UNIVERSITY of LIFE SCIENCES in Lublin

FACULTY OF VETERINARY MEDICINE

International Scientific Conference

"Human – Animal – Environment – our health, common health"

Book of Abstracts

Lublin, Poland

11-12 October 2024

University of Life Sciences in Lublin

Faculty of Veterinary Medicine

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IN VIVO AND IN VITRO STUDIES ON CANINE PLACENTAL FETO-MATERNAL COMMUNICATION; IMPLICATIONS FOR CANINE PLACENTA AS A TRANSLATIONAL MODEL

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The dog has an endotheliochorial placenta, developmentally placed between the less invasive placentation of other domestic animal species and the more invasive haemochorial placenta of humans and rodents. In invasive placentae the process of decidualization takes place involving morpho-functional differentiation during mesenchymal-epithelial transition of uterine stromal cells, increasing their hormonal activities to support embryo-maternal communication. Failure to decidualize leads to pregnancy loss.

Unlike in humans and rodents, where a decidual layer forms, dogs have decidual cells within the placental labyrinth, located near maternal vessels. These cells, along with maternal endothelial cells, evade trophoblast proteolytic activity and play a key role in the endotheliochorial canine placenta. Thus, decidual cells uniquely express the nuclear P4 receptor (PGR) and rely on luteal P4 for placental function, as the placenta lacks steroidogenic activity. Natural or antigestagen-induced disruption of PGR signalling induces parturition or abortion by activating feto-maternal communication and PGF2α release from the trophoblast.

Our studies combine *in vivo* observations with *in vitro* functional studies to investigate canine decidualization and feto-maternal interaction. We identified decidual cells forming a syndesmo-/deciduo-chorial barrier and 3D cellular network closely surrounding maternal vessels. We also linked decreasing PGR signalling with reduced availability of decidual cells during the progression of pregnancy. Using omics technologies and targeted studies, we established PGR-mediated signalling pathways, both *in vivo* and *in vitro* utilizing canine-specific decidualization model developed in our laboratory. The canine model of shallow trophoblast invasion offers valuable insights into conditions associated with aberrant trophoblast and decidua-trophoblast interaction in more invasive placentae.

Keywords: canine decidualization, feto-maternal interaction, P4 receptor

FEELING GOOD OR FEELING STRESSED? FACTORS AFFECTING THE ASSESSMENT OF STRESS RESPONSES IN ANIMALS

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The effect of an acute stressful stimulus on an animal is usually measured by the magnitude of the response of the hypothalamic-pituitary-adrenocortical axis (glucocorticoids and mineralocorticoids) and the sympathetic-medullary axis (heart rate and its variability, catecholamines), which defines a mild or severe stress response. However, if we analyse the same physiological response when the animal is exposed to rewarding stimuli, we can detect a high amplitude response, similar or even greater than the response to stressful stimuli.

Considering the possible combinations of stimuli and neuroendocrine responses, there may be methodological issues in defining whether a particular stimulus acts as an acute stress for the animal. Given that it may be difficult to assess the effect of variables that influence the stress response (social interactions, environment, individual experience, genetics and health status), it is important to consider the conditions that cause an organism's natural capacity for homeostatic regulation to be exceeded.

A stimulus that induces an acute stressful response should be characterised by the absence of an anticipatory response (unpredictable stimulus) or a delay in the recovery of basal homeostatic conditions (uncontrollable stimulus) of the neuroendocrine response. Therefore, to define a stimulus as stressful, it is neccesary to consider not only the magnitude of the neuroendocrine response, but rather evaluate the time it takes for the response to occur and the time it takes for the organism to return to baseline. These considerations are essential to assess whether the neuroendocrine response is adaptive or maladaptive.

Keywords: stress, neuroendocrine response

EXTRACT OF ONION VS BRAIN: TOWARDS A BETTER UNDERSTANDING OF ONIONS IMPACT ON THE INSULIN RECEPTORS IN THE RAT`S HIPPOCAMPUS

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Introduction: Onion (*Allium cepa* L.) is a source of natural phytochemicals with beneficial effects to health. One is quercetin, which has been found as neuroprotective preventing brain aging by inhibiting biochemical processes affecting neurodegenerative processes. The insulin, through its receptors (IRs), participates in the process in the creation of new synaptic connections and drives the memory processes of the healthy brain.

Aim of the study: The present study aimed to evaluate whether immunoreactivity of IRs in obese rats' hippocampus is changed after feeding with onion-rich diet.

Materials and methods: 8-week-old Wistar rats (n=32) were divided into four groups (n=8). Group K (control) received standard rodent food; Animals of: group K5-received onion extract with a concentration of 5% with feed; group F were with induced obesity, group F5 with induced obesity and fed with 5% onion extract. The diet supplemented with onion extract lasted for 12 weeks. After the euthanasia, formalin-fixed, paraffin-embedded sections of the brain were made. The sections were immunohistochemically stained with PAP method using a primary polyclonal antibody directed against IR/CD220. The obtained results were statistically analyzed with ANOVA.

Results: In the hippocampus of obese rats, the reduction of InRs-immunoreactive (IR) neurons and InRs-nerve fibres was observed. In obese rats fed with onion, an increased number of InRs-IR neurons/nerve fibres in the hippocampus was found. Feeding with onion of non-obese rats was not affected the immunoexpression of InRs either in hippocampal neurons or nerve fibres.

Conclusions: The altered numbers of IRs-IR neurons during feeding with onion suggests that the antioxidants present in the plant may potentially act as neuroprotective factors against obesity-evoked damages.

Keywords: onion, obesity, quercetin, insulin receptors, hippocampus

COMPARATIVE ANALYSIS OF BONE ADAPTATIONS TO LOCOMOTOR AND BEHAVIORAL STRATEGIES IN THREE DUCK SPECIES (ANATIDAE*: ANATINAE*)

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Introduction: Ducks (*Anatinae*) play a crucial role in wetland ecosystems, contributing to seed dispersal and nutrient cycling.

Aim of the study: This study investigates the skeletal adaptations of three duck species: the Mallard (*Anas platyrhynchos*), Tufted Duck (*Aythya fuligula*), and Green-Winged Teal (*Anas crecca*) to understand how these adaptations support their locomotion and habitat preferences.

Materials and methods: Bone samples (n = 6 from each species, both male and female) were cleaned and measured for length and weight. Dual-energy X-ray absorptiometry was used to determine bone mineral density (BMD) and bone mineral content (BMC), and mechanical properties were tested using a 3-point bending test.

Results: The findings demonstrate that each species exhibits unique bone characteristics suited to their specific behaviors and habitats. Mallards, known for their versatility, possess stronger and denser bones, which are advantageous for various environments. They show the highest Seedor index, indicating robust bone structure. Teals, being smaller and capable of rapid flight, have lighter and less dense bones, which are beneficial for quick movements in shallow wetlands but exhibit lower. Tufted Ducks, which are adapted for deep diving, have bones that are particularly strong and stiff, allowing them to forage effectively underwater.

Conclusions: Understanding these skeletal differences may provide insight into the evolutionary biology and biomechanics.

Keywords: avian biomechanics adaptation, flight behaviour, mallard, teal, tufted duck

CHANGES IN PLACENTAL THBS1, TGFβ1 AND SELECTED METALLOPROTEINASES DURING PREGNANCY AND PARTURITION IN COWS

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Introduction: During pregnancy, many changes occur in the female body to maintain appropriate conditions for the development of the placenta and foetus. During delivery, it is important to properly separate the placenta and remove it from the mother's body to avoid infection and other complications. One of the disorders is retained placenta, the aetiology of which is not fully known. It is believed that retention of foetal membranes may be related to abnormal remodelling of the extracellular matrix, which involves biologically active proteins and proteinases.

Aim of the study: This study describes the changes and relationship between thrombospondin-1 (THBS1), transforming growth factor beta1 (TGFβ1) and matrix metalloproteinases (MMP2, 3, 7) during bovine pregnancy (2^{nd}, 4^{th}, 6^{th} months; n=3/month), at normal parturition (NR; n=6) and parturition with foetal membrane retention (R; n=6).

Materials and methods: Placental tissue homogenates were subjected to Western blot analyses and ELISA for proteins quantification.

Results: Results showed significantly higher THBS1 concentrations in R group compared to NR group. Conversely, TGFβ1 concentrations were significantly lower in R group than in NR group in the maternal part of the placenta. MMP3 and 7 concentrations decrease with pregnancy progress between the 2^{nd} and 4^{th} month. During parturition, levels of MMP3 were significantly higher in R group compared to NR group. MMP7 levels decreased significantly in R group in the maternal part of the placenta.

Conclusions: We demonstrated the interdependencies between the examined proteins. Altered MMPs activity may be associated with retention of foetal membranes in cows. We also suggest the involvement of THBS1 in the activation of TGFβ1 in bovine placental tissues, leading to normal release of foetal membranes during parturition.

Keywords: bovine placenta, pregnancy, parturition, thrombospondin-1, transforming growth factor beta1, matrix metalloproteinases

THE INFLUENCE OF TRIVALENT CHROMIUM ON THE QUALITY AND STRENGTH OF BONE TISSUE IN THE FEMORAL NECK OF WISTAR RATS

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Introduction: Trivalent chromium plays an important role in the maintenance of normal carbohydrate, lipid and protein metabolism, and is found in food (meats, some vegetables) and in vitamin and dietary supplements.

Aim of the study: The purpose of the study was to investigate the influence of three different doses of trivalent chromium on the quality of the femoral neck.

Materials and methods: The experiment was performed on 40 healthy male Wistar rats which received, by gavage, during 90 days, a chromium sulphate in either a daily dose of 400, 600 and 800 ug/kg b.w. At the end of the experiment, after euthanasia, the right femurs were cleaned of soft tissue and scanned using the DXA method to determine the bone mineral density (BMD), and bone mineral content (BMC). Then, the pQCT method was used to determine the parameters of trabecular and cortical bone tissue. Finally, a strength analysis of the femoral neck was performed based on a bending test to investigate ultimate strength, work to the ultimate strength and the Young modulus.

Results: Trivalent chromium, in higher doses, significantly decreased BMD and BMC. Furthermore, values of Tb.vBMD and Tb.BMC measured by pQCT in the femoral neck were significantly lower in the experimental groups than in the control. What is more, trivalent chromium revealed a negative influence on the mechanical properties of sampled bone. Indeed, all doses of Cr (III) significantly decreased values of ultimate strength and Young modulus in the investigated femora.

Conclusions: The results of this experiment demonstrate that trivalent chromium is dose-dependent, and exerts a disadvantageous effect on the skeleton, as it decreases bone density and resistance.

Keywords: rat, trivalent chromium, bone, DXA, pQCT

THE TEST FOR STERILITY FOR MEDICINAL PRODUCTS USING THE ISOLATOR WITH HYDROGEN PEROXIDE VAPOUR DECONAMINATION TECHNOLOGY

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Introduction: The National Veterinary Research Institute (NVRI) in Pulawy is a member of General European OMCL Network (GEON). The NVRI as the Official Medicines Control Laboratory (OMCL) performs the batch release of immunological veterinary medicinal products and the quality control of medicinal products for veterinary use after introducing to the market.

Aim of the study: The test for sterility is required for immunological veterinary medicinal products manufactured in aseptic conditions and sterilised in immediate packaging at the end of production cycle.

Materials and methods: This test can be performed by direct inoculation or membrane filtration according to European Pharmacopoeia. Aseptic working conditions are required to conduct the test for sterility. Testing of medicinal products for which sterility is required must be performed in grade A zone. In standard cleanroom the test for sterility requires maintaining grade B environment in surrounding of grade A zone.

Results: Our laboratory uses the isolator with hydrogen peroxide vapour decontamination technology in order to assure appropriate conditions for performing the test for sterility. An isolator is a device designed for aseptic procedures in grade A zone, which can be also placed in lower grade environment. The isolator provides also monitoring of environment conditions required for the test for sterility.

Conclusions: Using the isolator results in reduced cost of hygiene standards, protective clothing for staff and monitoring of the working area which are maintained in lower grade. Decreased risk of contamination and better comfort of work for staff are other beneficial effects.

Keywords: immunological veterinary medicinal products, test for sterility, isolator, European Pharmacopoeia

PLAC1, A TROPHOBLAST-SPECIFIC CELL SURFACE PROTEIN, IS EXPRESSED THROUGHOUT PREGNANCY IN THE BOVINE PLACENTA

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Introduction: Characterization of changes in the protein profile is helpful in studying the mechanisms of control and regulation throughout pregnancy. Placenta-specific protein 1 (PLAC1) is a small secreted protein considered to be a molecule with a significant role in the development of the placenta and the establishment of the mother-foetus interface.

Aim of the study: The aim of the study was to evaluate the PLAC1 profile in healthy bovine placenta and plasma and its relation to early-mid pregnancy course.

Materials and methods: Placental samples from pregnant cows (1–6 months) were collected at a slaughterhouse (n=4/month), and blood samples were collected during routine veterinary procedures from non-pregnant (NP; n=5) and pregnant (P; 1–6 months; n=5/month) cows. Primary bovine epithelial cell cultures originating from maternal caruncles (n=5) and primary cell cultures originating from foetal cotyledons (n=1) were also used. RT-qPCR and Western Blot were employed to exhibit the expression pattern of PLAC1. Quantitative assessments of PLAC1 were carried out using ELISA.

Results: PLAC1 concentrations in the plasma of P cows were significantly higher ($p<0.05$) than those obtained from NP animals. The highest PLAC1 levels were observed for plasma from cows in the 2nd month of pregnancy. Relative quantification of PLAC1 transcripts in the placenta showed a significant decrease ($p<0.01$) in its expression in the 6th month of gestation compared to the 3rd month.

Conclusions: The results of this study suggest the involvement of PLAC1 in the development of bovine placenta. PLAC1 presence in the plasma of pregnant cows as early as the first month may make it a potential candidate as a pregnancy biomarker.

Keywords: bovine placenta, pregnancy, PLAC1

PLASMA ASPROSIN CONCENTRATION IN EXERCISED PUREBRED ARABIAN HORSES

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Asprosin has been recently discovered to be a novel hormone produced by white adipose tissue during fasting. Asprosin stimulates the liver to release glucose into the blood stream and promotes food intake, as well as inflammation. Elevated plasma asprosin levels have been reported in obesity and diabetes. Aerobic exercise training leads to a decrease in plasma asprosin level. The role of this adipokine in energy metabolism in horses is unknown so far. The aim of the study was to analyze the influence of two different exercise types on blood asprosin concentration in horses. Purebred Arabian horses were used in the study because horses of this breed are routinely submitted to two different types of exercise, such as long-lasting endurance rides and short-time intense racing exercise. The group of 27 horses were involved in the study. They were 13 individuals taking part in an official endurance ride, and 14 horses studied during routine race training session on racetrack. Blood samples were collected at rest and after the end of exercise. Plasma asprosin and cortisol concentrations were detected using ELISA tests and glucose were determined enzymatically. The results were analyzed using ANOVA, t-test and Pearson test. Plasma asprosin concentration remained unchanged in the studied horses, despite the exercise tests they performed induced significant changes in plasma cortisol and glucose levels. No significant correlation were found between the analyzed parameters. In conclusion, both short-term intense exercise and endurance exercise did not affect asprosin secretion in horses in the short term. Probably, asprosin is not involved in the regulation of energy metabolism in exercised horses. Thus, asprosin determination is not useful in monitoring horse exercise.

Keywords: asprosin, exercise, horses, monitoring, training

LOCALIZATION AND IMMUNOEXPRESSION LEVELS OF THE NEWLY DISCOVERED PEPTIDE PHOENIXIN IN THE GASTROINTESTINAL TRACT OF DOMESTIC CATTLE (*BOS TAURUS TAURUS*)

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Introduction: Phoenixin (PNX), discovered in 2013, is a peptide involved in various physiological processes, including food intake, reproductive function, and cardiovascular regulation. Derived from the enzymatic cleavage of SMIM20, PNX has two primary isoforms: PNX-14 and PNX-20, and acts through the GPR173 receptor.

Aim of the study: This study aims to investigate the immunolocalization and immunoexpression levels of PNX in the gastrointestinal tract (GIT) of calves and adult cattle, hypothesizing distinct patterns reflecting developmental differences in metabolic activity and gastrointestinal function. Understanding these patterns may provide insights into the roles of PNX in ruminant physiology and aid in optimizing livestock nutrition and health.

Materials and methods: Study material including gastrointestinal tract sections of two age groups, adults and calves of domestic cattle (n=6), was obtained from a slaughterhouse. Enzyme-linked immunosorbent assay (ELISA) and immunohistochemical (IHC) analyses were performed.

Results: The immunoexpression of PNX in the GIT of both groups of domestic cattle was confined to the cytoplasm of epithelial cells and was very sparse. ELISA showed the highest peptide levels in the rumen and reticulum of both groups, and intergroup differences with higher peptide levels in calves compared to adults in the abomasum, duodenum, and jejunum. Our study's observation that PNX is consistently present throughout the GIT supports its peripheral role, distinct from its central effects on food intake regulation.

Conclusions: The complexities of its interactions and the context-dependent nature of its effect highlight the need for precise delivery mechanisms and deeper understanding of its role in bovine health and nutrition.

Keywords: cow, ELISA, gastrointestinal tract, IHC, PNX, SMIM20

EFFECT OF A HIGH-FAT DIET ON THE LOCATION OF COLLAGEN IN THE AORTA IN FEMALE RATS

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Introduction: In recent years, much attention has been paid to the relationship between diet, obesity, metabolic disorders, and cardiovascular health. Obesity and its impact on the progressive development of disorders in the circulatory system and its connection with the increase in the development of diabetes have become the subject of research by many researchers.

Aim of the study: This study was aimed to determine the changes in aorta tissue collagen using imaging spectroscopic techniques: infrared and Raman imaging.

Materials and methods: The experiment involved 24 female rats divided into 3 experimental groups: group L received a low-fat diet, group S received a standard diet, and group H received a high-fat diet. The animals were euthanized after 8 weeks of the experiment and next proximal aortas were collected and fixed in formalin. Fragments of the aortas were dehydrated and embedded in paraffin blocks. The paraffin-embedded sections of the aortas were cut into 6 µm thick sections, deparaffinized in OTTIX PLUS, and mounted on aluminum-coated microscope glass slides. The tissue samples were analyzed using Fourier Transform Infrared (FTIR) and Raman spectroscopy. Aortic wall components, i.e. the type of collagen, elastin, lipids, and amides, were analyzed.

Results: Based on the FTIR analyses, the chemical distribution of selected bands for phospholipids and mature and immature collagen were examined at 1740, 1660, and 1690 cm^{-1}, respectively. The ratio of collagen maturity (1660:1690 cm^{-1}) was also calculated. Moreover, the FTIR spectra analysis showed the lowest peak of lipid, mature collagen, immature collagen, and amide I in the group receiving the low-fat diet (L), compared to the control group (S) and the group receiving the high-fat diet (H). It should be noted that the group receiving the high-fat diet had the highest lipid peak and mature-to-immature collagen ratio compared to the other groups. The aortas of rats from group S were characterized by the lowest ratio of mature to immature collagen, compared to groups L and H.

Conclusions: In conclusion, the application of a varied high and low-fat diet in female rats significantly affects the localization and amount of collagen, lipids, and amide I in the aorta, which may affect aortic function in nutritional and metabolic disorders.

Keywords: high-fat diet, aorta, collagen, FTIR

IN VIVO EVALUATION OF THE NEUROPROTECTIVE PROPERTIES OF PALMATINE ON PARVALBUMIN-IMMUNOREACTIVE NEURONS IN PENTYLENETETRAZOLE (PTZ)-INDUCED SEIZURE MICE MODELS

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Introduction: Epilepsy is a neurological disease in which GABA-ergic neurotransmission is impaired. PV is a calcium-binding protein responsible for the regulation of the Ca^{2+} homeostasis in GABA-ergic inhibitory interneurons.

Aim of the study: We studied whether exposure to PALM affects PV immunoreactivity in mice hippocampus during epilepsy. Palmatine (PALM) is an isoquinoline alkaloid that exerts marked central nervous system-directing properties.

Materials and methods: Male Swiss mice (20-30 g) were divided into four groups (n=8). Group 0(-) – control group; Group 0(+)- received 5% Tween + PTZ; PALM (20mg/kg) - administered *ip* 30 min before each PTZ treatment; PALM (40 mg/kg) - was administered *ip* 30 min before each PTZ treatment. After the euthanasia, formalin-fixed, paraffin-embedded sections of the brain were made. The sections were immunohistochemically stained with PAP method using a primary antibody directed against PV. The obtained results were statistically analyzed with ANOVA with Dunnett's post hoc test.

Results: In each animal, no less than one hundred parvalbumin-immunoreactive neurons (PV-IR) in fields and DG of the hippocampus of each group were counted, using Cell D software. Our results indicated that the administration of PALM in both doses decreased the mean numbers of PV-IR neurons in the hippocampal fields as compared to the control groups.

Conclusions: The results of the present study suggest that PALM may be a factor influencing Ca^{2+} homeostasis in CNS, and thus may compose a good strategy in the prevention of neurodegenerative diseases and provide the basis for further research into the possible neuroprotective role of bioactive compounds.

Keywords: palmatine, epilepsy, parvalbumine, hippocampus, mice

THE EFFECT OF WHEY PROTEIN SUPPLEMENTATION ON THE STRUCTURE OF THE SMALL AND LARGE INTESTINES IN RATS AFTER CADMIUM EXPOSURE

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Introduction: Cadmium (Cd) is a toxic metal that accumulates in the kidneys and liver, potentially causing ER+ breast cancer and osteoporosis. The absorption of cadmium in the gastrointestinal tract relies on its dynamic uptake and accumulation in the enterocytes of intestinal villi. Whey proteins, such as β-lactoglobulin and lactoferrin, exhibit antioxidant, immunomodulatory, and divalent metal chelating properties.

Aim of the study: Thus the aim of this study was to assess the effects of cadmium and whey proteins on the morphology of the small and large intestines in rats.

Materials and methods: Three-week-old Wistar rats were separated by sex into four groups (n=12): K+ (cadmium control), K- (negative control), S+Cd (diet with WPC80 and cadmium), and S (diet with WPC80). Cadmium was administered in drinking water at a concentration of 19.4 mmol/l for 10 weeks. Histomorphometric analysis was performed on intestine samples taken from the duodenum, mid-jejunum, ileum, and colon.

Results: Cadmium significantly reduced the thickness of the mucosa, villi height, and muscle layer in the small intestine while caused deepening of the crypts. In contrast, whey proteins increased mucosal thickness and villi height, offering protection against cadmium's harmful effects. In the large intestine, whey proteins significantly increased Goblet cell size, suggesting enhanced intestinal wall protection, while cadmium reduced the muscle layer thickness and Goblet cell size.

Conclusions: Whey proteins in the diet of rats exert a protective effect on the gastrointestinal tract when exposed to cadmium chloride.

Keywords: cadmium intoxication, whey protein, small and large intestines histology

WHEY PROTEIN SUPPLEMENTATION EXHIBITS PROTECTIVE EFFECTS ON LIVER MORPHOLOGY UNDER CONDITIONS OF CADMIUM CHLORIDE INTOXICATION

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Introduction: Cadmium is a highly toxic heavy metal that accumulates in the kidneys and liver, causing organs damage. Whey proteins have strong chelating and antioxidant properties due to sulfur-containing amino acids, mainly cysteine.

Aim of the study: This study aimed to assess the impact of whey protein supplementation on liver structure and morphometry in cadmium-intoxicated rats.

Materials and methods: Three-week-old Wistar rats were divided into four groups by sex (n=12): K+ (cadmium control), K- (negative control), S+Cd (diet with WPC80 and cadmium), and S (diet with WPC80). Cadmium was given in drinking water (19.4 mmol/l) for 10 weeks. Liver samples were analyzed histomorphometrically.

Results: In male rats intoxicated with cadmium, there was a significant reduction in mononuclear hepatocytes, an increase in multinucleated hepatocytes, necrosis, inflammation, enlarged portal veins, fibrosis, and fatty liver changes. WPC supplementation reduced hepatocyte surface area. In female rats, multinucleated hepatocytes and hepatocyte surface area increased in the S+Cd and S groups. WPC supplementation in the S+Cd group showed a protective effect by reducing ballooning, while the S group exhibited a localized inflammatory response and reduced hepatocyte size in males.

Conclusions: Cadmium administered in drinking water causes degenerative changes in the liver structure of rats, and the addition of whey proteins to the diet of cadmium-intoxicated rats of both sexes reduces the toxic effects of this element, as evidenced by an increase in the number or surface area of mononuclear hepatocytes, with histopathological changes being less pronounced.

Keywords: cadmium intoxication, whey protein, liver histology

SESSION OF THE PRECLINICAL VETERINARY SCIENCES

ANTIBIOTICS IN VETERINARY AND THEIR EFFECT ON HUMAN PATHOGENS

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It has been widely assumed that since their discovery, the prolonged and widespread use of antibiotics in veterinary and agricultural production has led to a wide variety of problems. Particularly worrisome has been the emergence and spread of human pathogenic antibiotic-resistant bacteria. In this sense, veterinary medicine has always been seen as a culprit.

Many years ago, Jacques Monod and François Jacob, who received the Nobel Prize in Medicine, published two essays, both of which achieved notable success. However, Monod's One („Chance and Necessity") may constitute a basis for understanding what happens to us. The germinal idea of this work comes from a phrase by Democritus "Everything that exists in the Universe is the result of chance and necessity" or in other words, everything that can happen, will happen. Therefore, resistance to antibiotics is the result of the existence of the antibiotics themselves, and of the ability of microbes to evolve; thus, the only thing that human activity can influence is, probably, the speed of the appearance of resistance.

In principle, the use of antibiotics in veterinary medicine should not have a greater influence on the evolution of resistance than their use in human medicine. Not so the use of antimicrobials as growth promoters, in which case the consequence would be much worse.

However, the data available seem to recommend the investigation of new antimicrobials or new anti-infective strategies, among which antimicrobial peptides and bacteriophage viruses should be highlighted.

Keywords: antibiotics, antibiotic-resistant bacteria

VIROLOGICAL RISKS IN THE WORLD – CURRENT STATE OF KNOWLEDGE AND PROJECTIONS

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A worrying element of climate change is the shifting boundary of ticks and insects, which are the most important element in the transmission of pathogenic viruses. Also have caused changes in mutation, survival and localisation of viral vectors and reservoirs.

This is particularly true for RNA viruses, characterised by genetic instability that leads to large changes in their virulence or adaptation to new hosts. The genetic variability of RNA viruses has allowed them to rapidly adapt, survive and multiply in an infected organism and evade the immune response, which plays a dominant role in new viral survival strategies.

In recent years, there has been a significant increase in the number of new dangerous viruses which include Bourbon, Heartland, Keystone and Oropouche. These new viruses of little known pathogenicity will pose a similar threat to animals and humans as Zika virus, Powassan virus, West Nile virus or SARS-CoV-2. Also noteworthy are the haemorrhagic fever viruses, the spread and pathogenicity of which have increased significantly in recent years, and in particular the dengue virus DENV-2, with some 400 million cases of the disease diagnosed annually worldwide.

Of particular note are the facts that emerging new viruses are characterised by the possibility of genetic reassortment, hiding from the immune system, increasing virulence, rapid spread and the ability to cross interspecies barriers. The observed phenomena call for new strategies for immunoprophylaxis and targeted therapy to limit the spread of new viruses and their unpredictable mutations and adaptability to new animals and human species.

Keywords: new emerging diseases, viruses

APPLICATION POTENTIAL OF BACTERIOCINS (POSTBIOTICS) PRODUCED BY BENEFICIAL BACTERIA

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Nowadays, our society has been focused on health condition in the framework of the “One Health concept”. Dominantly, this effort has been concerned on the use of natural substances to reach that interest.

Representatives of some beneficial bacteria can produce non-residual antimicrobial substances of proteinaceous character-bacteriocins which have been recently belonged to the postbiotic group. Postbiotics are defined as preparations of inanimate microbiota and/or their components that confers a health benefit on host.

The aim of our summary study has been to show beneficial application potential of postbiotics (produced by beneficial bacteria isolated and characterized in our laboratory) in different animals to optimize health status of host.

Bacteriocins used have been produced by our strains *Enterococcus faecium* of different origin deposited in the Czech Culture Collection in Brno (Czech Republic) as follows: AL41=CCM8558 (Ent M), CCM7419 (EntA/P), EF 412 (Ent 412), CCM7420 (Ent 2019), CCM4231, (Ent 4231), and Mundticin EM 41/3 by E. mundtii EM 41/3.

Application of postbiotics (bacteriocins-enterocins and/or mundticin) in broiler rabbits and horses lead to significant ($p<0.05$), ($p<0.001$) or mathematical reduction of coliforms, clostridiae or staphylococci. Moreover, especially in rabbits, significant increase of phagocytic activity (PA) was noted ($p<0.01$); in horses also increasing tendency of PA was noted after bacteriocins application. No oxidative stress was noticed. In broiler rabbits even count of *Eimeria* spp. oocysts was reduced; and in horses, parasitological status was influenced as well. Blood biochemistry was not negatively influenced, and in rabbits no negative effect on metabolic parameters and meat quality was noted.

Keywords: bacteriocins, application, postbiotic, health, influence

ASSESSMENT OF THE ACTIVITY OF BACTERIOCINS AGAINST *STAPHYLOCOCCUS* AND *MAMMALIICOCCUS* GENERA

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Introduction: The increase in antibiotic use has led to selection pressure among bacteria and the spread of drug resistance, and the need to develop new treatment strategies.

Aim of the study: The aim of the study was to assess the activity of bacteriocins isolated from different *Enterococcus* spp. and *Lactococcus* spp. in relation to bacteria belonging to *Staphylococcus* (including methicillin-resistant; MRSA) and *Mammaliicoccus.*

Materials and methods: The research was conducted on a total of 47 strains of *Staphylococcus* belonging to 16 different species and 3 species of *Mammaliicoccus.* Inhibitory activities of antimicrobial substances were checked using quantitative agar spot method and determined in arbitrary units per millilitre (AU/ml). The studies used concentrated or precipitated bacteriocins isolated from *E. faecium* (n=6), *E. mundtii* (n=1), *E. saccharolyticus* (n=1) and *Lactococcus lactis* (n=4).

Results: The highest inhibitory activity against the growth of indicator strains was shown due nisin (100-12800AU/ml). However, differences in the activity of this substance against individual *Staphylococcus* and *Mammaliicoccus* species were observed. Relatively low inhibitory activity of bacteriocins MK 2/8, MK 2/2 and MK 2/7 produced by the strains *L. lactis* and *E. saccharolyticus* were observed. Bacteriocins isolated from *E. faecium* and *E. mundtii* did not inhibit the growth of tested *Staphylococcus* and *Mammaliicoccus* with the exception of Ent412 and EntM in relation to *S. simulans* (activity 200AU/ml).

Conclusions: To sum up, bacteriocins isolated from the strain species *L. lactis*, especially nisin, as well as from *E. saccharolyticus* Es 3/11 D27, are characterized by higher inhibitory activity against tested bacteria compared to bacteriocins isolated from *E. faecium* and *E. mundtii*.

Keywords: bacteriocins, *Staphylococcus*, *Mammaliicoccus*

RISKS OF USING A VEGAN AND VEGETARIAN DIET IN HUMANS AND COMPANION ANIMALS OF DOGS AND CATS

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The available scientific literature shows that use of the restrictive diets can result in nutritional deficiencies of vitamin B12, iron, creatine, iodine, omega-3 polyunsaturated fatty acids (EPA and DHA), vitamin D, calcium, zinc and other essential nutrients. Pregnant women, breastfed and adolescent children are most at risk. Current research indicates that in humans, deficiencies of these key nutrients can result in impaired cognition, working memory, fluid intelligence and, as a result, a 10-15 point lower IQ. They are also associated with impaired mental health by increasing the risk of developing depression, ADHD, autism and dyspraxia.

Popularization of vegetarianism and veganism among people is a growing threat to companion animals of dogs and cats. Wanda McCormick, showed that 1% of vegetarians feed their dogs a vegetarian diet, but as many as 1/3 of vegans feed them a vegan diet. Other studies have shown that some vegans feed their cats a vegan diet. The recently observed implementation of restrictive vegetarian and vegan diets on these companion animals may expose them to serious health problems. Doubts are raised about the use of these diets, especially in pregnant females, during lactation and in the phase of rapid growth of young offspring. What is more, they should not be used in cats at all because cats are obligate carnivorous species.

15 YEARS OF CAMPYLOBACTER STUDY AT NATIONAL VETERINARY RESEARCH INSTITUTE IN PUŁAWY, POLAND

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Introduction: *Campylobacter*, especially *C. jejuni*, is among the most common causes of bacterial foodborne infections worldwide. Poultry has been shown to be the most important source of these bacteria and the main transmission route of *Campylobacter* to humans is handling contaminated food, especially of chicken meat. However, it has been described that ruminants, especially cattle, are also responsible for a high number of human *C. jejuni* (and to a lesser extent - *C. coli*) infections. *Campylobacter* has also been identified in pigs and pig carcasses that are often contaminated with feces at the slaughter and processing facilities during the evisceration process, which resulted with the presence of the bacteria in pork meat.

Aim of the study: Identification of *Campylobacter* contamination of bovine and pig carcasses during 2009–2023 and investigate the antimicrobial resistance of the obtained *Campylobacter* isolates.

Materials and methods: A total of 7,623 swabs from animal carcasses were collected during by official veterinarians in commercial abattoirs located all over Poland. Among them, 5,766 samples were of chicken origin, 646 from bovine carcasses and 1,211 from pig carcasses, respectively. *Campylobacter* was isolated according to the ISO 10272-1:2017 standard and confirmed as *C. jejuni* or *C. coli* with PCR. Antimicrobial resistance of the *C. jejuni* and *C. coli* isolates was tested as recommended by the European Union Reference Laboratory for Antimicrobial Resistance and the European Commission. The following antimicrobials were used: erythromycin, ciprofloxacin, nalidixic acid, streptomycin, and tetracycline. A microbroth dilution method was used to establish the minimum inhibitory concentrations (MICs) of *Campylobacter* isolates to antimicrobials with Sensititre® custom susceptibility plates, EUCAMP. The obtained results were evaluated using the Vision system.

Results: Among 7,623 samples tested, 3,482 (45.7%) were positive towards *Campylobacter*. Poultry carcasses were most contaminated, with 2,989 (51.8%) samples positive for the bacteria tested. However, pig carcasses were also often positive with *Campylobacter*, with 435 (35.9%) swabs, whereas only 58 (9.0%) bovine carcasses were contaminated with the bacteria tested. A total of 1,959 (56.3%) samples were positive for *C. coli* and the remaining 1,523 (43.7%) were contaminated with *C. jejuni*. *C. coli* predominated among chicken and pig carcasses, with 51.8% and 88.7% strains, respectively. On the other hand, among samples from bovine carcasses, *C. jejuni* was more common than *C. coli* (56.9% and 43.1%, respectively). Antimicrobial resistance analysis revealed that most strains, irrespective of the origin, were resistant to ciprofloxacin (97.5%) and nalidixic acid (83.0%), followed by tetracycline (64.7%) and streptomycin (30.4%). Only few strains were resistant to gentamycin (0.6%) and

erythromycin (4.6%), although higher percentage of erythromycin-resistant isolates were observed among *C. coli* than *C. jejuni*.

Conclusions: Food-producing animal carcasses are often contaminated with *Campylobacter*, both *C. coli* and *C. jejuni*. The most positive samples were found among poultry carcasses; however, *Campylobacter* was also often identified in pig carcasses, which suggests that these animals may be an underestimated reservoir for these bacteria. A high resistance rates of *Campylobacter* strains to streptomycin, quinolones and tetracyclines highlights the need for monitoring of chicken, bovine and pig carcasses towards such antimicrobials.

Keywords: *Campylobacter*, chicken, bovine and pig carcasses, prevalence, antimicrobial resistance, monitoring, Poland

RW5, A POLY (TRP-ARG) PEPTIDE: INSIGHTS THEIR ANTIMICROBIAL ACTIVITY AND MECHANISM OF ACTION

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Introduction: The rise of multidrug-resistant bacteria, coupled with the lack of new effective antimicrobials, is a global public health concern. In this context, the search of new molecules, either natural or synthetic is necessary.

Aim of the study: The aim of this work was to stud the antimicrobial activity and to explore the mechanisms of action of a synthetic Poly (Trp-Arg) peptide RW5.

Materials and methods: The antimicrobial activity of RW5 was investigated by the minimum inhibitory concentration (MIC), growth kinetics and time-kill assays in different bacterial strains. The action of RW5 on membranes was evaluated by measuring conductance in artificial lipid bilayers and by Sytox green assay. The action on efflux pumps was evaluated by the acridine orange (AO) accumulation assay. RW5-treated bacteria were visualized by atomic force microscopy (AFM) and transmission electron microscopy (TEM). The interaction of RW5 with linezolid or imipenem was evaluated by the checkerboard assay and growth kinetics.

Results: The MIC of RW5 was 32 µg/mL for *E. coli* and *P. aeruginosa* and 64 µg/mL for *S. aureus*. In *E. coli*, RW5 inhibited the growth for 13h at 16 µg/mL and for 10h at 8 µg/mL. In *P. aeruginosa*, RW5 inhibited the growth for 8h at 8 µg/mL, and for *S. aureus*, RW5 inhibited the growth for 9h at 32 µg/mL. Time-kill curves of *E. coli* showed a bactericidal effect at 32 µg/mL and a reduction of two-log at 16 µg/mL after 6h, while *in P. aeruginosa* a bacteriostatic effect was observed after 8h at 32 µg/mL. On artificial membranes, few transient conductance events were observed after peptide addition. In Sytox green assay, RW5 induced immediate membrane permeabilization in *E. coli* and *P. aeruginosa*. RW5 increased the ability of the bacterium to accumulate AO in *E. coli* 25922 and *P. aeruginosa* 27853. AFM and TEM images showed alterations on the bacterial surface and formation of blebs on the outer membrane of *E. coli.* A synergistic effect was observed with linezolid.

Conclusions: RW5 may be a promising peptide displaying good antimicrobial activity against E. coli and P. aeruginosa. Preliminary results suggest that the mechanisms of action of RW5 involve efflux pumps inhibition and alterations of bacterial membranes. Moreover, it can be used in combination with conventional antibiotics.

EVALUATION OF THE EFFECT OF FUNGAL EXTRACTS ON NEUTROPHILS IN THE CONTEXT OF BIOMATERIAL MODULATION

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Introduction: Biomaterials are playing an increasingly important role in medicine, used in implants, devices and other therapies. Their success depends largely on their interaction with the surrounding tissue, including neutrophils, key cells of the immune system. Modulating biomaterials with natural fungal extracts may be one way to alter the inflammatory response and increase implant biocompatibility.

Aim of the study: The aim of this study was to evaluate the effect of fungal extracts on neutrophils in the context of biomaterials modulation.

Materials and methods: Alcoholic extracts (70% ethanol) were made from the fruiting bodies of 8 mushroom species: *Phellinus hartigii, Phellinus robustus, Hypsizygus tessellatus, Armillaria ostoyae, Amanita rubescens Pers., Suillus luteus, Lepista nuda, Macrolepiota procera*. The addition of extracts was used to culture neutrophils obtained from porcine blood. Their effect on neutrophils was studied in vitro, assessing parameters such as reactive oxygen species (ROS) and nitrite (NO_{2^-}) production by biochemical assays (e.g. Griess reaction, Confer reaction).

Results: The fungal extracts tested showed effects on neutrophil activity. Some inhibited the production of ROS and NO_{2^-}, while others stimulated the cells to produce them.

Conclusions: Fungal extracts may be promising modulators of biomaterials, affecting neutrophil function and potentially modulating the body's inflammatory response to implanted materials. Depending on the type of extract (in this case, the species difference), the cellular response is different, which may be a prelude to further research into their use as immunomodulators in veterinary as well as human medicine.

Keywords: fungal extracts, immunomodulation, neutrophils, medical mushrooms, biocompatibility

ANALYSIS OF ALTERNATIVE RESEARCH MATERIAL IN VETERINARY TOXICOLOGY OF Hg

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Introduction: Nowadays most methods of veterinary diagnostics towards exposure to the Hg is based on the analysis of the basic research materials, such as blood and urea. The selection of the analyzed sample is dependent on the Hg toxicokinetics and the degree of invasiveness of the sampling method. On the contrary, the application of the alternative research material (e. g. hair, nails, feathers) is related to noninvasiveness, painlessness and accuracy of toxicological studies.

Aim of the study: The main research aim was the development and validation of Hg determination procedures in alternative materials (horse hair) by Cold Vapour Atomic Absorption Spectrometry.

Materials and methods: Samples of the horse hair from the neck and mane were sampled by the medical-veterinary procedure. They were decontaminated and then homogenized by the cryogenic mill. Hg was directly determined in the homogenized samples by the Cold Vapour Atomic Absorption Spectrometry after their high-temperature treatment.

Results: The application of the cryogenic mill could effectively homogenize studied samples. It influenced the high repeatability of the obtained results (below 10 %). Moreover, Hg content values in the studied alternative samples were higher than in the comparable blood samples.

Conclusions: The alternative research materials can be good candidates for noninvasiveness studies concerning exposure to the Hg. The application of the alternative research materials in the toxicological analysis can reduce costs of the analysis and ensure the long term monitoring of the Hg in the animal organisms.

Keywords: mercury, horse hair, cold vapour atomic absorption spectrometry, cryogenic homogenization

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IN VITRO INFLUENCE OF NEUROKININ B, DYNORPHIN A, AND KISSPEPTIN-10 ON GONADOTROPIN SECRETION BY ANTERIOR PITUITARY CELLS ISOLATED FROM PUBESCENT EWES

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Introduction: Kisspeptin-10 (KiSS-10), neurokinin B (NKB), and dynorphin A (Dyn A), produced by the KNDy subpopulation in the ARC region of the hypothalamus, play a relevant role in the endocrine regulation of the hypothalamic-pituitary-ovarian (HPO) axis during puberty. A detailed knowledge of their feature will provide a better understanding of the pathomechanism of e.g. delayed puberty or other neuroendocrine disorders leading to infertility.

Aim of the study: The aim of our experiment was to analyze the direct effect of KiSS-10, NKB, and Dyn A on gonadotropin secretion by pituitary cells isolated from pubescent ewes.

Materials and methods: Cells were incubated in McCoy's 5A medium without hormones (control) or with 10-11-10-7 M of KiSS-10, NKB or Dyn A. After 4-24 h of the experiment, the LH and FSH secretion were analyzed by ELISA using species-specific antibodies.

Results: Our results showed an increase in the LH and FSH secretion after the 4-24 h exposure to 10-11-10-8 M and 10-11-10-7 M of KiSS-10, respectively, compared to the control. Moreover, NKB and Dyn A at the concentration of 10-11-10-7 M caused the elevation of both gonadotropins secretion throughout the whole experiment.

Conclusions: We demonstrated that KiSS-10, NKB, and Dyn A directly affect gonadotropin secretion by ovine pituitary cells. However, a detailed explanation of the role of these neuropeptides in LH and FSH secretion in sheep and the determination of their impact on the regulation of the HPO axis during sexual maturation require further studies.

Keywords: neurokinin B, dynorphin A, kisspeptin, gonadotropin, puberty

THE EVALUATION OF INTERLEUKIN-6 LEVELS IN THE MILK AND SERUM OF COWS SUFFERING FROM SUBCLINICAL MASTITIS CAUSED BY *ENTEROCOCCUS FAECALIS*

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Introduction: Interleukin-6 (IL-6) is a pleiotropic cytokine that has many different functions, such as host defense through immune response, and regulation of inflammation. It is the main inducer of hepatic induction of acute phase proteins during infection. This cytokine is secreted by a variety of cells, including lymphocytes, monocytes, macrophages, epithelial cells and fibroblasts, and its expression is stimulated by invading bacteria.

Aim of the study: The aim of this study was to evaluate the concentration of IL-6 in the milk and serum of cows suffering from subclinical mastitis caused by *Enterococcus faecalis*.

Materials and Methods: Material for the study was collected from 20 Holstein-Fresian cows, including 10 cows with mastitis and 10 healthy cows. The concentration of IL-6 in the milk of the cows was determined by immunoenzymatic methods using the using kits for IL-6 from USCN Life Science INC., Houston, USA.

Results: The present study showed that IL-6 levels were significantly higher in milk and lower in serum of cows with subclinical mastitis caused by Enterococcus faecalis compared to healthy cows (122.9 pg/mL vs. 25.4 pg/mL, $p<0.001$; 20.5 pg/mL vs. 99.3 pg/mL, $p<0.001$, respectively).

Conclusions: This findings suggest that cows with mastitis caused by *Enterococcus faecalis* develop local immune response in mammary gland in response to the pathogen. We have reported an increase in the level of proinflammatory cytokine - IL-6 in milk but not in serum of cows with mastitis.

Keywords: cows, mastitis, *Enterococcus faecalis*, interleukin-6

THE IMPACT OF HYPERTHERMIA, DEHYDRATION AND OXALIPLATIN ON COLORECTAL CANCER CELLS (HT-29): A STUDY OF THE CELL DEATH MECHANISM

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Introduction: Peritoneal surface malignancies are a challenging problem in human and veterinary clinical oncology.

Aim of the study: Current treatments are ineffective, which led our research group to propose a new potential therapy involving three factors: hyperthermia, dehydration, and chemotherapy. This strategy can effectively inhibit proliferation and induce tumor cell death. Since the mechanism of cell death in this protocol is not known, we aimed to investigate it in this study.

Materials and methods: Human colorectal cancer cell line HT-29 was seeded in plates and grown for 48 hours before treatment. After incubation with oxaliplatin at a concentration of 92 mg/ml (1h 37°C), the cells were dehydrated by removing the medium and incubated at 45°C or 50°C for 45 minutes or in the reverse order. The CellEvent™ Caspase-3/7 Green Flow Cytometry Assay Kit (Invitrogen) was added. The INCUCYTE® S3 system was used to detect caspase-3/7 activity and cell membrane permeabilization. Data were collected every 4 hours for 2 days.

Results: Cells, after exposure to the presented procedure, changed morphology from typical epithelial cohesive to round shaped cells with clear boundaries. Significant changes occurred when dehydration followed by oxaliplatin exposure was applied: proliferation decreased significantly compared to the negative control (t48 : t0 ratio: 1 vs 1.6) and caspase-3/7 activity increased approximately 5, 4 and 1.5 times compared to the negative control, oxaliplatin followed by dehydration and dehydration alone, respectively. Permeabilization of cell membrane was also prominent for this procedure.

Conclusions: In conclusion, the presented procedure activates caspase-3 and thus induces apoptosis, which can be a new tool for peritoneal carcinomatosis treatment.

Keywords: hyperthermia, intraperitoneal chemotherapy, apoptosis, peritoneal malignancies, dehydration, HT-29 cell line

DOGS AS A RESERVOIR OF MULTIDRUG-RESISTANT *STAPHYLOCOCCUS AUREUS*

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Introduction: Bacteria of the genus *Staphylococcus* spp. are pose a threat to animals. *S. aureus* can cause infections or constitute a component of the skin and mucous membranes microbiota.

Aim of the study: The aims of the study were analysis of occurrence, the species molecular identification of *Staphylococcus aureus* isolated from dogs and the assessment of the level of drug resistance and virulence of these microorganisms.

Materials and methods: The research material was collected as swabs from 150 dogs from four different areas of body (skin, ears, anus, mouth). A total of 600 swabs were collected from animals. Standard selective-differentiating media dedicated to *Staphylococcus* spp. (Chapman and Baird Parker) were used to identify at the genus level. Then the species were confirmed by a multiplex-PCR. Drug resistance was assessed by Kirby-Bauer disc diffusion method. Then, resistance and virulence genes were identified using the PCR.

Results: A total of 101 coagulase-positive *Staphylococcus* spp. strains were isolated, including 22 isolates belonging to *S. aureus*. The isolated strains were resistant to eleven of thirteen antimicrobials. The majority of *S. aureus* strains were resistant to penicillin, which accounted for 72%. The strains were also resistant to cefoxitin, tetracycline, clindamycin, ciprofloxacin, erythromycin, gentamicin, linezolid, chloramphenicol, sulfamethoxazole/trimethoprim and nitrofurantoin. Additionally, 68% of *S. aureus* strains were resistant to more than one antimicrobial agent. All strains were susceptible to rifampicin and quinupristin/dalfopristin. The following resistance genes (mecA, mecC,erm A, erm B, cat pC221, cat pC194, tetK, tetM, blaZ) and virulence genes (seB, seE, PVL, Luk E-Luk D) were detected in *S. aureus* strains.

Conclusions: The dogs can be a reservoir of multidrug-resistant strains of *Staphylcoccus aureus*. Many strains of *S. aureus* have numerous resistance and virulence genes, which may pose problems in the diagnosis and treatment of animals.

Keywords: *Staphylococcus aureus,* drug resistance, virulence, dogs

OCCURENCE OF *PASTEURELLA MULTOCIDA* IN EUROPEAN BISON IN POLAND

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Introdution: *Pasteurella multocida* is a pathogen of numerous domestic and wild animals. Some of the capsular types are the etiological agents of severe pasteurellosis, such as bovine haemorrhagic septicaemia. This microorganism is also often found as a commensal in the upper respiratory tract of clinically healthy animals.

Aim of the study: The subject of the study was to determine the presence of *P. multocida* in clinical material from dead or eliminated between 2013-2023 European bison in Poland.

Materials and methods: Samples of internal organs were cultured on agar with 5% horse blood and MacConkey agar. Identification of *P. multocida* strains was performed on basis of morphological and biochemical characteristics. Multiplex PCR allowing simultaneous identification of species and determination of capsular type A, B, D or F, and next PCR used to identify strains *P. multocida* serotype B:2 causing haemorrhagic septicaemia, were applied.

Results: Twenty-seven strains of *P. multocida* were isolated. In animals with *P. multocida*, anatomopathological changes in the respiratory system were found at autopsy. In multiplex PCR the capsule type A was identified in twenty-five strains, type B in one strain and type D in one strain. The strain of B:2 serotype causing haemorrhagic septicaemia was detected in one European bison from the free-living population.

Conclusions: Isolation of *P. multocida* strains representing different biochemical profiles, with the capsule type A, as well as the occurrence of strains with the capsule type B and D, proves that this microorganism is a constant and constantly growing threat to the health of European bison.

Keywords: *Pasteurella multocida*, pasteurellosis, European bison

STUDY OF EUROPEAN BISON HEALTH RISKS ON THE EXAMPLE OF EPIZOOTIC MONITORING

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The migration of European bison not only causes conflicts related to the destruction of crops, but can also pose a potential epidemiological threat. All Polish E. bison populations live in areas that are directly adjacent to the areas of agricultural activity. The impact of anthropopression on E. bison habitats may increase the risk of transmission of infectious agents from humans and farm animals (spill-over) or in the opposite direction (spill-back). Such population growth was also possible thanks to the relocation of European bison to new places. Over the past 20 years, 53 new farms and free-living herds have been established in Europe. Therefore, knowledge about the threats of diseases in European bison is an important contribution to the protection of the species, but it is also important for understanding its role as a potential reservoir of zoonotic, endemic or emerging diseases.

From the year 2008, conducte extensive research related to the spread of infectious and invasive diseases in the populations of E. bison in Poland, with particular emphasis on zoonotic factors. Their completion resulted in broadening the knowledge on the spread of selected infectious and invasive agents in the E. bison population, with particular emphasis on those that may also pose a threat to humans, which in the XXIst century is of key importance, not only for the protection of species of rare animals, but also for the protection of public health. Along with climate change in our part of Europe, infections with vector-borne pathogens, such as arthropods (ticks, flies, mosquitoes), are also described more and more often. An example may be the occurrence of infections with bluetongue virus (BTV) and Schmallenberg virus (SBV) in Poland, transmitted by the the genus *Culicoides* spp., or the recently publicized problem of infestation with the parasite transmitted by flies, *Thelazia* spp. in Bieszczady. Wild animals an important element in the One Health concept, where their supervision is an important element of public health protection.

Infections and invasions with agents that have a zoonotic potential are an increasingly serious problem, because the development of international trade, overexploitation of the natural environment and tourism. The circulation of pathogens between free-living and domesticated animal populations and humans are challenges that epidemiologists in both human and veterinary medicine must meet. Managing populations of protected animals based on health monitoring and minimizing the risks of infectious and invasive diseases is the basis in urbanized areas such as Poland.

Keywords: European bison, One Health, zoonoses

SELECTED PARAMETERS OF IMMUNE RESPONSE AND THEIR POTENTIAL USE AS DIAGNOSTIC MARKERS IN CANINE PERIANAL TUMOURS

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Introduction: Articles concerning canine cancers occurrence emphasise that the number of tumour bearing animals is constantly raising, especially in the regions of large urban agglomerations. The most common canine neoplasms are skin cancers and among them perianal tumours account for 15-17%. In most cases, tumours of perianal area derive from perianal glands and they occur mostly in aged male dogs (medium age above 10 years).

Aim of the study: The aim of the present study was to determine the profile of the immune response in male dogs with benign and malignant perianal tumours by immunophenotyping the selected leukocyte subsets using flow cytometry.

Materials and methods: Forty male dogs were divided into 2 investigated groups depending on the tumour malignancy degree. Flow cytometry was used for immunophenotyping the basic leukocyte subsets. Monoclonal antibodies for CD3, CD4, CD5, CD8, CD14, CD21, and MHC II surface antigens were applied.

Results: We found that in tumour-bearing dogs regardless of the malignancy degree the profile of the immune response was different from that of healthy dogs. Moreover, flow cytometric evaluation of specific leukocyte subpopulations revealed significant differences concerning particular lymphocyte subsets between groups with malignant and benign tumours.

Conclusions: These differences, in combination with other parameters, may have prognostic significance, and may enable more precise perianal tumour diagnostics and treatment monitoring.

Keywords: perianal tumours, flow cytometry, immunophenotyping, tumour immunology, canine leukocytes

PHARMACOKINETIC PROFILE OF DERACOXIB IN SELECTED ANIMAL SPECIES

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Introduction: The integration of pain management in veterinary practice, driven by heightened animal welfare concerns, extends to small ruminants and avian species where subtle and nonspecific behavioral signs pose challenges. Given that safety concerns with classical NSAIDs highlight the need for more targeted alternatives in these species, this study explores the pharmacokinetic (PK) properties of Deracoxib (DX), a COX-2 selective NSAID approved for use in dogs, following a single oral administration in sheep, goats and geese.

Materials and methods: The study employed an oral administration of DX at a dose of 150 mg/head (sheep and goats) and 4mg/kg in geese. Blood was drawn to heparinized tubes at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10, and 24 h. Plasma concentrations were determined after validating a high-performance liquid chromatography method, coupled to a UV detector. The PK parameters, including maximum plasma concentration (Cmax), time to reach Cmax (Tmax), elimination half-life (t1/2), and area under the curve (AUC), were evaluated through non-compartmental analysis.

Results: Results showed detectable DX in plasma up to 48 h and no significant differences in any PK parameters were noted between sheep and goats. Notably, t1/2 values were relatively long, at 16.66 h for sheep and 22.86 h for goats. Despite the fact that both species exhibited comparable drug exposure, high individual variability was noted within each species, suggesting to take into account individual variations in response to DX treatment, rather than species-specific considerations. In geese the results indicated a terminal half-life of 6.3 h and a Tmax of 1 h.

Conclusions: While refraining from claiming absolute safety based on a single dose, it is worth high lighting that further safety studies for DX in geese are warranted, suggesting a possibility for intermittent use. Further research involving pharmacodynamics and multiple-dose studies is necessary to comprehensively evaluate the profile of DX in these species. Additionally, conclusions regarding efficacy and suitability require more investigation, particularly to understand COX-2 selectivity and protein binding characteristics specific to small ruminants and birds.

Keywords: deracoxib, NSAIDs, pain management

AMPLIFYING OXALIPLATIN'S IMPACT ON HT-29 CANCER CELLS THROUGH DEHYDRATION

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Introduction: Intraperitoneal dissemination of cancer cells is a significant issue in human medicine and increasingly diagnosed disease entity in veterinary medicine, often resulting from metastasis of gastrointestinal and gynecological cancers. Despite various therapeutic strategies, patients with peritoneal metastasis (PM) face a poor prognosis due to rapid cancer progression, requiring new therapeutic options. Our research group proposed adding a dehydration factor to the established direct chemotherapy strategy.

Aim of the study: The aim of this study was to investigate the influence of the dehydration factor under normothermic conditions, combined with direct chemotherapy, on proliferation, caspase-3/7 activity, and cell membrane permeabilization in the human colorectal cancer cell line HT-29.

Materials and methods: HT-29 cells were seeded in 96-well plates 48 hours before the experiment. The cells were then treated with: dehydration, dehydration followed by exposure to oxaliplatin at 92 mg/ml for one hour, and the reverse order, all at 37°C. Partial dehydration was achieved by removing the culture medium for 45 minutes. To monitor the results, we used the CellEvent™ Caspase-3/7 Green Flow Cytometry Assay Kit (Invitrogen) to assess caspase-3/7 activity and cell permeabilization. The INCUCYTE® S3 Live-Cell Imaging and Analysis System was used for fluorescence detection, with data collected every 4 hours for 2 days.

Results: Changes in cell morphology were observed, with the most significant inhibition of proliferation in the dehydration followed by oxaliplatin treatment. Notably, caspase-3/7 activity was induced only in this protocol, while other treatments showed activity at control levels.

Conclusions: In conclusion, dehydration followed by oxaliplatin treatment activates caspase-3/7, suggesting a potential new therapeutic strategy for peritoneal malignancies.

Keywords: direct chemotherapy, oxaliplatin, caspase 3/7, peritoneal malignancies, dehydration, HT-29 cell line

LEUKOCYTE RESPONSE TO MILD OXIDATIVE/NITROSATIVE STRESS: PRELIMINARY STUDY ON OZONE INTERACTIONS WITH IMMUNE CELLS

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Introduction: Therapy with ozone activates the mechanisms that are responsible for protecting the body against the overproduction of reactive oxygen species (ROS).

Aim of the study: The research aimed to explain how mild oxidative/nitrosative stress generated by ozone affects the activity of key elements of the white blood cell system (WBC).

Materials and methods: Neutrophils and monocytes were isolated from porcine blood, and each of the separately isolated subpopulations was divided into three groups: control and two experimental groups. The first experimental group was exposed to an air stream for 10 minutes, and the second to an oxygen-ozone mixture with an ozone concentration of 30 µg/ml for 10 minutes. Cell suspensions were incubated in a 24-well plate at 37°C, 5% CO_2. After one hour, neutrophil activity was assessed by measuring generation of free radicals (NO and O_2•-). Free radical activity in monocytes was examined after 24 hours.

Results: The research showed that ozonation of the neutrophil suspension reduced the production of NO and O_2•-. Monocytes after stimulation with the oxygen-ozone mixture generated significantly lower amounts of free radicals in comparison with untreated cells.

Conclusions: In conclusion mild oxidative/nitrosative stress generated by ozone affects the activity of the studied elements of WBC system, which suggests that ozone therapy may have therapeutic potential in limiting excessive inflammatory response. This may prevent damage to tissues and organs resulting from excessive pro-inflammatory activity generated by leukocytes.

Keywords: ozone; white blood cells, neutrophil, monocyte, oxidative/nitrosative stress

APPLICATION OF THE ADSRRS METHOD TO DIFFERENTIATE ESCHERICHIA COLI STRAINS OBTAINED FROM RED FOXES (*VULPES VULPES*)

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Introduction: Standard monitoring of samples for drug resistance of strains obtained from animals includes a one-sample-one-strain analysis. Unfortunately, this approach does not reveal the full level of drug resistance of strains occurring in the environment. Literature research indicates that from a single research sample it is possible to isolate strains of *E. coli* bacteria that differ in phenotypic drug resistance. One of the new approaches to the stage of isolation of resistant strains is the use of several media supplemented with different antibiotics to obtain different strains from a single sample. When using this approach, there is a danger of overestimating the results. To avoid this, an effective method of eliminating repeated strains should be used. An example of such a method is ADSRRS-fingerprinting.

Aim of the study: The aim of the study was to compare the genomic profiles of *E. coli* strains using the ADSRRS-fingerprinting method.

Materials and methods: The research material included 23 strains of *E. coli* obtained from 7 anal swabs of red foxes. The strains were obtained as a result of direct isolation on MacConkey agar supplemented with tetracycline/streptomycin/chloramphenicol. The research procedure included: isolation of the genetic material of the strains, then restriction enzyme digestion with XbaI and BglII, ligation with appropriate adapters and PCR reaction. The amplification products were separated in 30% polyacrylamide gel.

Results: As a result, electrophoretic separation showed that all analyzed strains differed in terms of their genomic profiles. The individual profiles differed in both the number of bands and base pairs in the amplified fragments.

Keywords: ADSRRS-fingerprinting, *E. coli*, red foxes, drug resistance

QUALITY OF RAW COW'S MILK FROM RETAIL SALE

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Introduction: "Raw milk" is defined as milk produced by the secretion of the mammary gland of farmed animals that has not been heated to more than 40°C or undergone any treatment that has an equivalent effect. In Poland, the retail sale of raw milk intended for final consumers does not contravene the food law. Raw cow milk should meet the microbial criteria in terms of total bacterial count (TBC ≤ 100,000 CFU mL^{-1}) and somatic cell count (SCC ≤ 400,000 cells mL^{-1}). The fat and protein contents of non-standardised milk should not be lower than 3.5% and 2.9%, respectively. Moreover, food law obliges producers to provide reliable information on the composition of the product.

Aim of the study: The aim of this study was to assess the quality of raw cow's milk from retail sales.

Materials and methods: A total of 24 production batches of raw cow's milk were evaluated. TBC, SCC, and fat and protein contents were determined using Bactoscan FC, Fossomatic 5000 FC and MilcoScan 4000 (Foss Analytical, Denmark), respectively.

Results: Total bacteria count, somatic cell count, and the content of fat and protein were in the range of 1.6-4.6x106 CFU mL^{-1}, 509.1-664.4 thou. mL−1, 1.7-2.9% and 3.1-3.4%, respectively.

Conclusions: The study showed that raw cow milk from retail sale did not meet quality standards with regard to TBC, SCC and fat content (24/24). Additionally, the fat content was not consistent with the fat content declared in the packaging, indicating that the product was adulterated.

Keywords: raw cow's milk, retail sale, quality

MICROBIOLOGICAL STATUS OF CHICKEN HINDQUARTERS FROM RETAIL SALE - A PILOT STUDY

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Introduction: The microbiological status of chicken meat depends on the conditions under which the animals are reared, slaughtered, and processed. *Salmonella* spp. and *Campylobacter* spp. remain the most relevant hazards in the context of chicken meat safety. In addition to pathogenic bacteria, microbiological status is determined by the quantitative contamination of aerobic bacteria, *Enterobacteriaceae*, *Escherichia coli*, coagulase-positive staphylococci, and enterococci.

Aim of the study: This study aimed to determine the microbiological status of the hindquarters of chickens from retail sales.

Materials and methods: Six production batches of the product were tested for the presence of pathogenic bacteria and bacteria considered hygienic indicators. Microbiological tests were performed using standard methods for isolation, identification and enumeration of individual bacterial species.

Results: *Salmonella* spp. was not detected in any sample, while *Campylobacter* spp. was detected in four samples. Contamination of the samples by aerobic bacteria, *Enterobacteriaceae*, *E. coli*, coagulase-positive staphylococci and enterococci ranged from $6.7x10^4$ to $6.0x10^6$, from $3x10^3$ to $7x10^5$, from $1.5x10^3$ to $1.2x10^4$, from $1.9x10^3$ to $4.1x10^4$, and from $8.6x10^2$ to $7.9x10^3$ CFU mL^{-1}, respectively.

Conclusions: This study showed that chicken hindquarters were not safe with regard to *Campylobacter* spp. In addition, some batches of the product were highly contaminated with bacteria, which are considered hygiene indicators. Some bacterial contaminants can grow or survive during cold storage. Further research is needed to fully comprehend the dynamics of the growth of these types of contaminants and their impact on product shelf life.

Keywords: chicken meat, microbial load, meat quality, food safety

MILK AND REGIONAL CHEESES – MONITORING AND CHARACTERIZATION OF MICROBIOLOGICAL HAZARDS

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Introduction: *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter* spp. and other zoonotic agents were identified as the bacterial food safety hazards relevant to public health that are associated with persistence in the food processing.

Aim of the study: The aim of the study was to determine the frequency of occurrence of pathogens and their toxins such as *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter* spp., *E. coli* O157, *Yersinia* spp. and staphylococcal enterotoxins in regional milk and cheese.

Materials and methods: The study was carried out on the basis of the screening immunoenzymatic method with the use of mini Vidas analyser, and positive samples were confirmed with reference methods. Pheno- and genotypic characterization of the isolates was performed. The strains were sequenced.

Results: The presence of *L. monocytogenes*, *Campylobacter* spp., *Salmonella* spp. and *Escherichia coli* O157 in raw milk from dairy farms was found. The percentage of the positive samples was 7,18% for *Listeria monocytogenes*, 2,59% for *Campylobacter* spp., 0,47% for *E. coli* O157 and 0,12% for *Salmonella* spp. Molecular typing allowed the classification of the tested *Listeria monocytogenes* isolates into 22 clonal complexes (e.g. CC11, CC7, CC14 and CC37). The most frequently sequence types (ST) reported are ST451, followed by ST7, ST37 and ST91. *S. aureus* strains possessing staphylococcal enterotoxin genes accounted for 34.28% of all isolates. Eighteen sequence types of ST were distinguished among *S. aureus* isolates. The most frequently recorded are ST97 and ST133, followed by ST700.

Keywords: milk and cheese, microbiological hazard, pheno- and geno typing

RESULTS OF THE STUDY OF THE TOXICITY OF THE EXPERIMENTAL DISINFECTANT "RABITDEZ" DURING LONG-TERM SKIN APPLICATION ON WHITE RATS

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Taking into account the adaptation of microorganisms to disinfectants after their long-term use, the issue of developing a new and ecologically safe disinfectant, which will make it possible to carry out practical preventive work in the presence of rabbits, becomes urgent. Therefore, the work aimed to establish the toxicity level of the experimental disinfectant "RabitDez" during long-term dermal application on laboratory rats, which is planned for use on rabbit farms in the presence of animals.

The study of the dermal toxicity of the experimental disinfectant "RabitDez" during long-term use was carried out according to OECD No. 410 (Repeated Dose Dermal Toxicity: 21/28-day Study). White Wistar rats with a body weight of 240-260 g were used for the experiment. The experimental disinfectant "RabitDez" at a concentration of 2% was applied to the skin of animals of 3 experimental groups (5 rats in each) daily for 28 days: 1 experimental group - 0.5 ml/kg of body weight of the animal; 2 experimental group - 2.5 ml/kg of body weight, 3 experimental group - 5.0 ml/kg of body weight. Water was applied to the animals of the control group on the pre-prepared area of the skin.

After applying the disinfectant, hematological and biochemical studies of the blood of rats were performed, and the weight coefficients of the organs of the animals of the experimental groups were determined.

When studying the effect of the disinfectant on the body of laboratory animals, it was established that its 28-day use did not cause illness or death in laboratory rats.

At the same time, in experimental animals, an increase in the concentration of hemoglobin, the number of erythrocytes and leukocytes, the value of hematocrit, an increase in the content of total protein, the activity of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, creatinine kinase, the level of creatinine, albumin, and a decrease in the level of platelets, urea, and cholesterol were found against the background of a slight an increase in the weight ratio of the liver and a decrease in the weight ratio of the spleen, heart, and thymus.

It should be noted that the most significant difference in the level of the investigated indicators characterizing the physiological and biochemical status of animals was found in rats of the II and III groups.

Keywords: disinfectant, toxicity, rats, lethality, dermal toxicity, intragastric toxicity, blood, hematological parameters, biochemical parameters, organ weight ratios.

THE CURRENT INVASIOLOGICAL STATUS OF THE PATAGONIAN MARA BRED IN EASTERN POLAND

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Introduction: The Patagonian mara (*Dolichotis patagonum*), also called Patagonian cavy or Patagonian hare, is a large (8–16 kg) rodent of the family Caviidae, with a body length up to 69–75 cm. It has long and pointed ears, its hind legs are longer and more muscular than fore legs; the feet are flattened, resembling hooves. The mara moves with an ambling gait, it has a grey dorsal coat with a white spot on the haunch. It feeds mainly on green vegetation and fruit. The mara lives in monogamous unions for life. In the wild, it is found in semi-desert shrub areas in Argentina. It is among species classified as "Near Threatened" by the International Union for Conservation of Nature, and placed on its Red List of Threatened Species. For this reason, captive breeding is likely to play an increasingly important role in the future conservation of this species. In captivity, the animal can be found in zoos around the world. Currently, they are also kept in private breeding farms as long as their adequate welfare is maintained. However, breeders report serious health issues among maras. Despite increased interest in mara breeding, little information is available on their diseases, including parasitic infestations.

Aim of the study: The purpose of our study was to compare selected diagnostical methods and determine the parasitofauna of a mara kept in a private breeding facility in eastern Poland.

Materials and methods: Faecal samples were collected from adult (24) and young (10) maras directly from the environment just after defecation, in the morning. The samples were examined using the following methods: flotation, McMaster chamber, Stoll chamber and the sedimentation-flotation method with own modification.

Results: In the young maras, oocysts of the genus *Eimeria* predominated with varying infestation levels of 100–400 OPG. In the adults, eggs of nematodes of the family Trichostrongylidae and the genera *Trichuris* and *Capillaria* were detected. Prevalence differed depending on the method applied. In the McMaster quantitative method, only eggs of nematodes of the family Trichostrongylidae were diagnosed, and the infestation level varied from 50 to 550 EPG.

Conclusions: In summary, it was found that young maras are mainly exposed to protozoa of the genus Eimeria, while adults are exposed to nematodes of the family Trichostrongylidae. The preferred method for the parasitological diagnosis of maras is the sedimentation-flotation method with own modification.

Keywords: Patagonian mara, *Dolichotis patagonum*, parasitofauna, diagnosis

THE OCCURRENCE OF *S. LUPI*-LIKE EGGS IN A RETROSPECTIVE ANALYSIS FEACES EXAMINATION OF DOGS FROM POLAND

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Introduction: Spirocercosis is a disease occurring predominantly in Canidae, caused by *Spirocerca lupi* belongs to the order *Spirurida* of the family Spirocercidae. The heteroxenous life cycle *S. lupi* includes intermediate (coprophagous beetles) and paratenic hosts. Adult nematodes are located in nodules in the esophageal wall causing sarcoma in some cases. Typical clinical signs are regurgitation, vomiting and dyspnoea. Primary radiological lesions include an oesophageal mass, usually in the terminal oesophagus, spondylitis, and undulation of the aortic border. Diagnosis can be made by stool examination, showing characteristic small elongated eggs (11–15 × 30–38 µm) that contain L1 larvae. Due to the high specific gravity of *S. lupi* eggs, it is recommended to use both flotation methods using fluids with a SG above 1.25, as well as sedimentation methods.*S. lupi* is a cosmopolitan species, occurring mainly in regions with a warm climate, especially tropical and subtropical. The most cases of canine spirocercosis are found in Israel, Greece, Turkey, India, Pakistan, the southern regions of the United States, Brazil, Kenya and South Africa. In Poland, the invasion has so far been described only in wolves based on the analysis of the faeces of the examined animals.

Aim of the study: Retrospective analysis (2019-2024) of the occurance of *S. lupi*-like eggs in dogs from Poland based on the of feaces examination.

Materials and methods: The study was conducted based on the database of the veterinary laboratory VetDiagnostyka, the study analyzed the results of fecal examinations from 6966 dogs over 0.5 years of age, exclusively from Poland. Both sedimentation and flotation results were taken into account.

Results: In the analyzed database, 3 eggs were found that matched S. lupi eggs in morphology and size. All eggs contained a developed larva and were of the size: 14.5 x 36 µm; 11.3 x 31 µm; 14.4 x 36 µm. The overall prevalence was 0.043% (CI ±95% 0.005 – 0.091)

Conclusions: Based on thise analysis, it can be assumed that dog spirocercosis occurs in Poland, but very rarely. It should also be remembered that stool testing techniques have limited sensitivity and the actual prevalence of S. lupi may be higher. Nevertheless, to be certain, dog feces tests should be confirmed by PCR techniques and imaging diagnostics.

Keywords: *Spirocerca lupi*, dog, parasitology

CADMIUM (Cd) AND LEAD (Pb) CONCENTRATION IN THE KIDNEYS IS LOWERED IN BROILER CHICKEN FEED MIXTURES CONTAINING 20% RYE

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Aim of the study: The study aimed to determine the transfer of cadmium (Cd) and lead (Pb) to the kidneys of broiler chickens fed mixtures containing 20% of rye grains.

Materials and methods: The study was conducted on 32 broiler chickens that were divided into 4 groups: the control group (gr. K - standard feed for broilers), gr. II (feed with 20% rye), gr. III (standard feed with xylanase), gr. IV (feed with 20% rye and xylanase). The content of Pb and Cd was determined in feed and kidneys samples of broiler chickens using atomic spectrometry.

Results: In feed Cd content (mg/kg DM) amounted to 0.028 in gr II, 0.031 in gr. I, 0.034 in gr. IV and 0.052 in gr. III. The Pb content (mg/kg DM) found in fed samples were: 0.052 in gr. III, 0.062 in gr. II, 0.085 in gr. IV and 0.28 in gr. K. The lowest content of Cd in mg/kg WM. was found the kidneys in group IV (0.06), higher in the group III (0.19) and group II (0.17) and the highest in the control group (0.28). The lowest Pb content in the kidneys was found in group IV (0.27), it was higher in the control group (1.48), and in group II (3.02) , while the highest in group III (4.06).

Conclusion: Feeding broiler chickens with mixtures containing 20% of rye the reduces the transfer of Cd and Pb from feed to organs.

Keywords: rye, cadmium, lead, kidney

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BOVINE MILK WHEY PROTEINS DIET SUPPLEMENTATION POSITIVELY INFLUENCE FEMUR BONE PROPERTIES IN CADMIUM INTOXICATED FEMALE RATS

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Aim of the study: The study aimed to evaluate the protective effect of bovine whey protein and casein inclusion in the diet of rats intoxicated with CdCl2 in drinking water.

Materials and methods: 24 mature female Wistar rats were kept in standard laboratory conditions for 10 weeks. Rats were randomly divided into four equal groups: gr. 1 (+ve control) was administered CdCl2 in drinking water (19.4 mmol/l), gr. 2 (-ve control) was administered drinking water without CdCl2, these two groups were fed a basal diet for rats for 10 wks. The rats from gr. 3 and gr. 4 were fed a basal diet for the first 5 weeks of the experiment, while for the consecutive 5 weeks were fed a whey protein and/or casein supplemented diet (5.8%). Rats had free access to water and feed and were killed by a CO2 overdose at the end of the experiments. Femur bones were isolated and frozen for further analysis. The right femur mid-diaphysis was subjected to measurements. A 3-point bending test was conducted and the yield strain, yield stress, breaking strain, and breaking stress were analyzed.

Results: Cd intoxicated rats didn't show significant differences in the values of femur mass, yield load, ultimate load, stiffness, MRWT and Young modulus. However, the femur length differed significantly from the negative controls (37.1±0.5 vs. 36.5±0.6 mm) and the vertical diameter was bigger in Cd treated rats (3.31±0.13 vs 3.19±0.11 mm).

Conclusions: Bovine whey protein and casein diet supplementation positively influenced rat femur bone properties.

Keywords: Cd, whey protein, casein

ANATOMY AND PHYSIOLOGY OF EDIBLE SNAILS *CORNU ASPERSUM* SSP. IN THE VETERINARY ASPECT

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Edible snails *Cornu aspersum* ssp. are the most frequently farmed species of land molluscs. Their rapid growth rate makes their breeding profitable. Farm farming exposes these animals to numerous threats, including infectious diseases and parasite invasions. This study presents practical aspects of the anatomy and physiology of the described snails in terms of their diagnostics and early detection of pathological changes.

Keywords: *Cornu aspersum*, farmed snails

WPŁYW SUBSTANCJI HUMUSOWYCH NA MIKROBIOTĘ ŚCIÓŁKI DROBIOWEJ ORAZ OBECNOŚĆ GENÓW OPORNOŚCI NA ANTYBIOTYKI

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Przeprowadzone doświadczenie skupiło się ocenie wpływu substancji humusowych - biowęgla i torfu na mikrobiotę ściółki drobiowej oraz określenie wpływu tych dodatków na liczebność wybranych ARB, ARG i integronów (MGE). Badania przeprowadzono w Zakładzie Doświadczalnym Żywienia Zwierząt (UPP). Układ doświadczenia obejmował 3 grupy doświadczalne. Każdy kojec wyścielony został 5 cm warstwą standardowej ściółki trocinowej. Do dwóch z nich została dodana zgodnie z rekomendacjami 15% domieszka węgla drzewnego (GrW) oraz torfu (GrT). Kojec bez dodatków ściółkowych stanowił grupę kontrolną (GrK). Kojce obsadzono 10 jednodniowymi brojlerami rasy Ross-308. Chów kurcząt trwał 35 dni i odbywał się w optymalnych warunkach mikroklimatycznych. W trakcie odchowu, kurczęta nie były poddawane antybiotykoterapii. W doświadczeniu przeprowadzono analizę fizykochemiczną, mikrobiologiczną oraz molekularną ściółki. Oznaczenie mikrobiomu wykonano metodą sekwencjonowania nowej generacji (NGS). Typy *Firmicutes*, *Actinobacteria* i *Proteobacteria,* stanowiły dominującą grupę bakterii podczas całego cyklu produkcyjnego. DNA uzyskane z izolatów *E.coli i Enterococcus spp*, posłużyły również do badania liczby wybranych ARG za pomocą qPCR. Wśród ww. bakterii wskaźnikowych, odnotowano wzrost względnej liczby kopii genów (*bla*$_{TEM}$, *vanA* i *tetW ermB* oraz *sul1* i *sul2*), zarówno w GrW oraz GrT. Integrony, czyli struktury, kształtujące wielolekooporność bakterii, odnotowano w obu grupach doświadczalnych jedynie w 10 (27%) izolatach *E.coli*. W miarę upływu czasu odchowu, nieznacznie zwiększał się odczyn oraz wilgotność ściółki, przy czym najniższe pH odnotowano w GrT (pH<6,1). Analiza metagenomiczna nie wykazała związku między rodzajem podścieliska (GrK, GrW, GrT) a społecznością bakterii. Odnotowano natomiast zależność między różnymi społecznościami bakterii a czasem trwania doświadczenia. Rozwój mikrobioty był w głównej mierze determinowany przez warunki fizykochemiczne w ściółce. Zmiany liczby kopii ARG i MGE, były powiązane z biomasą bakterii. 15% dodatek torfu i węgla drzewnego do podścieliska, poprawiał właściwości fizykochemiczne ściółki, ale nie był skuteczny w ograniczaniu antybiotykooporności.

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SESSION OF THE CLINICAL VETERINARY SCIENCES

CANINE VIABILITY EVALUATION AT BIRTH: THE APGAR SCORE

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Because of the high perinatal mortality rate in dogs and the important role played by birth and neonatal adaptation in newborn puppy survival, the availability of practical methods for assessing neonatal viability and providing adequate assistance is pivotal.

The assessment of newborn puppy viability can be performed by evaluating the APGAR score (AS), in which five clinical parameters are measured with the use of only simple instruments (stethoscope, timer), providing a final score indicative of the viability class. The AS systems specific for the newborn dog has been firstly described in 2009, and after then used by many authors.

Although the most relevant strengths of the AS evaluation in newborn dogs are its practicality and its role as a short (and long) term prognostic factor, from a clinical standpoint, the AS is very useful for classifying the viability of each newborn allows tailored assistance/resuscitation, improving survival. During neonatal assistance/resuscitation, the AS is also useful to assess the response to assistance/resuscitation. Recognized factors influencing the AS, besides the breed and the body size, are the time of measurement after birth, the anesthetic protocol for puppies born by Cesarean section (CS), and the type of whelping (spontaneous, assisted, emergency CS, elective CS).

In conclusion, although further useful tools for neonatal assessment and assistance will be provided in dogs as in humans, at present, AS represents a simple, practical, and irreplaceable clinical tool for the viability evaluation in canine neonatology.

Keywords: newborn dog, viability assessment, APGAR score

IMPORTANT ASPECTS OF REPRODUCTIVE EFFICIENCY IN DAIRY COWS

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In order to ensure a constant milk production, cows need to give birth to offsprings on a regular basis. This can only be achieved in a dairy herd with fertile cows. Reproductive performance is influenced by a multitude of factors which can be grouped in the following categories: genetics, feeding, husbandry and management.

Due to a negative genetic correlation between milk yield and reproductive traits, a constant decline in reproductive performance has been observed over the last decades. However, adaptations in animal breeding programs accompanied by improvements in reproductive management could level off or even reverse this negative trend.

Reproduction costs energy. Therefore, proper animal feeding is of utmost importance (enough energy, rumen compatible). Cows undergo a period of negative energy balance (NEB) in early lactation. When NEB is severe and long-lasting, it has direct negative effects on reproductive organs and function. In addition, it is a risk factor for common production diseases which in consequence have a negative effect on reproductive performance.

Improper housing conditions can cause stress due to overstocking, low social status in the herd, bad animal handling and regrouping which in turn have a negative impact on reproduction. Also heat stress, unhygienic conditions, low cow comfort and lameness have been linked to a lowered fertility.

Hormonal sychronisation programs and automated activity monitoring can lead to marked improvement in the service rate improving reproductive performance. Targeted reproductive management is a new concept using all available herd data to customize reproductive actions to individual cows or group of cows.

Keywords: cattle, reproductive efficiency, dairy cows

EVALUATION OF THE ANESTHESIA PROTOCOL FOR CESAREAN SECTION USING INHALATION AND EPIDURAL ANESTHESIA

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Introduction: Despite significant progress in anesthetic protocols for cesarean sections (CS), the mortality rate of newborn dogs remains high. Assessment of the puppies' viatality and the need for intensive care relies mainly on the Apgar scale. Furthermore, sedatives and anesthetics that offer maternal comfort often have adverse effects on fetuses.

Aim of the study: Developing an anesthetic protocol for CS that is optimal for both mother and newborns.

Materials and methods: The bitches undergoing elective CS were enrolled into this study. Females were assigned into: Gr I (Isoflurane), Gr IE (Isoflurane, Epidural) and GR IECo (Isoflurane, Epidural, fluid Coload). Umbilical blood was collected immediately after the puppy’s removal from the uterus but before placental detachment. The Apgar scoring system (AS) was used to score neonatal health at 0, 5 and 20 min after birth.

Results: All pups had mild acidosis with elevated pCO2, HCO3- on the lower border, lowered BE and elevated lactates. The initial Apgar score results were similar in all groups. However, the subsequent measurements revealed differences between both groups. Puppies from the IECo group received the betst AS scores at 5 and 20 min. In the IE group, up to 81% of females experienced episodes of hypotension, which was mitigated by crystalloid infusion, reducing episodes of hypotension to 33% in the IECo group.

Conclusions: The results showed that the optimal anesthesia protocol for the mother and the newborns included induction with propofol, maintenance with an inhalation anesthetic, epidural anesthesia, and fluid coload to prevent intraoperative hypotension.

Keywords: cesarean section, anesthesia, dogs

ANALYSIS OF C-REACTIVE PROTEIN IN CANINE MASTITIS

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Introduction: Mastitis is an inflammation of the mammary gland in female dogs. C-reactive protein (CRP) is a diagnostically useful marker of inflammation. The level of CRP increases in the course of inflammation.

Aim of the study: The aim of the study was to determine CRP in milk and plasma for canine mastitis diagnosis.

Materials and methods: 45 bitches were included in study, and divided into two groups: group I (control, n=10), group II contained four subgroups suffering from mastitis acuta (n=9), galactostasis (n=6), mammae congestion (n=3), mastitis gangrenosa (n=3). Blood and milk samples for CRP analysis were collected from both groups. CRP was determinated by Parra et al., 2005.

Results: Higher serum CRP concentrations were detected in bitches suffering from clinical (11.4 [5.2–81.7] µg/mL) and subclinical (21.9 [8.1–67.9] µg/mL) mastitis, when compared with healthy dogs (5.6 [3.9–8.0] µg/mL) ($p<0.05$ and $p<0.01$, respectively). In a similar manner, higher milk CRP concentrations were detected in bitches suffering from clinical (6.5 [2.8–9.2] µg/mL) and subclinical (11.3 [2.9–20.7] µg/mL) mastitis in comparison with healthy dogs (2.1 [0.1–2.5] µg/mL) ($p<0.05$ and $p<0.01$, respectively).

Conclusions: Determination of CRP in milk and serum can be useful for canine mastitis diagnosis.

Keywords: CRP, dogs, mastitis

COMPARISON OF A NEWLY DEVELOPED LAPAROSCOPIC TECHNIQUE WITH CYSTOSCOPIC ABLATION IN THE TREATMENT OF URINARY INCONTINENCE DUE TO UNILATERAL INTRAMURAL ECTOPIA OF THE URETER IN FEMALE DOGS

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Introduction: Ureteral ectopia is a rare disorder of the urinary tract, that is opening of one or both ureters elsewhere than in the urinary bladder triangle. Currently the least invasive way of treatment of urinary incontinence due to ureteral ectopy is endoscopic surgery. Classic surgical methods are feasible with incomparably less damage to the site of surgical access using endoscopy. The endoscopic techniques used in the above-mentioned pathology include the recently described laparoscopic approach (which is possible regardless of the sex of the affected dog and the type of ectopia) and the cystoscopic method (can only be used in the case of intramural ectopia). Cystoscopic laser ablation of the ectopic ureter is a removal of the wall between the ectopic ureter and the urethra as well as the urinary bladder, until the proper opening of the ureter into the bladder is achieved. An alternative for this cystoscopic ablation is a recently developed by the authors laparoscopic technique of inserting the ectopic ureter opening to the lumen of the urinary bladder. The laparoscopic ureteroneocystostomy is performed using the three-trocar technique and a basic tool set for the laparoscopic procedures.

Aim of the study: The aim of this work was to compare both techniques for treating urinary incontinence resulting from unilateral intramural ectopy of the ureter.

Materials and methods: The study included 20 female dogs with unilateral ectopy. The laparoscopic technique was used in 14 of them, and the cystoscopic approach in 6.

Results: Regardless of the method used, no short- or long-term serious postoperative complications were observed. The mean time of the laparoscopic procedures was 76 minutes, and 32 minutes for the cystoscopic ablations.

Conclusions: Comparing those two methods we concluded, that the laparoscopic approach is a very good alternative for the cystoscopic ablation in the treatment of unilateral intramural ectopy of the ureter.

Keywords: ectopia, ureter, laparoscopy, laser

PROGRESSIVE RETINAL ATROPHY IN DOGS IN TERMS OF CHOROIDAL DISORDERS

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Introduction: Progressive retinal atrophy (PRA) is a group of genotypically non-homogenous hereditary diseases reported in over a hundred dog breeds. PRA directly impacts the quality of vision, eventually leading to complete blindness.

Aim of the study: The aim of the study was to determine the thickness of the choroidal layers in dogs diagnosed with PRA using SD-OCT.

Materials and methods: The research was conducted on 47 mixed-breed dogs, divided into two groups: 22 dogs suffering from PRA, aged 2 to 12 years, and 25 healthy dogs aged 2 to 12 years – as a control group. All dogs underwent detailed ophthalmological examination, Measurements of choroidal layers: RPE-Bruch membrane-choriocapillaris complex (RPE-Bm-CC) with tapetum lucidum in tapetal fundus, medium-sized vessel layer (MSVL), large vessel layer with lamina suprachoroidea (LVLS) and whole choroidal thickness (WCT) were performed dorsally (D), ventrally (V) in the distance 5000-6000 µm, temporally (Temp) and nasally (Nas), in the distance 6500-7000 µm to the optic disc. The measurements were conducted temporally and nasally both in tapetal (TempT, NasT), and nontapetal fundus (TempNT, NasNT).

Results: In all dogs diagnosed with PRA, there was a statistically significant thinning of the MSVL in all regions (median(µm)) - control, PRA, D (32.25) (24.75), V (24.20) (15.00), TempT (49.00) (34.20), TempNT (30.00) (15.92), NasT (35.00) (22.67), NasNT (30.00) (18.52). Statistically significant thinning of LVLS and WCT – D (118.17) (163.00), V (51.00) (75.83), TempT (125.00) (164,00), TempNT (62.00) (86.00), NasT (94.67) (130.33), NasNT (70.67) (97.42) were found in all nontapetal regions.

Conclusions: PRA in mixed-breed dogs is associated with choroidal abnormalities, such as thinning of the LVLS and the WCT in nontapetal regions, and of MSVL in all regions of the fundus.

Keywords: progressive retinal atrophy, optical coherence tomography, choroid

SIDE WALKER IN HORSES: ONE SYMPTOM, DIFFERENT DIAGNOSIS – BASED ON CLINICAL CASES

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Neurologic side walker syndrome in horses may have various etiologies that might range from musculoskeletal, neurological, to a combination of conditions resulting in a specific gait. The syndrome is poorly understood and scarcely reported in the literature.

In case of side-walking horses, the trunk, pelvis, and pelvic limbs drift to one side, while the thoracic limbs are usually normal. The movement of the thoracic and pelvic limbs is, therefore, specifically incoherent.

The side walker requires examination of the CNS area and exploration the pathomechanism of changes visible in the radiological image. Imaging diagnostics is important to explain the nature of the observed neurological deficits in animals.

The study is based on two clinical cases of horses which were admitted at the Clinic of Animal Surgery of the University of Life Sciences in Lublin, Poland, with neurological symptoms – sidewinder gait. Clinical examination, blood test, CT scan of head and neck as well as postmortem autopsy confirmed two entirely different diagnosis.

In case of the first horse, the changes observed posthumously and macroscopically indicated an advanced and intense chronic inflammatory process in sinus and meninges with brain infection. In case of the second horse, glioma invading the cerebellum and spreading to the guttural pouch was notified.

We can draw then the following conclusions:

- the side walker can be considered in differential diagnosis as a potential cause of non-specific neurological symptoms,
- it seems also appropriate to perform a CT scan to determine the cause of the side walker symptom.

CARDIAC TUMORS IN DOGS AND CATS: A RETROSPECTIVE CASE REPORTS (2022-2024)

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Introduction: Tumors involving heart have been considered infrequent in dogs and cats. They occur both as primary and as metastatic lesions and can be classified according to histological characteristics. The most common type of cardiac tumor is hemangiosarcoma.

Aim of the study: The study was conducted to determine the incidence of heart tumors in the dog and cat population, and to characterize the associations between the occurrence of tumor and age, sex or breed.

Materials and methods: The study regarded 814 dogs and cats referred for cardiology consultation due to the presence of murmurs during auscultation, signs of heart failure and before anesthesia. Each patient has a thorough medical history and echocardiography. In some animals, cytological or histopathological examination was performed.

Results: Cardiac tumors were diagnosed in 11 dogs (1.35% total population). The following tumors were reported: hemangiosarcoma (6 dogs), chemodectoma (2 dogs), mesothelioma (2 dogs), pericardial neoplasia (1 dog). Chemodectoma have been associated with French Bulldog. Cardiac tumors occured in dogs, aged 7 - 14 years. The interpretation is limited by the small group of dogs with cardiac tumors.

Conclusions: The incidence of cardiac tumors in population of dogs and cats is low. In veterinary practice, this diagnosis is still an incidental finding. However, the greater availability of imaging studies and increasing middle-aged canine and feline population (due to a longer life expectancy) may have contributed to the increased diagnosis of cardiac tumors in everyday veterinary practice and may influence prognosis, treatment and outcome.

Keywords: cardiac tumors, chemodectoma, dogs, hemangiosarcoma, mesothelioma

EFFECT OF PROBIOTIC SUPPLEMENTATION DURING THE TRANSITION PERIOD ON PROTEIN METABOLISM, MAMMARY GLAND STATUS, MILK COMPOSITION AND PERFORMANCE IN DAIRY COWS

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Introduction: Dairy cows experience significant metabolic changes as a result of calving, the start of lactation and changes in their diet. During this period, a decrease in appetite, stress, immunosuppression, and negative energy balance are associated with a high incidence of perinatal metabolic diseases, infectious diseases and mastitis.

Aim of the study: The aim of the study was to determine the effect of probiotic supplementation during the dry period and early lactation on general health, protein metabolism, the occurrence of mastitis, milk composition and milk yield.

Materials and methods: The study included 12 dairy cows (6 experimental groups and 6 control groups) from a herd of 62 cows. For cows of the experimental group, a probiotic containing *Lactobacillus plantarum*, *Lactobacillus casei* 5 x 10^6 cfu/ml, *Saccharomyces cerevisiae* 3 x 10^3 cfu/ml at a dose of 100 ml 1 x daily starting from 2 months before parturition to 3 months after parturition was fed daily. The cows of the control group received feed without a probiotic. Blood for testing was collected for 8 weeks and 2 weeks before the calving and 1-2 weeks, 3-4 weeks, 7-8 weeks and 11-12 weeks post partum. Haematological examination and examination of protein metabolism parameters were performed.

Results: In the cows of the experimental group, a significant increase WBC, RBC, the concentration of haemoglobin and hematocrit was revealed in 1-2 weeks after calving, an increase in serum protein and globulin levels in 3-4 weeks after calving. In the experimental group, 2 cows suffered from mastitis, while in the control group, mastitis affected 4 cows. In cows in the experimental group, the fat content in milk at 1 and 2 months after parturition was higher, while milk yield was 12% lower.

Conclusions: The use of a probiotic during the transition period improves the overall health of cows, affects protein metabolism, reduces the number of cases of mastitis and increases the fat content of milk.

Keywords: transition period, probiotic, mastitis, protein metabolism

Cr, As, B, Al, AND Co LEVELS IN PRESCRIPTION AND NON-PRESCRIPTION CAT AND DOG FOODS

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Introduction: Providing the adequate elements ingredients and avoiding undesired potentially toxic metals in pet foods are essential for pet food industry. As chromium (Cr), cobalt (Co), and boron (B) involve in many metabolic processes, arsenic (As) and aliminium (Al) are considered as a toxic elements and reveal a potential health risks for cats and dogs.

Aim of the study: In this study, it was aimed to determine was aimed to determine aimed to determine the Cr, As, B, Al, and Co levels in cat and dog foods and compare their concentrations in in prescription and non-prescription food types.

Materials and methods: A total of 84 cat foods included gastrointestinal (n=8), renal (n=12), urinary (n=8), obesity (n=16) and non-prescription (n=40); and 152 dog foods included gastrointestinal (n=20), renal (n=8), urinary (n=8), obesity (n=4), hepatic (n=4), dermatologic (n=20), joint (n=12), non-prescription (n=76) foods from different flavors and brands were used. In each food group, Cr, Co, B, As and Al concentrations were measured by ICP-OES.

Results: Mean Cr and Al concentration in dog foods were significiantly higher compare to cat foods ($p<0.001$). In renal dog diets, mean Cr was lower than obesity ($p<0.05$), dermatologic ($p<0.05$) and non-prescription diets ($p<0.001$); mean Al was lower in urinary than gastrointestinal ($p<0.05$) and non-prescription diets ($p<0.01$); was higher in non-prescription than urinar diet ($p<0.01$).

Conclusions: Cr and Al levels in prescription and non-prescription diets may differ significiantly in dog foods. As a toxic element, dog food seems to be more contaminated with Al than cat foods.

Keywords: pet foods, elements, prescription, cat, dog

THE INFLUENCE OF PROBIOTICS ADMINISTRATION ON SELECTED PARAMETERS OF THE IMMUNE SYSTEM IN THE PERIPHERAL BLOOD OF HOLSTEIN CALVES

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Introduction: In cattle breeding, effective prevention of calf diseases is a major challenge. For the first weeks after birth, the calf relies on immune protection obtained from colostrum, the quality of which is not always sufficient. Recently, there has been increasing interest in the use of probiotics as feed additives. The use of probiotics as non-specific immune stimulants is becoming increasingly popular, but their effect in calves requires further investigation.

Aim of the study: The objective of the study was to assess the phagocytic activity and intracellular killing of phagocytic cells and selected leukocyte subpopulations in calves after the use of a probiotic.

Materials and methods: The study included 20 calves divided into 2 groups (10 calves each). The experimental group consisted of calves receiving a probiotic as a feed supplement. In the control group, the calves did not receive the probiotic. In addition, the animals in the experimental group were derived from cows that had also received a probiotic during pregnancy. The material for the study was blood taken from the calves four times: 48 hours after birth and then 21, 60 and 120 days after birth. Lymphocyte immunophenotyping was performed using a flow cytometer, and the phagocytic activity of granulocytes and monocytes and the intracellular killing capacity of neutrophils were assessed. Serum amyloid A levels were also measured using a commercial ELISA kit.

Results: Phagocytic activity and phagocyte killing were shown to be significantly higher in experimental group calves at 48h postpartum. These differences may mean that the administration of probiotics to pregnant cows had a significant effect on increasing immune cell activity not only in the cows themselves, but also in the calves born to these cows. The use of probiotics as a nutritional supplement in calves after birth is also justified, as calves receiving the probiotic showed significantly higher phagocytic and phagocyte killing activity and a higher percentage of TCD4+, BCD21+, BCD25+ lymphocytes and β2 (CD18+) and αM (CD11b+) integrins, throughout the experiment, compared to control calves.

Conclusions: Probiotics have an immune-stimulating effect in Holstein calves.

Keywords: calves, probiotics, neutrophils, fagocytosis

PRIMARY RETROPERITONEAL CYSTIC MYXOID LIPOSARCOMA IN A DOG – CLINICOPATHOLOGICAL FINDINGS

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Introduction: Soft tissue sarcomas (STSs) are a heterogeneous group of tumors that arise from mesenchymal tissues. Most STSs are solitary tumors in middle-aged to older dogs. Myxoid liposarcomas are locally aggressive and classified as STSs of fibroblast and lipoblast origin with an abundant myxoid matrix. The most frequently reported location of myxoid liposarcoma is the subcutaneous tissue in dogs, however, it can also be diagnosed in the abdominal cavity.

Aim of study: To report the clinical characteristics in a dog with histologically confirmed STS originating from the retroperitoneal space.

Material and methods: A 15-year-old Siberian Husky dog presented with a 2-month history of abdominal distension, dysphagia, chronic inflammation in blood and difficulty moving. Ultrasonography revealed a nonuniform echogenic mass occupying the abdominal cavity. On X-ray picture tumor had filled the entire abdominal cavity with displacing adjacent organs and tumor mass effect. After surgical excision, the tumor was pedunculated, encapsulated, multicystic, measured 35×30×15 cm, weighed 8 kg, and was submitted for histopathological examination. Tumor invasion was not observed. Although the dog was euthanized due to abdominal hemodynamic disturbances 227 days after surgery, its general condition maintained until death.

Results: Immunohistochemically, tumor cells were positive for vimentin and S-100 protein and negative for cytokeratin. The tumor mass was diagnosed as cystic myxoid liposarcoma with chondroid metaplasia.

Conclusions: Primary retroperitoneal myxoid liposarcoma is a rare canine neoplasia that has a significant impact on abdominal hemodynamics and may remain undetected until the tumor becomes very large.

Keywords: canine, retroperitoneum, myxoid liposarcoma, immunohistochemistry

SERUM IRON LEVEL IN BITCHES UNDERGOING SPAYING

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Introduction: The serum level of iron (Fe) can be used as one of the criteria of assessing the health status of dogs, effectiveness of ovariohysterectomy as a method of spaying in female dogs as well as the course of the postoperative period.

Aim of the study: The aim of the study was to evaluate the dynamics of serum Fe level in dogs undergoing ovariohysterectomy (OHE).

Materials and methods: Seventeen bitches of different breeds, ages, body weight, and body condition score were included into the study. Blood samples were collected before surgery (D0), 3 (D3) and 10 (D10) after surgery. Iron concentrations were assayed using the immunoturbidimetric test designed for determinations in humans and validated for dogs.

Results: Before surgery, the level of Fe was within the normal physiological limits for dogs and revealed 31.24 µg/L (±12,98). These recorded values were within the normal physiological limits. 3rd day after OHE the level of Fe significantly decreased compared with D0 and revealed 15.57µg/L (±9,16) ($p < 0.05$).

Conclusions: The local inflammatory process resulting from tissue discontinuity leads to a reduction in serum levels of iron. Fe could be classified as a marker of severe injury (surgery procedure).

Keywords: iron, dogs, ovariohysterectomy

THE MOST COMMON DENTAL CASES OCCURRING IN DOMESTIC RABBITS (*ORYCTOLAGUS CUNICULUS*)

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Rabbits are very often kept as companion animals. They are characterized by constant tooth growth (hypselodontism). Dental problems are common reason for veterinary visits.

We can distinguish between congenital and acquired defects. Congenital defects occur mostly in young rabbits and mainly affect the incisor teeth. Acquired defects may affect each tooth or many teeth at the same time.

The most common congenital defects are overgrowth of the crowns of the cheek teeth (so-called exostosis or dental spines). They predominantly affect teeth P2-M1 in the mandible and P1-M1 in the maxilla. Improper tooth growth may lead to injuries to the cheek mucosa and tongue.

Another acquired defect is retrograde hypertrophy and deformation of the apexes inside the sockets. It most often affects the maxillary incisors. By causing obstruction of the nasolacrimal duct, it may lead to lachrymation and inflammation within the structures of the duct and the eye. Acquired defects also include periapical abscesses of tooth roots. Abscesses most commonly occur in the final phase of chronic inflammation of the periodontal space. They cause deformation of the skull bones and require surgical treatment involving the extraction of a tooth or teeth and the removal of an abscess.

Dental treatment includes dental corrections, surgical and pharmacological treatment. An X-ray examination of the oral cavity under general anesthesia is also necessary. Due to the sensitivity of rabbits to antibiotics that penetrate well into bones (toxic effect), treatment is long-term. Moreover, due to the constant growth of teeth, some patients require dental corrections even every month for the rest of their lives.

Keywords: rabbits, dental problems, abscesses

CANNABINOIDS IN THE COMPLEMENTARY TREATMENT OF CANINE MAMMARY TUMOURS – PROGRESS AND PROMISE

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Introduction: Cannabinoids are a family of unique compounds synthesised by *Cannabis sativa* (marijuana). Different plant-derived cannabinoids and cannabis-based pharmaceutical drugs have been the subject of research for their potential antitumor activity. The mechanism of action of cannabinoids is through CB1 and CB2 receptors. Several tumour types express cannabinoid receptors in a manner related to the degree of anaplasia and grade of the tumour.

Aim of the study: The aim of the study was to investigate the CB2 receptor immunoexpression in benign and malignant canine mammary tumours (CMT) in comparison with immunoexpression of the MIB-1 proliferative marker and a grade of malignancy.

Materials and methods: Samples of formalin-fixed, paraffin-embedded canine normal mammary gland (n=5), mammary adenomas (n=5), and mammary adenocarcinomas (n=15) were immunohistochemically labelled and examined. Monoclonal antibodies directed against CB2 receptor and MIB-1 were used. The number of positive cells and the intensity of the reaction were taken into account in order to assess CB2 and MIB-1 immunoreactivity. Histological malignancy grade was asseseed based on mitotic counts, nuclear pleomorphism, and tubule formation.

Results: CB2 immunoexpression was evident in all cases, although differences in the number of positive cells and the intensity of the reaction were shown. CB2 immunoexpression was higher in mammary adenocarcinomas and correlated with the MIB-1 index and histologic malignancy grade.

Conclusions: Our results are consistent with other cannabinoid studies in humans and animals and offer preliminary insight into the use of cabaminioids for the complementary treatment of CMT.

Keywords: cannabinoids, CB2 receptor, canine mammary tumours

COMPARISON OF SERUM PROTEIN PROFILES OF *BORRELIA BURGDORFERI*-POSITIVE BERNESE MOUNTAIN DOGS AND DOGS OF OTHER BREEDS USING MALDI-TOF TECHNIQUE

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Introduction: Lyme disease is a multi-organ disease caused by *Borrelia burgdorferi* sensu lato transmitted by *Ixodes* ticks. Bernese Mountain Dogs more often test positive for antibodies against Bb in rapid diagnostic tests without showing any clinical symptoms of the disease.

Aim of the study: This study aimed to determine if the proteome analysis of healthy BMDs' serum could identify specific proteins responsible for positive seroreactivity for Bb. The serum protein profiles of BMDs with positive Bb reactions in snap tests were compared to those of other breeds using MALDI time-of-flight (MALDI-TOF) mass spectrometry.

Materials and methods: The study involved five groups of dogs:
1. Six BMDs with Bb-positive snap test results and clinical borreliosis symptoms.
2. Ten Bb-positive BMDs without borreliosis symptoms.
3. Ten clinically healthy BMDs with negative Bb snap tests.
4. Five dogs of various breeds, Bb-positive with borreliosis symptoms.
5. Ten healthy dogs of different breeds, Bb-negative in snap tests.

Results: Serum samples from all dogs underwent proteomic testing. Mass spectrometry revealed additional protein fractions of approximately 7,630 and 15,260 kDa in all Bb-positive serum samples, regardless of symptoms. These proteins were absent in Bb-negative serum samples, suggesting these fractions can differentiate between seropositive and seronegative dogs for B. burgdorferi.

Conclusions: Consequently, these protein markers may be used to indicate seropositivity in dogs.

Keywords: *Borrelia burgdorferi* s.l., *Ixodes* ticks, Bernese Mountain Dogs, MALDI-TOF

CHANGES IN CHOSEN IMMUNOLOGICAL AND HEALTH PARAMETERS IN CALVES BORN BY LAME DAIRY COWS

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Introduction: Lameness in cattle is a multifactorial health problem, which have impact on cows behaviour and welfare. The presence of stress in lame cows has significant influence on development of fetus. Lameness in cows can cause poor transfer of colostrum nutrients by newborn calves. The results of this condition are also low levels of serum immunoglobulins and cells involved in calves immune response.

Aim of the study: The aim of the study was to evaluate health and physiological parameters of calves born by cows with lameness.

Material and methods: The research was conducted on 45 Holstein-Fresian calves born by 35 cows with various degrees of lameness 1(healthy cows), 3, 4 and 5. The birth body weight and average weight gain during the first month of rearing were determined in calves. We also verified the results of passive immunoglobulin transfer (total IgG level), acute phase response parameters, like haptoglobin and SAA conc. and proinfammatory response by evaluation of chosen cytokines level.

Results: The results confirmed that lameness degree in pregnant cows causes significant decrease of birth weight of calves (average 1.9 kg). The ABW was 39.3 ± 2.2 kg for calves born by lame cows, and 40.8 ± 3.6 kg for calves born by cows without lameness. These calves had also lower average weight gains during the first month of life. The significant differences ($p \leq 0.05$) were observed in IL-1, IL-6 concentraction in calves born by cows with lameness. Changes of Hp levels in calves born by cows with lameness pointscale 3-5 were significant ($p \leq 0.05$) in comparison to calves born by healthy cows. The SAA results were not statistically important. Passive immunity transfer at 48 hours of birth in calves born by cows with lameness was lower and more ferequently observed (7/22 calves). No significant differences was observed in cortisol concentration among calves born by cows with lameness. We also observed reduction of IgG conc. (<50 mg/L) in colostrum obtained form higly lame cows (4-5). In those groups of cows we detected higher frequency of twin calves birth (7 twins/15 cows).

Conclusions: We confirmed significant influence of lameness in cows on immunological parameters in calves i.e. colostrum immunoglobulin transfer, cytokines and Hp level. High correlation (r=0.7) between degree of lameness to calves birth weight, confirms the significant impact on fetus development.

Keywords: lameness, cattle, dairy cows

DETECTION AND GENETIC CHARACTERIZATION OF EQUINE RHINITIS VIRUSES AMONG HORSES IN POLAND

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Introduction: Equine rhinitis A (ERAV) and B (ERBV) viruses are respiratory pathogens with worldwide distribution.

Aim of the study: The objective of the current study was to estimate the prevalence of infections with ERAV and ERBV among Polish horses and to determine genetic variability within them.

Materials and methods: Virus-specific quantitative RT-PCR assays were used for the detection of ERAV and ERBV in nasal swabs collected from 621 horses from 16 national horse studs located throughout Poland. Viral RNA from virus-positive swabs was used as a template in a nested RT-PCR assay. Amplified products were subjected to electrophoresis, purified and ligated. Then, ligated products were transformed into competent cells. Plasmid DNA was isolated from five separate insert-positive clones and sequenced using Sanger method. A phylogenetic tree was constructed using the maximum likelihood method with 1000 bootstrap replicates in MEGA11. The median-joining haplotype networks were generated in PopART version 1.7.

Results: Overall, none of the nasal swabs were positive for ERAV, whereas ERBV was found in 11/621 (1.78%) samples. Out of 68/621 (11.0%) horses sampled with clinical signs of respiratory disease, the RNA of ERBV was detected in one (1.47%) swab. Based on phylogenetic and network analysis, the ERBV sequences clustered within seven groups, with further sub-clustering within group 1. Analysis of molecular variance indicating that the sequences variability was correlated with individual horses and studs from which samples were collected.

Conclusions: The report presents the first detection of active ERBV infections among Polish horses.

Keywords: Equine rhinitis viruses, ERAV, ERBV, horses, sequence analysis, haplotype network

STĘŻENIE WYBRANYCH CHEMOKIN (CXCL8, CCL2, CXCL12) W OSOCZU SUK Z NOWOTWORAMI ZŁOŚLIWYMI GRUCZOŁU SUTKOWEGO BEZ PRZERZUTÓW ORAZ Z PRZERZUTAMI

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Nowotwory złośliwe gruczołu sutkowego suk wykazują wiele podobieństw do raka piersi i często dają przerzuty do regionalnych węzłów chłonnych oraz narządów wewnętrznych, głównie płuc. W ostatnim czasie liczne badania wskazują, że w progresji raka piersi i innych nowotworów złośliwych u ludzi istotną rolę odgrywają chemokiny i ich receptory. Szczególne znaczenie w postępie choroby nowotworowej i procesie formowania się przerzutów przypisuje się chemokinom CXCL8, CCL2 i CXCL12. W odniesieniu do nowotworów złośliwych gruczołu sutkowego u suk brakuje badań nad potencjalną rolą tych chemokin w progresji choroby i procesie przerzutowania. Celem prezentowanych badań była ocena stężenia chemokin (CXCL8, CCL2 i CXCL12) w osoczu suk ze złośliwymi nowotworami gruczołu sutkowego bez przerzutów i z przerzutami. Badania przeprowadzono na 25 sukach z nowotworami (15 zwierząt z nowotworami bez przerzutów i 10 z przerzutami) oraz 10 sukach zdrowych. Stężenie badanych chemokin określano przy użyciu testów ELISA swoistych dla psów. Średnie stężenie CXCL8 w osoczu, zarówno u suk z nowotworami złośliwymi gruczołu sutkowego, dającymi przerzuty, jak i bez przerzutów, było istotnie wyższe niż u suk zdrowych. U suk z przerzutami stężenie CXCL8 było istotnie wyższe niż u suk bez przerzutów. Średnie stężenia CCL2 i CXCL12 u suk z przerzutami były istotnie wyższe niż u suk zdrowych i wyraźnie (choć statystycznie nieistotnie) wyższe w porównaniu do suk bez przerzutów. Uzyskane wyniki wskazują, że progresja nowotworu złośliwego w gruczole sutkowym suk i tworzenie przerzutów są związane ze zwiększoną produkcją chemokin CXCL8, CCL2 i CXCL12, co z kolei prowadzi do wzrostu ich stężenia w osoczu krwi.

Słowa kluczowe: chemokiny, CXCL8, CCL2, CXCL12, nowotwory złośliwe, gruczoł sutkowy, suki.

TUBERCULOSIS DUE TO *MYCOBACTERIUM BOVIS* AT FARMER-CATTLE INTERFACE ON THE FARM, POLAND

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Introduction: Tuberculosis (TB) is one example of a zoonotic disease. In 2020, TB was second only to COVID-19 in the top ten infectious causes of death in human. Research results confirm that human TB has been encountered on all continents for millennia. Tuberculosis is caused by bacteria (mycobacteria) belonging to the *Mycobacterium tuberculosis* complex (MTBC), which differ in morphological and biochemical properties, gene sequences and drug resistance features. This is the first documented case of common source of infection with *Mycobacterium bovis* in Poland.

Aim of the study: The aim of this study was to confirm transmission between farmer and cattle in one farm and provide a high-level overview of what is known about tuberculosis transmission.

Materials and methods: The patient had a pulmonary form of TB accompanied by a cough with haemoptysis and a high fever, which intensified mainly in the evenings. At the same time, the disease was also confirmed in 20 out of 25 cows on the farm. The clinical specimen (sputum) was examined in accordance with the European Union (EU) laboratories' methodology. Tissue materials (retropharyngeal, mandibular, bronchial, mediastinal, mesenteric, and supramammary lymph nodes) from animals were verified with the guidelines for the laboratory diagnosis of bovine tuberculosis (BTB).

Results: All *Mycobacterium bovis* isolates represented one spoligotype, SB0120 assigned by www.Mbovis.org and Bov_1 482 assigned by SITIVIT. The results of mycobacterial interspersed repetitive unit variable number tandem repeat (MIRU-VNTR) evaluation showed the same genetic pattern.

Conclusions: Diagnostic and financial limitations in identifying *M. bovis* and *M. caprae* in human medical laboratories and the number of cases of *M. bovis* and *M. caprae* tuberculosis in most countries of the world do not reflect the actual case number. Although the location of BTB outbreaks and possible routes of BTB transmission are known, the epidemiological map of this zoonotic disease may be mostly underestimated.

Keywords: tuberculosis, *Mycobacterium bovis*, cattle, zoonoses

ENCEPHALITOZOONOSIS IN GUINEA PIGS

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Encephalitozoon spp. are pathogenic fungi classified in the genus *Microsporidia*. They produce spores that have a very long survival time in the environment. Their presence has been found in the secretions and excretions of many wild and domestic mammalian species as well as birds, reptiles, amphibians and fish.

The main vectors of the fungus are companion animals that are commonly kept in home, mainly rabbits. In the rabbit population, it has been reported that about 70% of animals are carriers of *Encephalitozoon cuniculi*. It should be noted that all *E. cuniculi* genotypes described so far, are able to infect both immunocompetent and immunodeficient people. Thus, with the increasing popularity of rodents and rabbits as companion animals, the risk of infection with this pathogen in human is also increasing.

The clinical signs of encephalitozoonosis in both animals and humans are non-specific. The most frequently observed disorders in animals are problems with urinary tract, neurological symptoms and visual impairment.

The study included 100 guinea pigs that were patients of the Small Mammal Ambulatory at the University of Life Sciences in Lublin. In 73 of them, the PCR test was positive. The aim of this study was to determine the prevalence of *E. cuniculi* infection in guinea pigs and how symptoms may indicate the development of encephalitozoonosis in this species. The research results showed that: urinary, nervous and ocular symptoms were most frequently observed in animals. The high prevalence rate in domestic cavies indicates that they may be a source of infection for humans as well as rabbits.

Keywords: *Encephalitozoon cuniculi*, guinea pigs, PCR, encephalitozoonosis

CRICKET PARALYSIS VIRUS (CrPV)

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Cricket paralysis virus (CrPV) was discovered in wild Australian crickets (*Teleogryllus commodus* and *Teleogryllus oceanicus*) and then became the cause of a fatal disease in domestic crickets (*Acheta domesticus*). The virus is non-enveloped, spherical, 27 nm in diameter, and its genetic material is single-stranded RNA. It causes the death of up to 95% of the population in cricket farms, although it has also been found in other insect species. It causes asymptomatic infections in bees. The route of infection is the tissues of dead crickets (cannibalism), water contaminated with feces, litter and feed. The disease spreads quickly, causing falls a few days after infection. Symptoms include loss of coordination, then characteristic lying on the back with symptoms of progressive paralysis and then death. Insect bodies are pale yellow, dissection often reveals tissue liquefaction. The disease causes very large losses in insect breeding; unfortunately, no treatment methods are known, although diagnostic methods are constantly developing.

Keywords: cricket, cricket paralysis virus, CrPV

ASSESSING THE IMPACT OF PROPOFOL AND ISOFLURANE ON THE CANINE CHOROID WITH THE USE OF SD-OCT

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Introduction: SD-OCT is a non-invasive method that facilitates cross-sectional, in vivo imaging of the choroid, an anatomical structure with a primary function of supplying oxygen and nutrients to the retina.

Aim of the study: The study aimed to determine the impact of propofol and isoflurane on the canine choroid as a consequence of their effect on the cardiovascular system.

Materials and methods: The study was performed on 9 beagles aged 4-6 years. The dogs were anaesthetized with propofol i.v. followed by isoflurane. All dogs underwent cardiovascular and ophthalmic examinations including SD-OCT before administration of the anaesthetic agents (Control), during 40-minute propofol infusion (Propofol), during 35-minute isoflurane administration (Isoflurane) and 10 minutes after isoflurane discontinuation (Recovery). Measurements of choroidal layers: RPE-Bruch membrane-choriocapillaris complex with tapetum lucidum in tapetal fundus, medium-sized vessel layer, large vessel layer with lamina suprachoroidea and whole choroidal thickness (WCT) were performed dorsally, ventrally, temporally and nasally to the optic disc. The measurements were conducted temporally and nasally both in tapetal, and nontapetal fundus. Echocardiography was performed and mean arterial pressure, blood flow through the aorta and pulmonary artery were measured.

Results: The study showed a statistically significant ($p<0,05$) decrease in diastolic blood pressure in Propofol [Mean±SD] (61.67±11.87), and both systolic (105.33±17.36) and diastolic (63.00±14.87) blood pressure in Isoflurane comparing to the Control (systolic 124.33±16.92) (diastolic 80.78±22.44). Changes in blood pressure during anaesthesia with propofol and isoflurane were not accompanied by a statistically significant difference in the thickness of the individual choroidal layers and WCT.

Conclusions: Propofol and isoflurane do not affect the thickness of choroidal layers and WCT.

Keywords: propofol, isoflurane, anaesthesia, choroid, SD-OCT

SESSION OF YOUNG SCIENTISTS

THE HORSE, A MULTI FUEL DEVICE: ENERGY SOURCES AND UTILIZATION

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Just like all other animals, the horse is a thermal machine, which stores, processes and uses energy for all maintenance processes and for all production. In addition to this, the horse has the possibility of using multiple substrates as energy sources. This feature is very useful in work, since depending on the type of exercise, some energy sources will be more effective than others.

In this talk, the main energy sources used by the horse will be examined, the implications of using different energy substrates for work, and the importance of nutritional factors in the energy transformations that take place in the organism.

NUTRACEUTICAL POTENTIALS OF GREEN MICROALGAE

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Green microalgae are single-celled eukaryotic organisms that, in recent years, are becoming increasingly important in the nutraceutical, cosmetic, and pharmaceutical fields because of their high content of bioactive compounds. In recent years we have been involved in some projects dedicated to characterizing the chemical profile and evaluating the antioxidant and anti-inflammatory effect of some microalgae obtained from the freshwater lakes of the Ecuadorian Highlands, such as *Ettlia pseudoalveolaris* and *Chlamydomonas agloeformis*. Furthermore, we also characterized and evaluated its interaction with drug metabolism and a possible protective effect against chronic obstructive pulmonary disease (COPD) of *Chlorella vulgaris*, that is a microalgae already widely known and used as a food source.

FENTANYL: PAIN-KILLER OR MAN-KILLER?

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Fentanyl is a potent agonist of the mu opioid receptors 100 times more potent than morphine. It possesses a strong analgesic activity and it is used in controlling pain in dogs. Its use in veterinary medicine is almost always extra-label.

Its routes of administrations are intravenous, subcutaneous, transmucosal (human derived) and recently a veterinary-only transdermic formulation has been issued. The differences in these formulations bring to pronounced differences in fentanyl pharmacokinetics and consequently differences in dose regimens. The therapeutic index of this drug is not wide and caution should be used in its administration.

Fentanyl belongs to the class of opioid drugs and can produce tollerance and addiction. Indeed, over last two decades fentanyl has been known from the general population more for its recreactional drug (heroin replacement) and for its affordable cost and strong effects has had a wide and quick way in to the black market. Currently, the United States faces the most serious situation related to fentanyl overdose, commonly referred to as the opioid epidemic and European countries are following. Nowadays, fentanyl is considered as the number one cause of death for adults aged 18–45.

IMPACT OF PHYTOBIOTICS ON OXIDATIVE-ANTIOXIDATIVE STATUS IN LAYING HENS: A COMPARATIVE STUDY

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The use of phytobiotics in poultry nutrition is gaining increasing interest due to their potential to improve production efficiency and animal health.

This study aimed to determine the effects of various phytobiotics on the oxidative-antioxidative status in the liver, breast muscle, and plasma of laying hens. Laying hens were divided into four groups: T1 (control group fed standard feed), T2 (feed enriched with medium-chain fatty acids C6 to C9), T3 (feed with encapsulated essential oils: carvacrol, cinnamaldehyde, and capsaicin), and T4 (feed with a mixture of polyphenolic compounds). Tissues and plasma were collected during slaughter and analyzed for malondialdehyde content, retinoids, ubiquinone, and catalase activity.

Phytobiotics are expected to exhibit anti-inflammatory effects, with polyphenols showing the strongest antioxidant activity. While medium-chain fatty acids may improve antioxidant status through indirect metabolic mechanisms, polyphenolic compounds and essential oils demonstrate the most potent and direct antioxidant effects. Detailed results will be presented during the session proceedings. This research contributes to our understanding of how different phytobiotics influence the oxidative-antioxidative status in laying hens, potentially leading to improved poultry nutrition strategies.

Keywords: phytobiotics, laying hens, antioxidants, oxidative stress, medium-chain fatty acids, essential oils, polyphenols

VIRAL AND PROTOZOAN DIARRHOEA IN DOGS AND CATS - THE ABC OF DIFFERENTIAL DIAGNOSTIC

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Parvoviruses, coronaviruses and protozoans (*Giardia* spp., *Cryptosporidium* spp.) are established causes of diarrhoea in dogs and cats maintained as pets in metropolitan and village zones in Eastern European countries, including Poland. Veterinarians have a variety of diagnostic methods that can be used to make a differential diagnosis of the causes of diarrhoea. These methods have their advantages and limitations, knowledge of which is essential in order to perform an appropriate diagnosic procedure.

The aim of presentation is to discuss whether the immunochromatographic rapid tests and real-time PCR methods are able to detect the presence of viruses and protozoans in feces collected from dogs and cats.

Recent articles presenting results of own researches are stating that immunochromatographic tests are not able to detect the presence of vaccine viruses in faeces collected from dogs and cats, vaccinated respectively against CPV and FPV. On the other hand they showed good performance for detecting these pathogens in animals infected with field strains of these viruses.

These topics will be discussed in presentation, as it is an important area of consideration for clinically working veterinarians. The presentation will discuss recommended time to perform particular diagnostic methods and their limitations.

Keywords: CPV, CCV, FPV, FCoV, protozoan, immunochromatographic tests, PCR

STABILITY OF THE GENETIC MATERIAL OF *BORRELIA BURGDORFERI* SENSU LATO SPIROCHETES AFTER STORAGE AT LOW TEMPERATURES

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Introduction: The spirochetes *Borrelia burgdorferi* sensu lato complex are zoonotic pathogens causing Lyme disease. The main vector of Borrelia spirochetes in Europe are ticks of the genus *Ixodes ricinus*. Poland is considered an endemic area for Lyme disease.

Aim of the study: The aim of this study was to assess the durability of the genetic material of *Borrelia* spirochetes isolated in 2018-2023 and to determine its suitability for further research after storage at low temperatures.

Materials and methods: The study material consisted of isolates of the genetic material of *Borrelia burgdorferi* sensu lato complex isolated from *Ixodes ricinus* ticks in a commercial diagnostic laboratory. In the next stage, isolates stored at a constant low temperature of -80ºC were used to assess the suitability of the genetic material. Amplification of the genetic material by polymerase chain reaction (PCR) was performed in 40 cycles using specific primers. Visualization of the results was performed on a 2% agarose gel.

Results: Re-amplification showed the presence of genetic material of the spirochetes *Borrelia burgdorferi* sensu lato in the following number of samples: in 2018 (37/57; 61.4%), in 2019 (22/51; 43.1%), in 2020 (22/48; 58.3%), in 2021 (16/26; 61.5%), 2022 (7/10; 70%) and 2023 (4/5; 80%).

Conclusions: The obtained results allowed us to hypothesize that a longer period of storage of genetic material at a constant, low temperature reduces the suitability of isolates for further testing. It is suggested that, to confirm the hypothesis, a larger number of samples should be tested.

Keywords: *Borrelia burgdorferi* sensu lato, *Ixodes ricinus*, Lyme disease, PCR, vector-borne diseases

EVALUATION OF THE USEFULNESS OF *LUCILIA SERICATA* IN TOXICOLOGICAL ANALYSIS OF HEAVY METALS

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Introduction: *Lucilia sericata*, commonly known as the common green bottle fly, was selected due to its role in forensic entomology and potential applicability in bioindication studies.

Aim of the study: The study aimed to evaluate the potential of *Lucilia sericata* at different developmental stages - pupae, larvae, and imagos - as alternative materials for toxicological analysis.

Materials and methods: The research involved analyzing the accumulation and retention of heavy metals in these stages, comparing their effectiveness to traditional biological matrices such as blood and urine. Methodologies included sample preparation, chemical analysis, and statistical evaluation of data.

Results: Preliminary findings suggest that *Lucilia sericata* stages offer promising attributes for toxicological studies, including ease of collection, transportation, and storage. Further optimization of analytical protocols and comprehensive validation are necessary to establish their reliability and applicability in routine toxicological assessments.

Conclusions: This study contributes to expanding the range of biomonitoring tools available in environmental and forensic toxicology.

Keywords: *Lucilia sericata*, heavy metals, toxicology, bioindication, biomonitoring

EVALUATION OF THE USEFULNESS OF SKIN DERIVATIVES AS AN ALTERNATIVE BIOLOGICAL MATERIAL IN TOXICOLOGICAL ANALYSIS

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Currently, dominant diagnostic methods in veterinary medicine rely on blood and urine analysis, which come with specific limitations regarding the toxicokinetics of elements and the invasiveness of sample collection. Additionally, hair, nail, and feather samples can be easily collected, transported, and stored, reducing analysis costs.

Alternative diagnostic materials also enable retrospective monitoring of heavy metals due to the presence of sulfur-containing proteins that bind these elements. Analysis of such samples is characterized by low measurement uncertainty compared to bodily fluids. Despite numerous advantages, alternative diagnostic materials are not widely used in routine toxicological analysis, mainly due to issues with exogenous contamination and limited sample mass. Furthermore, optimized procedures for determining xenobiotics in these materials are lacking.

The main objective of the project was to develop and validate procedures for the determination of selected heavy metals and metalloids in alternative diagnostic materials collected from companion and wild animals, and to evaluate their effectiveness in comparison to traditional diagnostic materials such as blood and urine.

Keywords: alternative matrices, toxicology, heavy metals, metalloids

The project was funded by the Ministry of Science and Higher Education under the „Student Scientific Clubs Create Innovations” Programm.

POLYMORPHISMS OF THE 5'-UNTRANSLATED REGION OF THE GLUTATHIONE S-TRANSFERASE P1 GENE OF DOGS WITH MAMMARY TUMORS

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Mammary tumors account for more than half of neoplasms in female dogs worldwide. Early diagnosis of tumors increases the effectiveness of treatment of sick animals and requires studying of new tumor markers. These can be single nucleotide polymorphisms (SNPs) of genes particularly the glutathione S-transferase P1 gene (GSTP1).

The aim of the study was to identify SNPs in the GSTP1 of dogs (*Canis lupus familiaris*) with mammary tumors, compare the results with healthy animals and determine the association with the occurrence of tumors. The clinical stage of the disease was determined using the TNM classification of tumors, their types were determined histologically. DNA was extracted from the blood of dogs, the gene region was amplified using PCR, and the resulting products were sequenced by the Sanger method. The study focused on the 5' untranslated region (5'-UTR) and the proximal part of the promoter of the GSTP1. At the current stage of the research 7 SNPs and 3 deletions in 5'-UTR were identified. These can lead to sites of splicing enhancement proteins changing. No polymorphisms were detected in the studied part of the promoter. A comparative analysis of polymorphisms of 5'-UTR GSTP1 and tumor types is being conducted to determine possible correlations between them.

Keywords: cancer, canine, glutathione S-transferase, mammary gland, PCR.

The research was financially supported by the Ministry of Education and Science of Ukraine (0118U003495) and the Visegrad Fund (62410196).

CHANGES IN THE ACTIVITY OF BRUSH BORDER ENZYMES OF FALLOW DEER'S (*DAMA DAMA*) FED WITH BASKET WILLOW (*SALIX VIMINALIS*)

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Introduction: A species of deer called fallow deer (*Dama dama*) is now widespread in many countries, including Poland, and its population is constantly growing, both in breeding and in the wild. Fallow deer are ruminant animals that live in deciduous forests, but they can also be found in mixed and coniferous forests. A significant part of their diet consists of graminoids, grasses and herbs. In this experiment, animals were provided with leaves and upper segments of willow branches as their food source. Fallow deer may choose *Salix* sp. as a dietary option due to its desirable taste and nutritional value.

Materials and methods: Thirty fallow deer at the age of 9 months were randomly divided into control (Con) and experimental (Salix) groups. Standard breeding nutrition was used for two winter seasons. In the control group, summer feeding was based on pasture fodder. During summer grazing, the experimental group (Salix) additionally had free access to feeding plots planted with willow bark (*Salix viminalis*). The experiment was terminated by slaughtering the animals at the farm, with 6 from each group. Immediately after slaughter, the length of the intestines was measured. The intestines were divided into five sections - the duodenum, the proximal part, the middle part, the distal part and ileum. Samples were taken for further analyses and frozen at -80ºC. The intestinal mucosa was homogenised and then the total protein content and the activity of brush border enzymes (dipeptidase, aminopeptidase A and N) were determined.

Results: A statistically significant increase of aminopeptidase A and aminopeptidase N activity was observed in duodenum, proximal, middle part of intestine in deer of experimental (Salix) group compared to control animals. Analysis of aminopeptidase A and N activity in distal part of jejunum and ileum was observed tendency to increased activity in animals of experimental group.

Conclusions: The addition of willow bark (*Salix viminalis*) to the feed of fallow deer stimulates the activity of intestinal brush border enzymes, supporting the ability to digest and absorb food.

Keywords: fallow deer, *Salix viminalis*, brush border enzymes

DRUG RESISTANCE PROFILES OF COAGULASE-NEGATIVE STAPHYLOCOCCI ISOLATED FROM COWS

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Introduction: The uncontrolled use of antibiotics has led to an increase of drug resistance in pathogenic bacteria including *Staphylococcus*.

Aim of the study: The aim of the study was to determine drug resistance profiles of coagulase-negative *Staphylococcus* isolated from the nasal cavity and milk of cattle in Poland.

Material and methods: Material for research included nasal swabs (n=83) and milk samples (n=56) collected from cows from 9 herds in Poland. Species identification was performed using MALDI-TOF mass spectrometry. The disk diffusion method was used to assess the susceptibility of strains to drugs using the following antibacterial substances: penicillin, chloramphenicol, gentamicin, cefoxitin, nitrofurantoin, mupirocin, sulfamethoxazole/ trimethoprim, enroflaxacin, ciprofloxacin, erythromycin, rifampicin, linezolid, clindamycin and tetracycline.

Results: A total of 44 *Staphylococcus* were isolated, classified into 9 species (*S. haemolyticus*, *S. xylosus*, *S. arlettae*, *S. succinus*, *S. chromogenes*, *S. vitulinus*, *S. simulans*, *S. sciuri*, *S. candimenti*), with dominance *S. xylosus* (50%). Four *Staphylococcus* species (*S. xylosus*, *S. haemolyticus*, *S. arlettae*, *S. succinus*) showed resistance to 6 antibacterial substances. The highest percentage of *Staphylococcus* resistance was recorded for penicillin (15%), erythromycin (11%) and tetracycline (6%). Four strains belonging to *S. xylosus* were multidrug-resistant. They were characterized by the following resistance profiles: PEN, ERY, TET (n=1), ERY, KLI, TET (n=2) and ENR, ERY, KLI, TET, PEN (n=1). All *Staphylococcus* were susceptible to gentamicin, cefoxitin, nitrofurantoin, mupirocin, sulfamethoxazole/ trimethoprim, ciprofloxacin, rifampicin and linezolid.

Conclusions: To sum up, cows may be a reservoir of potentially pathogenic, multidrug-resistant Staphylococci, which, due to the ease of transfer of strains (and resistance determinants) between hosts, may raise concerns for public health.

Keywords: *Staphylococcus*, multidrug-resistance, cattle

THE APPLICATION OF ALTERNATIVE RESEARCH MATERIAL IN VETERINARY TOXICOLOGY

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Aim of the study: The aim of the project was to develop and validate procedures for the determination of Cd and Pb in alternative materials (skin derivatives) using atomic absorption spectrometry methods.

Materials and methods: The research material consisted of horse hair taken from the neck area and mane hair and blood serum obtained from horse blood obtained as part of a veterinary medical procedure. In order to determine the absorbed metals, the hair and fur were decontaminated. A cryogenic grinder was used to homogenize hair coat samples. The concentrations of Cd and Pb were determined by electrothermal atomic absorption spectrometry after prior mineralization. The use of liquid nitrogen ensured effective homogenization of hair coat samples.

Results: The content of Pb and Cd in the hair coat was significantly higher than in the blood serum of the tested animals. Validation parameters for the determination of Cd and Pb in hair coat: detection limit, quantification limit, recovery, accuracy and repeatability/precision of the method were 0.001 µg/g, 0.002 µg/g, 91.6%, 8.4% and 0.56% for Cd and 0.001 µg/g, 0.002 µg/g, 109%, 9%, respectively and 1.2%, respectively, for Pb.

Conclusions: Alternative materials may provide a good non-invasive indicator of animal exposure to heavy metals. Advantages of the alternative material: they do not require special conditions during transport and storage, which reduces the costs of toxicological analysis; provide long-term (retrospective) monitoring of the presence of heavy metals in animals' bodies; they ensure low measurement uncertainty related to short-term changes in the content of the substances being determined, compared to body fluids, e.g. serum.

Keywords: toxicology, heavy metals, cadmium, Cd, lead, Pb

The project was funded by the Ministry of Science and Higher Education under the „Student Scientific Clubs Create Innovations” Programm.

CITROBACTER FREUNDII ISOLATED FROM ORNAMENTAL FISH IN POLAND

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Introduction: Bacteria belonging to the species *Citrobacter freundii* are Gram-negative bacilli widespread in the environment and considered to be the etiological factor of diseases in humans and animals, including fish.

Aim of the study: The aim of this study was the isolation, identification, assessment of antibiotic sensitivity and checking the presence of virulence genes in 20 *Citrobacter freundii* isolates from diseased ornamental fish.

Materials and methods: The isolated bacteria were initially identified to the species level by MALDI-TOF-MS method. The obtained results were supplemented by a method based on sequencing of a fragment of the 16S rRNA gene and analysis of biochemical profiles using the API-20E system. The sensitivity profile of the tested isolates to 15 antibiotics from 8 different groups was also examined. Based on the PCR method, the presence of five *C. freundii* virulence factors and three tetracycline resistance genes (coding efflux pumps) was verified.

Results: The identification methods used confirmed with a very high probability that all isolated bacteria belong to the species *Citrobacter freundii*. The tested strains showed a high level of resistance to the antibiotics, which means that as many as 8/20 can be classified as multidrug-resistant (MDR) bacteria. Additionally, genetic tests confirmed the presence of virulence and antibiotic resistance factors in DNA material isolated from bacteria. Only the hlyA gene encoding the ability to produce α-haemolysins was not confirmed.

Conclusions: The obtained results indicate that diseased ornamental fish may be a source of pathogenic strains, so further monitoring of *C. freundii* in this environment is needed.

Keywords: antibiotic resistance, virulence factors, fish isolates

COMMON MISTAKES IN KEEPING RABBITS AS COMPANION ANIMALS

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Domestic rabbits (*Oryctolagus cuniculus*) are becoming increasingly popular as companion animals. Despite this, many people are unaware of the specific needs of these animals, which leads to numerous mistakes in their care.

The most common mistakes involve providing appropriate housing and diet for rabbits. The most beneficial form of housing is free-range or minimizing the time spent in the cage. Aquariums do not provide adequate ventilation or space. Systematic cleaning of the cage is crucial to avoid many diseases. Rabbits can be trained to use a litter box, which additionally helps maintain cleanliness. It is a mistake to use cat litter, shavings, or straw.

Many people use water bottles, which is a mistake, as they do not allow rabbits to drink in a physiological manner. A better solution is a bowl. Lack of designated meal areas and the presence of a hay rack and bowls can lead to food contamination with droppings. A rabbit's diet should be based on plant fiber, which enables proper digestion. Key dietary mistakes include feeding grains, drops, grain sticks, and bread. These products do not meet basic dietary requirements and can lead to dental and digestive system diseases.

Not trimming the nails can cause pain for rabbits. Lack of regular brushing can lead to the formation of hairballs, which can even be fatal.

Rabbits require specialized medical care. Lack of regular visits to the veterinarian can lead to overlooking diseases, as rabbits naturally camouflage symptoms of illness.

Keywords: rabbits, common mistakes, diet and care

NUTRITION AND CARE OF THE DOMESTIC CAVY (*CAVIA PORCELLUS*) AS A DENTAL PATIENT

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The guinea pig (*Cavia porcellus*) is kept as a companion animal. One of the most common reasons for visiting a veterinarian is loss of appetite, often accompanied by drooling and teeth grinding. These are typical symptoms of dental disease, which is frequently diagnosed in these rodents.

In addition to performing a dental procedure on such a patient, it's important to introduce an appropriate diet, depending on the type and stage of the disease. Improper feeding is one of the main reasons for acquired dental diseases. Owners often reach for treats that aren't suitable for rodents, such as grain sticks, limestone fragments, sweet vegetables, and fruits. Meanwhile, the diet of a guinea pig should be rich in plant fibers, essential for the proper functioning of the digestive system and maintaining the proper microflora. Animals with dental problems have difficulty eating, which results in receiving much less fiber from their food. In more advanced stages of the disease, pain and discomfort also prevent the intake of an adequate amount of fiber-rich foods, such as hay and dried herbs. If the animal lives in a herd, care should be taken to feed it separately or ensure that it does not consume less food than other individuals. In advanced stages of dental disease, it's important to introduce a diet that involves feeding grated vegetables and emergency food several times a day, which allows the animal to receive the necessary nutrients. It's necessary because chewing food is difficult, and in some cases, impossible.

Keywords: proper nutrition, guinea pig diet, dental diseases

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