was referred to the Clinic because of vaginal bleeding and polydipsia. The patient had a previous diagnosis of degenerative mitral valve disease ACVIM stage C, and was stable under standard therapy. An enlarged and painful abdomen was observed during examination. Haematology and biochemistry profile showed signs of inflammation, most probably due to infection, and increased BUN and creatinine levels. Abdominal ultrasound showed dilation of the uterus with anechoic area and right uterine horn filled with content.

The patient was premedicated with midazolam (0.25 mg/kg IV) and methadone (0.15 mg/kg IV). General anaesthesia was induced with propofol (3 mg/kg IV), followed by endotracheal intubation. The patient was connected to the anaesthetic machine with a rebreathing circuit and maintenance of the anaesthesia was provided by a combination of oxygen and isoflurane. Pain management was provided by the use of fentanyl boluses (2.5 µg/kg IV). Patient monitoring included capnometry, non-invasive arterial blood pressure measurement, pulse oximetry, and ECG. Intraoperative findings were unilateral segmental aplasia of the left uterine horn and right horn filled with content and uterine body tumour. Ovariohysterectomy was performed with subsequent pathohistology. The pathohistology findings were: squamous metaplasia and cystic degeneration of the endometrial glands, uterine adenomyosis and squamous aplasia of the left uterine horn and left ovary.

Postoperative care included pain management, antibiotic therapy, control of renal parameters and constant monitoring of breathing frequency with attention to the occurrence of oedema due to cardiac problems. The patient was discharged to home care two days after the procedure.

In conclusion, this was a complex case of unilateral uterine horn aplasia, pyometra and uterine tumour complicated with a heart disease that in the end had positive outcome.

THERAPEUTIC POTENTIAL OF FAECAL MICROBIOTA TRANSPLANTATION IN DOGS

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Intestinal dysbiosis is a disruption of microbiota homeostasis caused by an imbalance in the complex ecosystem of intestinal microflora. Restoration of the microbiome has been a promising addition to treatment of various gastrointestinal disorders. Its modulation can be achieved by faecal microbiota transplantation (FMT), from a healthy donor into the gastrointestinal tract of an ill recipient. The aim of this study was to investigate efficacy of FMT on the clinical recovery of puppies with parvovirosis.

A total of 20 puppies (2–5 mo) where distributed into 2 equal groups: first group received a standard protocol treatment for parvoviral infections (STD) and the other group received FMT in addition to the standard therapy (STD+FMT).

Faeces for the FMT procedure were obtained by spontaneous defecation from healthy donors. They had no history of gastrointestinal disorders or administration of antimicrobials in the last year and underwent detailed clinical and laboratory diagnostic procedures. Donor faeces were divided into 10-g aliquots diluted in ringer lactate solution with the addition of glycerol. Transplants were administered into the proximal rectum every other day until improvement of faecal consistency.

In the group that received FMT+STD as a treatment protocol, resolution of diarrhoea was significantly faster ($p < 0.01$) compared to the STD group (median, 3.00; range, 1–7; mean, 3.8; SD, 1.69 days in the FMT group; median, 6.00; range, 5–15; mean, 7.5; SD, 3.1 days in the STD group). The average number of FMT applications needed for the improvement of the faecal consistency was 2.4 (range 1–4). The FMT procedure of the puppies in the STD+FMT group was not linked to any clinical abnormalities and therefore is considered safe.

In conclusion, faecal microbiota transplantation contributed to faster resolution of diarrhoea in puppies with parvoviral infection. Further large-scale studies of FMT therapeutical potential in treatment of various gastrointestinal disorders are needed.

RETROSPECTIVE STUDY OF LOCOMOTOR SYSTEM PATHOLOGY IN CROATIAN SHEPHERD DOGS – PRELIMINARY RESULTS

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The Croatian Shepherd Dog (CSD) is one of the seven national breeds of Croatia. Historically CSD was used as the herding dog and guard of property. In the last decades, due to its performance, CSD has proven to be very successful in sports such as agility and flyball. The aim of the study was to find the incidence and distribution of locomotor system pathologies that can affect the work and sports abilities of CSD.

This preliminary study included 60 dogs, who were admitted to the Veterinary Teaching Hospital University of Zagreb in the period 2017–2020. Data from the archives of the Radiology Department were used, inclusion criteria were referral for radiography of the vertebral column and/or appendicular skeleton. There were 45 males (75%) and 15 females (25%), mean age 7.1 (0.5–15) years. The aetiological classification of diagnoses included traumatic, degenerative, and neoplastic groups as causes of changes in the locomotor system.

The results show that traumatic (49.2%) and degenerative (41.3%) aetiology were the most described pathology of the locomotor system, with lower incidence of neoplastic (4.8%) respectively. The traumatic aetiology included mostly long bone fractures (64.5%), followed by intervertebral disc protrusions (12.9%), luxations (9.6%), and soft tissue contusions (12.9%). Degenerative changes found were mostly spondylosis of different degrees (40%), sacralization (33%), and degenerative joint disease (26.6%). Hip dysplasia was noted in 2 dogs and one dog had elbow dysplasia. The neoplastic aetiology group consisted of three dogs diagnosed with long-bone osteosarcoma.

This preliminary study indicates that CSD is not predisposed to common orthopaedic genetic disease, confirming their good potential as sport competition dogs.

INCIDENCE OF TESTICULAR TUMOURS IN DOGS UNDERGOING ELECTIVE ORCHIDECTOMY

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Most of the testicular neoplasia (TN) in dogs have benign biological behaviour but some can lead to endocrinological disbalance. On the other hand, if there is a suspicion for TN, there is a strong indication for orchidectomy. Orchidectomy (ORCH) is also treatment of choice for prostatic hypertrophy (PH), cryptorchidism (UDT) and it is the best prevention for unwanted and uncontrolled mating. There is a strong connection between the mid-aged male dogs (6 y+) which underwent ORCH and histopathological changes of the testicular tissue. Dogs older than 6 years are more prone to develop Leydig cell tumour (LCT), Sertoli cell tumour (SCT), Seminoma (ST) or a combination of these tumours. The aim of this study is to analyse histopathological findings of the testicular tissue after elective ORCH. Fifty healthy dogs (age 1–17 y, M = 8.3 y) were included in our research which underwent ORCH due to PH, UDT or owners wanted the ORCH. None of the dogs included in this study were suspected of TN since they were healthy on preoperative examination (clinical examination, thoracic and abdominal radiography, blood results, ECG).

Dogs were divided into groups by age (1–5 y = 15 dogs; 6–10 y = 17 dogs; 11–15 y = 15 dogs and > 15 y = 3 dogs). If the dog had only one altered testicle upon histopathology result, it was classified into a group depending on the dominant histopathological change.

Normal testicular tissue was noted in 13 dogs, atrophy with or without tissue degeneration in 15 dogs, ST in 10 dogs, LCT in 6 dogs, melanoma in 1 dog, "Mix 1" group (ST + LCT) in 2 dogs, "Mix 2" group (ST + SCT) in 2 dogs and "Mix 3" group (SCT + LCT) in 1 dog.

These results showed that mid- to old-age dogs have a high possibility to have degeneration of testicular tissue and even some type of a TN. Based on these results, we can conclude that for middle aged dogs (6 y+) there is a medical indication for ORCH as a good prevention of further testicular disorders.

ENTERAL NUTRITIONAL SUPPORT USING GASTROSTOMY AND OESOPHAGOSTOMY TUBES IN DOGS AND CATS

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Enteral nutrition is a therapeutic procedure that prevents further development of malnutrition and enables faster recovery. Enteral feeding using gastrostomy (G) and oesophagostomy (E) tubes is indicated in anorectic patients in need of prolonged nutritional support with simultaneous contraindication for naso-oesophageal tube placement. The aim of the conducted study was to determine the frequency and indications for placement of E and G tubes in dogs and cats. Additionally, to determine the duration of enteral nutrition, enteral formulations used for feeding, and complications of enteral feeding.

The research has been conducted by retrospective data collection from a University clinic, Faculty of Veterinary Medicine, University of Zagreb, between January 2015 and January 2020, and included 36 dogs and cats.

Tubes were placed more often in dogs (75%) than in cats (25%). In dogs, E-tubes were placed more frequently (66.67%) than G-tubes (33.33%), while in cats only E-tubes were used. The most common indications for E-tube placement were jaw trauma (mandibular fracture and cleft palate, neoplasms of the oral cavity and pharynx), while for G-tube the main indication was oesophageal lesion due to foreign body. The nutritional protocol and duration of feeding were determined on an individual patient basis, using polymeric liquid diets and diluted canned diets. The duration of enteral nutrition was on average 6.18 days (E-tubes) and 4 days (G-tubes). Mild to moderate complications occurred in 29.63% with E-tubes and 44.44% with G tubes and included tube and bandage displacement, stoma obstruction, vomiting and regurgitation. Severe complications (3.7%) included asphyxiation and pneumothorax.

There is the predominant use of E-tubes in cats due to the high rate of surgical indications such as jaw trauma. Enteral nutritional support using E and G-tubes provides a safe nutrition route when indicated with relatively rare severe complications.

COMPARISON OF LEFT ATRIAL VOLUME IN WORKING AND NON-WORKING BELGIAN MALINOIS DOGS

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Left atrium (LA) is an important and valuable marker of cardiovascular function. Athlete's heart is a well-known phenomenon, and it describes structural and functional changes of the heart prompted by intense training to accommodate a state of enhanced cardiovascular performance. It is associated with chamber enlargement and increased left ventricular mass and similar findings were described in studies in dogs. Since LA is a three-dimensional structure, studies have shown that volume is more accurate than linear measurements in estimation of LA size. Studies in human medicine showed that indexed LA volumes in trained athletes are higher, but no study evaluated the effect of intense physical activity on atrial volumes in dogs. The aim of this study was to determine if there is a difference in LA volumes between working and non-working Belgian Malinois dogs.

Sixty-five healthy dogs (37 female and 28 male dogs) aged 1 to 10 years with the mean weight of 27.9 kg were included in our study. Dogs were divided into two groups: 34 dogs that had more than three hours of intense physical activity/day were classified as working dogs, and 31 dogs with less than three hours were classified as non-working. In all dogs a complete echocardiographic exam was performed and volume of LA in 2- and 4-chamber views was calculated using Simpson's method.

Atrial volumes in working dogs were significantly higher than in non-working dogs, both in 2-chamber (18.8 ml [12.5–30.6] vs. 16.3 ml [12.1–29.8], $p = 0.006$) and 4-chamber (18.95 ml [12.7–31.2] vs. 16.3 ml [12.3–31.2], $p = 0.003$) views. It is known that under changed physiologic states during intense physical activity marked remodelling of the heart occurs. Similar changes in terms of LA remodelling have also been observed in this study.

In conclusion, LA remodelling and increase in volume is a physiologic response to intense physical activity in dogs.

RELIABILITY OF ANALYSIS OF CANINE URINE PRESERVED WITH BORIC ACID

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One of the few preanalytical problems related to urinalysis is preservation of urine. The aim of this study was to estimate acceptability of analysing a urine preserved with boric acid after 24 and 48 h, respectively.

The study included random 11 voided fresh dog's urine samples. The main conditions were urine volume of 10 mL and absence of contaminants in urine sediment. Urinalysis included strip test and corrected specific gravity (SG) value. After centrifugation (5 min, 400 g, 4°C), in the urine supernatant, urinary protein and creatinine concentrations were measured. On the 1st day, the urinalysis was made with unpreserved urine. Afterwards, urine samples were drawn into the Sarstedt Urine Monovettes with boric acid (boric acid preparation concentration 15 mg/mL), stored at 4°C and analysed after 24 and 48 h. Measuring of leukocytes, nitrite, urobilinogen, protein, pH, haemoglobin, ketone, bilirubin and glucose was made semiquantitatively on Siemens Clinitek Status+ analyser with Multistix 10SG strips. SG was measured quantitatively with a Reichert hand-held refractometer. Urinary protein and creatinine concentrations were measured quantitatively with Abbott Architect plus c4000 analyser with Abbott reagents.

After 24 and 48 h, bilirubin, ketone, urobilinogen, and nitrite values were unchanged. SG, urinary protein and creatinine concentrations were slightly unstable (0.2–3%; $p > 0.05$). Haemoglobin values were unstable (22%; $p > 0.05$) after 24 and 48 h. Leukocyte and pH values were lower after 24 h and glucose values after 48 h, compared to the initial analysis. The test strip protein values were lower after 24 and 48 h (−111%; $p > 0.05$). The change in pH values was statistically significant ($p < 0.05$).

In conclusion, strip test values are reliable during 48 h for bilirubin, ketone, urobilinogen and nitrite, and 24 h for glucose. SG, urinary protein and creatinine concentrations are reliable for 48 h. Haemoglobin, leukocyte, pH and protein values are reliable only in a fresh urine.

/ / / / / FARM ANIMALS

HEPATIC FATTY ACID PROFILE OF BROILER CHICKEN SUPPLEMENTED WITH PHENOLIC COMPOUNDS AND LINSEED OIL

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Nowadays, the implementation of phenolic compounds in animal feed is gaining significant interest. Linseed oil is used as animal feed additive to increase the content of beneficial n3 fatty acids in edible tissues. Nevertheless, n3 fatty acids are prone to oxidation, therefore possible dietary strategies do decrease the oxidation of fatty acids are important in increasing edible meet stability. The aim of our study was to investigate the possible effects of polyphenolic compounds mix on the liver fatty acid profile.

Eighty male broilers (Ross 308 strain) were allocated to four dietary treatments: control, linseed (2% linseed in diet), polyphenol (0.5% of gallic acid, tannic acid and thymol in 25:25:1 ratio) and polyphenol + linseed (0.5% of gallic acid, tannic acid and thymol in 25:25:1 ratio and 2% linseed in diet). The fatty acids were analysed using gas chromatography. Results were analysed using the GraphPad Prism 8 program. ANOVA and the post-hoc Tukey test were applied to determine statistical differences between the group. Significant differences were considered at $p < 0.05$.

As expected, linseed oil supplementation significantly increased the content of linolenic (ALA), eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids in the liver tissue. Polyphenol group had increased content of n3 fatty acids as well as EPA and DHA compared to the control group. The polyphenol + linseed group had higher content of ALA compared to the linseed group. Polyphenols could increase the content of beneficial EPA and DHA when linseed oil is not supplemented. But, when linseed oil is added, polyphenols increase the content of ALA via their antioxidative properties, but due to the limited conversion of ALA into higher n3 fatty acids, the content of EPA and DHA remains unchanged.

The inclusion of phenolic compounds had significant influence on the content of n3 fatty acids in the chicken liver. Therefore, we assume that polyphenolic compound could influence meat oxidative stability in the broiler chicken fed linseed oil.

HEALTH ASSESSMENT OF NORTHERN PIKE (*ESOX LUCIUS*) FROM THE MREŽNICA RIVER, CROATIA

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Northern pike (Esocidae family) is an important fish for aquaculture and the fishing industry in Croatia. Despite its commercial significance, there is only limited information on the health status of this species in natural waters.

Here we report the results of an initial evaluation of the health status of wild northern pike collected from 3 locations along the Mrežnica River. In the spring of 2020, a total of 9 specimens (30–67 cm in length, an average body weight of 925 g) were captured by electrofishing and examined as part of a fish health and ecotoxicological study, conducted under the Croatian Science Foundation project, "Metal-binding biomolecules and health disturbances of freshwater organisms exposed to industrial wastes" (IP-2019-04-2636). For each pike, the presence of gross external and internal lesions was documented, and samples were processed for virological, bacteriological and parasitological examination. Additionally, liver and kidney samples were fixed in 10% neutral-buffered formalin and processed for histology. The 5-µm thick sections were stained with haematoxylin and eosin (H&E), periodic acid-Schiff (PAS) and Mallory's trichrome.

No viruses or pathogenic bacteria were detected. However, a number of protozoan and metazoan parasites were observed, including ciliates (*Trichodina* sp.), myxosporeans (*Myxidium lieberkuehni*), monogeneans (*Dactylogyrus* sp. and Gyrodactylus sp.), intestinal nematodes, leeches (*Hemiclepsis marginata*) and crustaceans (*Argulus foliaceus*). Histologically, different changes were evident in the liver and kidney of several fish. Histopathological changes in the kidney were associated with *Myxidium* infection. In the liver, changes of unknown aetiology were mostly characterised by hydropic degeneration, vascular congestion, granuloma formation, lytic necrosis and necrosis of single hepatocytes.

These results suggest that special attention should be given to the health status of wild northern pike.

ANTIMICROBIAL SUSCEPTIBILITY AND BIOFILM FORMATION ABILITY OF *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED FROM TIBIOTARSAL JOINTS OF BROILER BREEDER PULLETS

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Staphylococcus aureus is a common causative agent of arthritis and synovitis in poultry. The birds with localised infection in the tibiotarsal joints and proximal flexor tendons show signs of lameness, which excludes them from reproduction and can lead to death from starvation and dehydration due to inability to walk.

In this study, we investigated antimicrobial susceptibility and biofilm-forming ability of *S. aureus* strains isolated from Ross 308 broiler breeder pullets from two flocks. The daily mortalities were submitted to necropsy, sampled, and the swabs were bacteriologically examined. After the overnight aerobic incubation at 37°C, the bacterial cultures were identified based on the morphology and biochemical characteristics. In total, eleven strains of *S. aureus* from different birds with swollen tibiotarsal joints and lesions in the gastrocnemius tendons were detected and further analysed. Antimicrobial susceptibility was determined by disk diffusion assay using amoxicillin, cephalexin, doxycycline, enrofloxacin, florfenicol, flumequine, lincomycin-spectinomycin and sulphonamides. The inhibition diameters were interpreted according to the EUCAST standards. Biofilm-forming ability was examined using the adherence quantitative assay on microtiter plates. Each strain was tested in ten replicates.

The results showed all strains were resistant to sulphonamides, while seven strains (63.64%) were resistant to cephalexin. The biofilm quantification assay showed ten strains (90.91%) were moderate biofilm producers, while one strain (9.1%) was a weak biofilm producer. Due to previous problems with reovirus infection, which is also clinically manifested with arthritis, tenosynovitis and lameness, staphylococcosis was considered a secondary infection.

Although the results showed low antimicrobial resistance and predominantly moderate biofilm-forming ability of the investigated strains, continuous surveillance is recommended due to high zoonotic potential of the species.

MICROMYCETES ON THE HEALTHY CALVES SKIN

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In animals, more than 300 species of pathogenic microscopic fungi have been known. They comprise three types of organisms: dermatophytes, non-dermatophytes and yeasts. In cattle, the most common superficial mycoses are dermatophytosis and dermatomycosis that arise when the epithelial barriers and defences of the skin are weakened. The yeast cutaneous infections are caused by *Malassezia*, *Candida* and *Trichosporon* species. The main purpose of this study was to identify fungal microflora of the healthy bovine skin and thus determine the representation of individual micromycetes.

The survey was carried out on 50 calves with clinically healthy skin image. The samples were collected from external ear canal, from the skin and hair of neck. Samples were inoculated on Sabouraud's dextrose agar with chloramphenicol (SCH), Modified Leeming-Notman agar medium (MLNA), Modified Candida-Chrom agar with Tween 40 (HIT) and Dermatophyte test medium (DTM). The plates were incubated at 25ºC and 32ºC for 1–4 weeks and examined at 2–3 day intervals for fungal growth. Identification was based on macroscopic, microscopic, biochemical and physiological characteristics.

Dermatophytes in 50 examined calves were represented by *Trichophyton* (6%) and *Microsporum* (4%). From the yeasts only *Candida* spp. was identified in 10 calves (20%). Non-dermatophyte fungi composition was as follows: 72% animals were positive for *Mucor*, 66% for *Penicillium*, 50% for *Aspergillus*, 40% for *Rhizopus*, 38% for *Alternaria*, 38% for *Absidia* and 10% for *Cladosporium*.

Our results show that micromycetes were present on healthy bovine skin. Animals diagnosed with pathogenic micromycetes did not show any clinical signs of skin disease. In some cases, pathogenic micromycetes may be the part of skin microflora and only after the skin immunity is weakened, does they multiply with subsequent emergence of dermatophytosis or dermatomycosis.

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MILK PROTEOMICS IN SIMMENTAL COWS WITH SUBCLINICAL AND CLINICAL MASTITIS

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Bovine mastitis is a serious disease with great impact to dairy industry and animal welfare. This study was aimed to characterise milk proteome in naturally occurring subclinical and clinical mastitis.

Milk samples from healthy Simmental cows (H, n = 10) were compared to those with subclinical (SC, n = 12) and clinical mastitis (CL, n = 10) using tandem mass tag (TMT) proteomics. Data were analysed using Proteome Discoverer software (version 2.3., Thermo Fisher Scientific).

237 proteins were changed in abundance in both SC and CL showing different change patterns.

The greatest fold changes were found in haptoglobin, serum amyloid A, beta-defensin, and serpins B3 and B1 with significant increase in both SC and CL compared to H although without significant difference between SC and CL. Proteins showed a significant increase in SC compared to H and a further significant increase in CL included alpha$_2$-macroglobulin, immunoglobulin heavy chain, pregnancy zone protein and cathelicidin-3. There were also proteins that did not increase in SC but increased in CL including a different variant of alpha$_2$-macroglobulin, calponin cathelicidin-1 and apolipoproteins A1 and A2. Some milk proteins decreased in response to mastitis, i.e. proteins that showed no change in SC but demonstrated a significant decrease in abundance in CL compared to healthy milk with the greatest reduction being shown by thrombospondin along with alpha$_{S2}$-casein, beta-lactoglobulin and beta-casein among others. Proteins showed significant decreases in SL and further decreases in CL included an ATP-binding transporter, mucin-1 and butyrophilin.

Alteration of abundance patterns of milk proteins indicate multiple protein interaction involved in complex reactions of host immune defence, acute phase response, coagulation cascade and amino acid metabolism. Identification of milk proteome is a valuable strategy for revealing potential biomarkers to be used in mastitis diagnostics, disease monitoring and therapeutic possibilities.

THE EFFECT OF 2500 MHz MICROWAVE RADIATION ON THE SPERM MOTILITY AND VELOCITY PARAMETERS OF *IN VITRO* IRRADIATED SEMEN OF BREEDING BOARS

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With the development of technology, there is an increase in public concern about the negative effects of radiofrequency electromagnetic radiation (RF-EMR), especially of device that operates at the frequency of 2500 MHz. It is presumed that the male reproductive system could be one of the most affected by negative effects. The data on the effect of RF-EMZ on the reproductive system of male domestic animals are scarce. The aim of this study was to investigate the effect of electromagnetic radiation with the frequency of 2500 MHz on sperm motility and velocity parameters of *in vitro* irradiated semen of breeding boars.

The ejaculates of 12 breeding boars, Landrace and Pietrain breed, aged 1.5 to 3.5 years were sampled by manual fixation of the penis. Freshly diluted semen of each boar was divided into a control (n = 12) and an experimental sample (n = 12) that was irradiated. The experimental samples were exposed to continuous non-ionizing electromagnetic radiation of 2500 MHz in gigahertz transverse electromagnetic chamber for 2 hours at an electromagnetic field strength of 10 V/m. In the control and experimental groups, the motility and sperm velocity parameters were determined using computer assisted sperm analyser. Following sperm velocity parameters were analysed: curvilinear velocity, straight-line velocity, average path velocity, amplitude of lateral head displacement, linearity, wobble, straightness and beat cross frequency.

Significant decrease ($p < 0.001$) in the proportion of progressive sperm motility was recorded in the experimental group. Also, in the experimental group a significant decrease in the share of progressive motile sperm, in the share of the straightness, in the share of the linearity index, in the values of the straight-line velocity and the beat cross frequency was recorded.

According to the results of this study, it can be concluded that EMR frequency of 2500 MHz has a negative effect on sperm motility and velocity parameters in irradiated semen of breeding boars.

INFLUENCE OF SEASON OF BIRTH ON THE AGE AND LITTER SIZE AT FIRST LAMBING IN ROMANOV EWES

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Seasonal pattern of reproduction in sheep is one of the main obstacles to achieving sound reproductive management, especially in ewe lambs at the beginning of their breeding activity. Romanov ewes are known for their prolificacy, partial aseasonality and early maturity. In this context, the information whether the season of ewe's birth has influence on the indicators of early reproductive efficiency seems particularly important.

A total number of 249 ewes of Romanov breed from one farm in Croatia were analysed according to their birth date, age at first lambing (AFL) and litter size at first lambing (LS). Ewe lambs aged 5 to 7 months were joined with rams regardless of the season. Once joined, rams were kept with ewe lambs permanently. For this analysis, animals were divided according to their birth date: spring-born (87), autumn-born (42), and winter-born (120). Statistical analysis was performed using Kruskal–Wallis test.

The overall mean of AFL was 380.6 days. Ewes born in spring were the youngest at first lambing (330.9) which was significant ($p < 0.05$) compared to those born in autumn (460.4) and in winter (388.7). Significant differences ($p < 0.05$) were also observed between AFL of autumn-born and winter-born ewes. The average LS was 1.89. Sheep born in spring, autumn and winter lambed on average 1.71, 1.93 and 2.00 lambs, respectively. Differences in LS between spring and winter-born ewes were significant ($p < 0.05$).

The results suggest that the season of birth significantly influence AFL and LS. Ewe lambs began their breeding activity during the natural breeding season typical for this latitude and climate, regardless of their age and time of joining with rams. This indicates a pronounced seasonality of reproductive activity, despite the breed being considered aseasonal. The explanation for this might be that ewe lamb can reach puberty only after experiencing alteration from long to short days. The season of birth might be a possible predictor of ewe's early maturity and production.

BLOOD SERUM OXIDATIVE STATUS OF LIKA PRAMENKA LAMBS FED WITH THE BUTTON MUSHROOM (*AGARICUS BISPORUS*) DIETARY SUPPLEMENT

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Mushrooms contain a variety of active substances and, among others, could provide functional organic selenium (Se). Se is an incorporated element into selenoprotein glutathione peroxidase (GSH-Px), a significant factor in protecting cells from oxidative stress. GSH-Px measured in blood adequately reflect their level in other tissues and systemic oxidative status. The aim of this study was to determine whether intake of the dry and raw fruiting bodies of white button mushrooms influences blood serum oxidative status in Lika pramenka lambs.

The study was conducted on 45 lambs of the Lika pramenka sheep breed at the age of three months in the Crkvina, Karlovac County, Croatia. The lambs were randomly divided into three groups, with 15 animals in each group (8 females and 7 males). Each group was fed for 6 weeks: control (basal diet), experimental I. (basal diet with 15% of raw *Agaricus bisporus*) and experimental II. (basal diet with 1.5% dried *Agaricus bisporus*). Blood was collected from the jugular vein using vacuum tubes without anticoagulants. The activities of GSH-Px, superoxide dismutase (SOD), and the concentration of malondialdehyde (MDA) were determined in the obtained serum.

The differences in oxidative status between groups were not statistically significant, although there were slightly higher serum GSH-Px and MDA values found in lambs fed with 15% of raw white button mushroom supplements.

It can be concluded that white button mushroom supplementation does not affect the lamb's serum oxidative status.

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TRANSFER LEARNING: KEY ENABLER FOR RAPID DEVELOPMENT OF AI APPLICATIONS IN PATHOLOGY

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Deep learning (DL) has great potential for automating routine tasks and quantifying diagnostic parameters in veterinary pathology. However, some obstacles remain. To train deep neural networks, a large amount of labelled data is needed. Labelling is not only a time-consuming task, but it also requires domain expertise, making the whole process much more expensive. In addition, DL models often suffer from low generalization and, for example, are unable to keep the same level of performance across slides digitised with scanners from different manufacturers. By using a method called "transfer learning" these obstacles can be bypassed. Instead of starting from scratch, transfer learning is using models that already have certain "knowledge" for solving related problems.

To create a DL model that will be able to label canine mastocytoma's mitotic figures digitised on our "in-house" developed microscope slide scanner, we used an open-source dataset of canine cutaneous mast cell tumour containing 44,880 annotations of mitotic figures. This dataset was acquired with a different scanner (ScanScope CS2, Leica) and, in order to make the two datasets as similar as possible, we removed two colour channels from the images, keeping only the green colour channel, which had the most similarity across both datasets. Also, as images acquired with our scanner were in significantly higher resolution, we were forced to blur our images with a gaussian filter.

After training a ResNet50 neural network, the DL model had high sensitivity and low specificity. On our slide sized 9753 megapixels (30 GB uncompressed file size), the model detected 2671 mitotic figures of which 30 were true positive (this was proven by visual confirmation by a veterinary pathologist). We were not able to detect any false negatives, thus alleviating the need to manually search and label the whole slide.

Transfer learning has proven to be a successful method of automating the process of creating deep learning models.

INFLUENCE OF DIFFERENT RODENT MODELS OF NON-ALCOHOLIC FATTY LIVER DISEASE ON METABOLISM OF IMPORTANT POLYUNSATURATED FATTY ACIDS AND INFLAMMATION

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Metabolic disease, such as non-alcoholic fatty liver disease (NAFLD), is characterised by simple to severe steatosis, and consequent hepatitis, fibrosis, and cirrhosis. Furthermore, it is related to an increase of the inflammatory markers, as well as changes in the content of tissue fatty acids, and their corresponding ratios. High-fructose diet and cafeteria diet are widely used dietary rodent models of NAFLD which involve obesity, glucose intolerance, and inflammation. The aim of our investigation was to determine differences between these two models in the metabolism of important fatty acids and inflammatory markers in the liver and white adipose tissue (WAT).

Thirty Wistar rats (15 male and 15 female) were divided into the control group (CON), the high fructose group (HF, 15% of fructose in drinking water) and the cafeteria dietary group (CAF, 50% basal diet and 50% cafeteria diet). The rats were sacrificed after 20 weeks of dietary treatment, and hepatic and WAT samples were obtained. The expressions of inflammation gene markers were quantified by RT-qPCR. The analysis of hepatic and white adipose tissue fatty acid composition was performed by gas chromatography. For statistical data analysis, GraphPad 8 was used.

The hepatic and WAT fatty acid profile showed significant variations depending on the diet and sex. Generally, in the treated rats significant increase in monounsaturated fatty acids and decrease in n3 and n6 polyunsaturated fatty acids (PUFAs) was observed. The CAF diet induced more pronounced decrease in both n3 and n6 PUFAs. In both diets, the concentration of n6 docosapentaenoic (n6 DPA) acid was increased. The expression of the inflammation gene markers (TGFβ, TNFα and IL6) was significantly increased in both diets and sexes.

Two dietary models of NAFLD led to characteristic changes in the hepatic and white adipose tissue fatty acid profiles, in comparison to the control group and to each other. These findings could play an important role in the interpretation of the experimental results.

HISTOSTITCH: IMAGE STITCHING ALGORITHM FOR BRIGHTFIELD MICROSCOPY

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In pathology, digitalization of whole slides at high numerical apertures is based on the acquisition of overlapping tiles, usually in a matrix layout. Using feature-based or Fourier-based algorithms, translations between neighbouring images can then be computed, enabling us to accurately "stitch" tiles and form a digitised slide. Feature-based algorithms are regarded as "gold standards" for image stitching tasks, but they are prone to errors in feature-poor images (e.g. fatty tissue). In commercial solutions, this problem is minimised with high-precision mechanical stages and by constraining feature search area, but this leads to higher hardware costs, especially in multi-slide systems.

In this paper we present HistoStitch, an algorithm that can model stage mechanics and minimise stitching errors by predicting possible tile positions, therefore constraining final global positions inside a range of possible values. This algorithm was successfully used with our "in-house" developed microscope slide scanner, Marvin, with CoreXY mechanical stage that can produce up to 200 pixels (30 µm) error in positioning. To quantify and compare our solution with other image stitching algorithms, we manually stitched a sample of 100 images. With a known baseline, we then stitched the same dataset with our HistoStitch and the MIST algorithm developed by the National Institute of Standards and Technology (NIST).

Both algorithms had no problem finding correct translations on the x-axis, with an average positioning error of < 1 pixel and no visible overlaps between stitched images. On the y-axis, however, due to much higher stage positioning variability, the MIST algorithm failed to find correct translations, with an average positioning error of 147 pixels per image.

This paper shows that using smarter stitching algorithms, we can successfully compensate for high positioning errors and reduce the costs of microscope slide scanners, making digital pathology more widely accessible.

HYPOTHYROIDISM IN PREGNANT RATS AFFECTS PROLIFERATION AND CELL DEATH OF GROWTH PLATE CHONDROCYTES IN PUPS

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Overt and subclinical maternal hypothyroidism affects cartilage and bone extracellular matrix formation during foetal endochondral bone development. Less is known about the effect maternal hypothyroidism has on cell proliferation and death of growth plate chondrocytes.

This study aimed to evaluate the expression of proliferation (Ki67), autophagy (LC3) and apoptosis (caspase 3) markers in the epiphyseal cartilage of the tibia in pups from subclinical and overt hypothyroid does, in their early postnatal development.

Hypothyroidism was induced with propylthiouracil using low (1.5 mg/L) for subclinical (H1) and high dose (150 mg/L) for overt (H2) form. Propylthiouracil was administered through drinking water in pregnant Albino Oxford does from the first day of gravidity and during lactation. Control (C) group was not treated. Six 7-day-old male pups from each group were euthanised. Histological examinations were performed on paraffin sections of the proximal tibial growth plate. Immunohistochemistry was used to assess the expression of Ki67, LC3 and caspase 3. The number of Ki67 positive chondrocytes was evaluated in the resting and proliferating zone using Image J. The expression of LC3 was performed by counting the number of positive dots in chondrocytes.

The number of Ki67 positive chondrocytes was higher in both hypothyroid groups, while the number of LC3 positive dots per cell was lower indicating that the chondrocyte cell cycle in hypothyroid animals is faster and that the basal level of autophagy is reduced due to frequent mitosis. Compared to controls, caspase 3 expression in the terminal hypertrophic chondrocytes was reduced in H1 and completely absent in H2 pups which might indicate impaired differentiation. We conclude that both forms of maternal hypothyroidism in rats lead to accelerated proliferation, slowed autophagy and compromised apoptosis of terminal hypertrophic chondrocytes in the early infantile period and a delay in cartilage to bone transition.

INFLUENCE OF DOCOSAHEXAENOIC ACID ON INFLAMMATORY MARKERS IN WHITE ADIPOSE TISSUE OF RATS FED HIGH FRUCTOSE DIET

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The incidence of obesity and its related comorbidities has become an important health problem worldwide. Excessive intake of fructose in diet leads to adipose tissue accumulation and dysfunction, which is largely associated with increased oxidative stress and systemic low-grade inflammation. Therefore, the current study investigated the ability of a diet with a higher amount of docosahexaenoic acid to reverse the metabolic consequences of a high fructose diet, with sex as an additional variable.

Thirty Wistar rats (15 male and 15 female) were divided into a control group (CON), a high fructose group (HF, 15% of fructose in drinking water), and a DHA group (15% of fructose in drinking water and basal diet supplemented with EPA and DHA). All dietary treatments lasted for 20 weeks. The expression of TNFα, TGFβ and NRF2 genes was assessed by RT-qPCR. Adiponectin protein abundance was determined by western blotting. Fatty acid composition was determined by gas chromatography. GraphPad 8 software was used for the statistical analysis.

Dietary treatments induced changes in the fatty acid profile among all treated groups, as well as between the experimental groups. A decrease in the content of DHA and EPA, and decreased EPA/ARA ratio point to susceptibility to low-grade inflammation in fructose treated rats. Moreover, the expression of inflammatory gene markers TGFβ and TNFα was significantly increased in the HF group, as was the expression of antioxidant gene marker NRF2. In addition, adiponectin protein abundance was significantly decreased in rats fed the HF diet. Sex did not significantly influence observed changes. All aforementioned changes were prevented in the group fed the DHA diet.

These findings could have important consequences for other tissues and animal health in general, due to the fact that the adipose tissue is a reservoir of polyunsaturated fatty acids for other tissues.

DIGITAL 3D ANATOMICAL MODEL OF THE CANINE STOMACH

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In the last 5 years, digital 3D anatomical models have been developed and used for teaching and studying at the Faculty of Veterinary Medicine, University of Zagreb (FVMUZ). The main purpose of these models was to establish new tools for the students' self-study. Nowadays, 3D models and anatomical specimens are combined in practicals. Furthermore, models proved to be useful for the study during the COVID-19 pandemic and online teaching. To maintain the high-quality study, we designed a new digital 3D anatomical model – the dog's stomach.

A canine healthy stomach was received from the pathology course in 2021. Stomach was dissected and the inside was washed with tap water. The stomach was preserved with 5% alum and left in it for 6 days. Afterwards, it was air dried in its anatomical position with air pump with the air-pumping tube inserted through the cardia for 4 days. Finally, it was filled with polyurethane foam to preserve the shape. The stomach was scanned with a CT scanner (Siemens SOMATOM go.Now) at the FVMUZ. A digital 3D model of the stomach was created with the software *3D Slicer*. Small errors of the model were fixed with *Blender* software. Additionally, the stomach was photographed from all angles, and photographs were applied to the model in *Blender* software to get the real texture of the stomach.

The model was labelled with 17 anatomical terms according to *Nomina Anatomica Veterinaria* in the *Blender* software. For the correct position of anatomical structures, *Illustrated Veterinary Anatomical Nomenclature* was used. *FinalMesh* software was used for the export of the model in WebGL format suitable for online view. The model is now available for free on the FVMUZ website (http://wwwi.vef.hr/3datlas/ostali_modeli/zeludac/).

Digital 3D anatomical models are new teaching tools in animal anatomy that improve the spatial orientation of students in the animal's body. However, they cannot fully replace the dissection and study on whole carcasses, body parts or organs.

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PROTECTIVE ROLE OF *AGRIMONIA EUPATORIA* L. IN BISPHENOL A INDUCED CYTOTOXICITY

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Agrimony (*Agrimonia eupatoria* L.) is traditionally used in folk medicine for antioxidant, anti-inflammatory, antimutagenic, antiviral and hepatoprotective effects in treatment of gastrointestinal tract infections and also as prevention against undesired effect of drugs or toxicants. Bisphenol A (BPA) is a substance dangerous for human health frequently occurring in environment, entering the organisms in food, air, dust, and water.

The aim of this study is to reveal the potentially protective role of ethanolic extract of agrimony against the cytotoxic effect of BPA *in vitro*, using intestinal porcine epithelial cell line (IPEC-1) as the model organism. Lyophilised ethanolic extract of agrimony at concentration 250 µg/mL was used. BPA was tested at concentrations 12.5, 25, 50, 100, and 200 µg/mL using two *in vitro* assays alone and in combinations. For the monitoring of the cell response after exposition to tested substances, xCELLigence system (Real-Time Cell Analyser (RTCA) was used. Cell changes in adherence and proliferation were expressed as cell index and recorded in curves during the whole time of treatment (48 h). In addition, the colorimetric MTS test was used for the measurement of the metabolic activity of cells.

The protective role of agrimony against cytotoxicity caused by BPA was observed after cell treatment with agrimony in combination with lower concentrations of BPA (12.5, 25 and 50 µg/mL). An improvement in cell adherence was observed in cells treated with these combinations in comparison to cells treated with BPA alone. On the other hand, metabolic activity was slightly improved in cells treated with combination of agrimony and BPA at higher concentrations (50 a 100 µg/mL). The positive effects of agrimony are attributed to a high amount of flavonoids, tannins, aromatic acids, triterpenes, coumarins, terpenoids, glycosides, and vitamin B and K. This supports our assumption that agrimony can protect a model organism against cytotoxicity caused by BPA.

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HISTOLOGICAL EVALUATION OF CHONDRO-DIFFERENTIATED cAD-MSC SPHEROIDS

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Adipose derived mesenchymal stem cells are multipotent cells that in vitro differentiate into adipocytes, chondrocytes and osteoblasts depending on the differentiation media used. We investigated properties of chondro-differentiated canine AD-MSCs (cAD-MSCs) cultured in three dimensional structures, spheroids, using routine haematoxylin-eosin stain (HE) as well as three special stains.

AD-MSCs from four young healthy female donors were incubated for 21 days in chondro-differentiation media at 37°C, with 5% CO_2 and 95% humidity. Formalin-fixed paraffin embedded (FFPE) spheroids were sectioned and stained with HE. After histological evaluation of slides, special stains Alcian Blue 8GX, Masson's trichrome and Von Kossa were performed.

Extracellular matrix (ECM) in spheroids stained positively for proteoglycan aggrecan and collagen fibres with Alcian Blue and Masson's trichrome stain, respectively. Multifocal areas of mineralization were observed in HE stained sections which stained positive with Von Kossa stain.

Positive staining for aggrecan showed successful chondro-differentiation and viability of cAD-MSCs in vitro. Since Masson's trichrome stains mainly collagen type 1, we can presume that the predominant type of collagen in spheroids is type 1 which is the most common ECM protein produced by mesenchymal cells. Further immunohistochemical analysis of collagen types should be carried out. Areas of mineralization indicate ossification of early cartilaginous tissue which could be related to cultivation duration.

Routine and special histological staining of FFPE spheroids enabled a more detailed and morphologically accurate representation of cells and their products inside a 3D cell culture. It is a useful method for evaluating differentiation efficacy and also opens new questions such as types of collagen present which should be further investigated.

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HORMESIS EFFECT IN CHICKEN EMBRYOS AFTER LOW-DOSE IRRADIATION

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Hormesis is a dose-depend-response effect where a low-dose phenomenon stimulates protective mechanisms of a biological system while a high-dose inhibits it. The effects of low-dose radiation are very difficult to observe and highly controversial. On the basis of our previous measurements, we study the hormesis effect of low-doses gamma radiation by analysing the activity of the antioxidant GSH-Px, as a first-line defence in cells, and the concentration of the lipid peroxide (malondialdehyde, MDA), as a measure of the harmfulness of ionizing radiation, in chick embryonic livers.

Fertilised eggs of COBB 500 broiler breed were irradiated with gamma-rays of ^{60}Co source on the 19th day of incubation with the doses of 0.05, 0.15, 0.3, 0.5, and 0.8 Gy and the dose rate of 0.0117 Gy/s. GSH-Px activities and MDA concentrations have been measured at 1, 3, 6, 12, and 24 hours after irradiation.

Activities of GSH-Px were significantly increased for doses of 0.15 and 0.3 Gy, compared with control values. The temporal dynamics analysis revealed a statistically significant almost linear increase of activity of GSH-Px within 24 hours for doses from 0.05 to 0.5 Gy. The analysis of dependence activity on doses at 12 and especially at 24 hours after radiation showed a sharp increase at very low doses, relatively steady activities between 0.05 and 0.3 Gy, and a decrease at the control values at higher doses. A combination of all three analyses showed that there was no recognizable consistent dependence of MDA concentrations on radiation doses.

We could assume that in conditions of our experiment low radiation could have a beneficial effect on chicken embryos because there is an increase of the GSH-Px activity while MDA concentration is steady. Our results were obtained from three different analyses. As we have shown earlier, those results are more reliable.

We found clear and reliable evidence of hormesis effect by low-dose radiation in chick embryos on the 19th day of incubation at 12 and 24 hours after irradiation.