

The 43rd Symposium of Veterinary Medicine: Animal Welfare

**Koret School of Veterinary Medicine,
The Hebrew University of Jerusalem, Israel**

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INVITED LECTURES

Hen Welfare – Where Are We Going?

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Conventional cages for laying hens were widely adopted in the second half of the 20th century and led to improved bird health in comparison with the backyard systems they replaced. However, cages also became symbolic of factory farming due to the extent to which they restricted bird movement. In Europe, following legislation which came into force in 2012, cages were replaced by colony cages, indoor barn or free-range systems, but the mix of housing systems continues to evolve. In 2019 over 1.6 million EU citizens signed a petition (“End the Cage Age”) calling for the end of colony cages. Simultaneously, a new Scientific Opinion on laying hen welfare has been commissioned, and both initiatives will underpin further planned changes in European legislation. Transitions from cages to cage-free systems are also now taking place in other countries around the world. Despite these dramatic trends some producers and governments show hesitancy or reluctance to follow suit, claiming that cage-free systems are harder to manage and result in uncertain health and welfare outcomes. This presentation will summarise research taking place to address these concerns, including rearing practices and design features that reduce the risk of fractures, and the effectiveness of management packages designed to reduce injurious pecking and bird mortality. It will also review the welfare benefits associated with greater behavioural freedom. Cage-free systems have a high potential to provide good welfare but transitions must be accompanied by a strong improvement strategy, where the best emerging science is integrated into agreed standards.

The Combined Effect of Filial Imprinting, Environmental Enrichment and Music on Chickens

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Filial imprinting is defined as the following response of precocial chicks to the first object they encounter after hatching. Precocial chicks can be imprinted both visually and aurally. They are, therefore, imprinted to their parents, other chicks around them and to their immediate surroundings. Newberry (1995) defined environmental enrichment as an improvement in the biological functioning of captive (domestic, zoo, or laboratory) animals, resulting from modifications to their environment. The development of the auditory system in the chicken occurs primarily in ovo (Gao & Lu, 2008). Chicks will exhibit responses to sound at around day 10-11 in embryo. Being a precocial species, chicks hatch with adult-like auditory capabilities. In behavioral observations, chicks responded to classical music, a type of auditory enrichment at a level of 75dB sound pressure (Gvaryahu et al., 1989). Similar to humans and other species, chicks prefer harmonic consonant sound over dissonant sound intervals (Chiandetti & Vallortigara, 2011). Studies over the last 40 years demonstrate that the exposure of day old chicks to imprinting objects, environmental enrichment and music (IEM) causes the following: a decrease in the fearfulness of the chicks when in the presence of a novel object; they fed significantly more often than the controls when music was activated; TI duration was shorter; mean heterophil to lymphocyte ratio was significantly smaller in layer chicks; significant increase of body weight at 8 wk of age in meat strain chickens, and a significant improvement of feed:gain ratio in 7 day old meat strain chicks. Fluctuating asymmetry (differences in bilateral skeletal anatomy), used as an indicator of developmental stress (Knierim et al., 2007), was also reduced, by rearing chicks post-hatch with music. This further supports the positive impact of classical music during rearing. It is, therefore, of crucial importance, to expose chicks to IEM during the imprinting period. In addition, sound pressure must not rise above 85 dB.

Animal Welfare in the Intensive Management; A Lacuna in the Law of Animal Welfare

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The Animal Cruelty Act prohibits inflicting suffering on an animal without proper justification, however, any possession of an animal involves constraints on its well-being. The question of whether the harm to animal welfare is included in the act of abuse is examined according to the test of proper justification. Keeping animals under intensive management involves severe constraints on their conditions and a real harm to their well-being. The proper justification test will be determined whether for the purpose of the known possession, the harm done to the animals is justified or it comes as an abuse.

In the intensive interface, certain animals are injured far more than the majority, and the harm to them exceeds what is permitted under the general conditions of possession approved. Due to the intensive growth (sometimes without human hand contact) this excess damage is taken into account as “depreciation” in production, and as long as it does not affect profitability, it is ignored. This suggests that animal protection in the intensive interface is not individual; It is not the “animal” that is protected, but the “average animal” that is protected. This situation clearly contradicts the provision of the law: “A person shall not abuse an animal in any way” and is a fundamental defect in the law.

SCIENTIFIC ABSTRACTS

Metabolic Stress in Laying Hens and Its Immune-Mediated Resolution

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As an outcome of the intensive poultry husbandry in Israel, the energetic demands of layers selected to increase egg production are high. Consequently, energy deficiency is minimally tolerated and results in metabolic stress. We hypothesized that metabolic stress impedes homeostasis, and its resolution includes immune mediation. This hypothesis is based on our previous observations, in which homeostasis restoration was immune mediated in other types of stress. Accordingly, our current goal was to examine the effect of metabolic stress on homeostasis in layers and the involvement of the immune system in its restoration. We determined metabolic stress responses in sixty Lomman layers which were subjected to a 7-day food deprivation program. Physiological and immunological parameters were tested at 4 time points (n=60). We observed a negative energy balance which resulted in extensive lipolysis, and increased expression of a wide array of general stress related genes (GCR, HSP-70 and IL-6) as well as energy stress related genes (SIRT1). Additionally, we identified oxidative stress reagents (AGE) in the food deprived layers, unique to metabolic stress. Furthermore, *in vitro* exposure of peripheral blood leukocytes from layers to different dosages and exposure times of AGE, resulted in production pro-inflammatory cytokines (IL-6, IL-1 β) and oxidative reagents (lysozyme, transferrin) gene expression. We suggest that negative energy balance constitutes metabolic stress, and the pro-inflammatory immune response plays a major role in its resolution. Hence, understanding the mechanism by which the immune system assists in restoring homeostasis following the resolution of metabolic stress, would be beneficial to poultry husbandry and welfare.

Improving Rat Welfare by Development of Regional Analgesia Technique for Ophthalmic Procedures: A Preliminary Cadaveric Study

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Rats are commonly used as an animal model to study glaucoma, which is induced experimentally using invasive painful procedures. Peribulbar anesthesia (PBA) is frequently used in people and domestic animals prior to ophthalmic surgeries to provide excellent perioperative analgesia. The goal of this study was to develop a PBA technique adapted to rat anatomy, to improve welfare of animals used as a glaucoma model. Eighteen rat cadavers were used to establish the optimal injection technique and the volume of injectate required. Four injection techniques using lidocaine 2% and contrast agent (1:1 volume ratio) were compared using computed tomography (CT) imaging performed before and after injection. A board-certified radiologist, masked regarding the injection techniques used, reviewed all CT images and scored injectate distribution at 4 locations: extraconal, intraconal, around the optic nerve and at the orbital fissure (scale 0-8; 0=none, 8=excellent). Injectate distribution scoring (median; range) was significantly higher (i.e., better) using the dorso-medial (5; 2-6) and medial-canthus (4.5; 2-8) techniques compared with mid-ventral (3; 2-5) and ventro-lateral (2; 1-3) techniques. A volume of 0.1 mL/100 g body weight provided overall good distribution, while a volume of 0.2 mL/100 g produced excessive exophthalmos. Presence of intracranial contrast was observed following all technique. In conclusion, PBA using the dorso-medial or medial-canthus techniques are most likely to provide local anesthesia. The intracranial contrast observed could be the result of post-mortem vessel leakage. Results of this preliminary study will be used to assess the efficacy and safety of these techniques in experimental rats.

The Challenges Faced by Free Ranging Fruit Bats (*Rousettus aegyptiacus*)

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To expose the natural challenges faced by free-ranging fruit bats (*Rousettus aegyptiacus*), we performed a retrospective analysis of nearly 1500 cases reported by a bat rescue NGO over 25 months, from all over Israel. All cases of stranded bats found and reported, were evaluated and categorized according to date, place, sex, age, and cause of incidence. Healthy bats are not reported. The analysis of the data showed a very clear increase in all types of morbidity during the wintertime, with more than twice the number of cases in comparison with the summertime, over two consecutive years. Moreover, we found that the number of abandoned pups peaks during the spring till autumn is in correlation with the known reproduction season. We characterized two prominent types of morbidity, one in the form of bacterial illness due to *Staphylococcus aureus* and the other associated with feet deformation in addition to other major anthropogenic related challenges. Out of all cases, 69% were categorized while 31% could not be diagnosed properly and are therefore marked as other. Within the diagnosed cases, the vast majority (79.7%) were due to Injuries, 17.4% were due to illness, and only 2.9% were attributed as feet problems. We analyze the reasons driving winter mortality and conclude that winter weather and specifically temperature are the main cause for this mortality. We hypothesize that this *R.aegyptiacus* fruit bat, of tropical origin, is facing major seasonal difficulties at the northern edge of its distribution, probably limiting its further spread towards the north.

Prevalence Survey of Feline Leukemia Virus (FeLV) and Feline Immunodeficiency Virus (FIV) Infections in Morbid *versus* Free Roaming Cats Presented for Neuter in Rishon Le Zion Israel

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Feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) are significant infectious diseases in free-roaming cats (FRCs). Ethical, Public Health and welfare issues arise when treating morbid or releasing to the surroundings FRCs from Trap-Neuter-Vaccinate-Release (TNVR) programs that are positive for FeLV or FIV. Assessing the prevalence of disease in the population is fundamental for conducting epidemiological studies. There are currently no comprehensive studies in Israel regarding FeLV or FIV prevalence in FRCs. In a survey conducted in Rishon LeZion Municipal Veterinary Clinic-out of 103 cats submitted for TNVR during March-April 2019 – random 30 FRCs trapped for TNVR were tested using serology antigen kit for FELV and FIV infections. 4/30 (0.13%) tested positive for FIV. 1/30 (0/03%) tested positive for both FIV and FeLV. All positive cats were males. During the time of the study, the Rishon Lezion municipal veterinary services treated 90 Morbid FRCs. 32 FRCs were tested for FELV and FIV antigen due to the suspicion of the attending clinician (the author). 20/32 (62.5%) were adult and castrated/spayed. 19/32 (0.59%) cats tested positive for FIV. 1/32 (0.03%) cats tested positive for FeLV. Total of 20/32 (62.5%) were positive either for FIV/FeLV. 12/19 (62%) of males, 7/13 (54%) of females tested positive for FIV. The study demonstrates a higher prevalence of FIV infections than FELV in FRCs in Rishon LeZion mainly in males and among the morbid FRCs. This factor needs to be addressed when practicing with FRCs in various situations in Israel.

Characterization of Birds and Horses West Nile and Usutu Virus Infections in Israel Using Serological, Genetic, Molecular, and Cell-Based Approaches

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West Nile virus (WNV), transmitted by arthropod vectors, mainly mosquitoes of the *Culex* species, may cause a severe encephalitis in avian species, equines and human. WNV outbreaks have occurred worldwide, since the late 1990's, and the virus is now presented in almost all continents. In Israel, WNV is considered endemic since the middle of the 20th century. Our study focused on analysis of genetic, molecular and virulence differences, which will hopefully promote understanding genetic contribution to geographical expansion and establishment to improve anticipation of future outbreaks. The initial work included extensive sampling of horses, wild avian and mosquitoes to identify and isolate the virus from different hosts and vectors. In 2018, WNV outbreak occurred in Israel and was accompanied by increased morbidity and mortality in wild birds and horses. WNV isolates collected from animals in 2018 and several decades before (all- of lineage 1) were used for phylogenetic analysis and comparative studies. Circulation of WNV in 2018 in wild birds prior to summer outbreak, was assessed retrospectively by molecular analysis of central nerve system and visceral tissues, WNV RNA was detected in 14/138 carcasses (10.15%). Seroprevalence for neutralizing antibodies against WNV and Usutu virus (USUV) in healthy horses in 2018 was 84.1% and 10.8% respectively. This is the first report describing exposure of horses in Israel to USUV.

Protozoan Parasites as a Cause of Abortions in Sheep in Israel over the Last Decade*

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*This abstract was awarded the second best abstract

Abortions in sheep flocks is a multifactorial problem which has considerable veterinary and economic consequences. In recent years, increasing attention has been given to the role of protozoan parasites as the cause of ovine abortions worldwide. Here, we review the serologic diagnoses of apicomplexan parasites in sheep (including aborted fetuses) in Israel in the last decade (2010–2019). The overall seroprevalence of *Neospora* and *Toxoplasma* was 67.4% (3238 of 4804 samples) and 46.7% (2489 of 5326 samples) respectively, with high rates of co-exposure (32.4% of 1877 positive samples). The seroprevalence of *Toxoplasma* was higher in aborting ewes than in pre-sale examinations (48.2% of 811 and 28.9% of 90, respectively, $P < 0.001$), while the seroprevalence of *Neospora* did not differ between samples from aborting ewes and from pre-sale examinations. Nevertheless, anti-*Neospora* antibodies were the most prominent finding diagnosed in aborted fetuses (22.9% of 31 fetuses). These results demonstrate that neosporosis is highly involved in abortions in sheep in Israel. However, the diagnosis of neosporosis or toxoplasmosis as the cause of abortion based solely on serology should be done with caution, since their seroprevalence can be high in ewes with or without history of abortion. A case example of an abortion storm in a sheep flock, in which high *Neospora* seroprevalence was found in aborting ewes, is described. In this flock, similar seroprevalence was found also in non-aborting ewes, leading to further investigation of the actual cause of abortions. This case study highlights the need of comprehensive epidemiological investigation for the diagnosis of the causative agent of abortion storms in endemic flocks when several pathogens are present.

In vitro Studies to Evaluate The Effect of Intramammary Sustained-Release Formulations (Chlorhexidine and Cetylpyridinium Chloride) and Blue Light on Bacteria Involved in Bovine Mastitis During The Dry Period

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Bovine mastitis is an inflammation of the udder of a cow primarily due to bacterial infection. Mastitis leads to reduction in milk production and quality. The treatment is primarily antibiotics which increase the risk of antibiotic resistance. The antiseptic agents, chlorhexidine and cetylpyridinium-chloride were tested against mastitis bacteria. The minimum inhibitory and bactericidal concentrations (MIC and MBC) were determined. The bacteria were cultured on Muller- Hinton plates and sustained release (SR) formulations were placed on the plates and incubated for 24 hours at 37°C. The formulations were transferred daily to new plates to determine the zones of inhibition.

In another trial, the same bacteria were exposed to blue light in order to determine its activity against these bacteria. The results showed that a single dose of the SR chlorhexidine was effective against *E. coli* for 28 days, *S. aureus* for 22 days, *S. haemolyticus* for 24 days, *S. chromogens* for 23 days, *Strep. agalactiae* for 11 days, *Strep. dysagalactiae* for 14 days, *Strep. uberis* for 14 days and *P. aeruginosa* for 5 days. Cetylpyridinium-chloride was effective against *E. coli* for 18 days, *S. aureus* for 14 days, *S. haemolyticus* for 18 days, *S. chromogens* for 19 days, *Strep. agalactiae* for 9 days, *Strep. dysagalactiae* for 12 days, *Strep. uberis* for 13 days and *P. aeruginosa* for 2 days. Blue light had no inhibitory effect. Use of antiseptics has the potential to provide a solution to the problem of frequent use of antibiotics and antibiotic resistance in the control of mastitis.

A Pilot Study for Fear-Free Client Education: Partial Results

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Fear Free[®] is a recent concept which main's objective is to change the different situations animals are exposed to and make the experience less stressful, by training professionals (veterinarians, assistants, trainers, groomers) how to guarantee that the patient will not suffer from fear, anxiety and stress (FAS) during the contact with them. Since visits to a veterinarian are very frequent, dogs and cats are often exposed to situations that can trigger a FAS response. The FAS situation is dangerous to all involved individuals due to the possibility of injuries, and, in addition, stressed animals are more susceptible to diseases increasing the frequency of the visits to the veterinarian. Previous studies shown misinterpretation of emotional signs in animals, turning hard to recognize the FAS signs. As part of creating a FAS guide for dog owners, it is important to first understand their knowledge about FAS signs, and for that a questionnaire was created based on the Fear Free[®] description of those signs. By now 164 dog owners answered the questionnaire and 67.72% of them believe that their dog is afraid of the veterinarian. Although there are several factors that lead to a FAS situation, only 17,47% of the owners associate FAS to smells and noise (at the veterinarian?) and 14,12% to pain due to injections or treatments. Some FAS signs seem to be very clear to dog's owners, but there are signs that can mislead the interpretation. More research is needed so the guide can be correctly based on owner's knowledge.

Thromboelastometry as a Prognostic Tool in Dogs with Naturally-Occurring Heatstroke

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Heatstroke (HS) is a devastating condition, with reported mortality rates of 40-50%. Excessive activation of the coagulation system, potentially culminating in overt DIC, is a prominent feature of HS. Thromboelastometry (TEM) is a viscoelastic diagnostic modality providing global assessment of the entire clotting process, enabling to determine whether the patient is hyper, normo, or hypo-coagulable. TEM has been previously validated in dogs; however, changes in TEM have not been described in dogs with HS. This study aimed to investigate changes in TEM and its utility as a prognostic tool in dogs with naturally-occurring HS at different time points from presentation until discharge to death. Forty-two dogs presented to the emergency service for HS were included. A previously reported HS severity score was calculated. A significant difference was documented in the median time from heat insult to presentation between survivors and non-survivors. Mortality rate was 31%, which was lower compared to previous studies. A worse HS severity score accurately predicated non-survival. Significant differences in several TEM parameters were documented, with non-survivors showing prolongations in clotting times, clot formation times, as well as a significantly lower alpha angle and AUC values ($P < 0.05$). These changes indicated that non-survivors were significantly more likely to develop a hypo-coagulable state that became apparent over the first 12-24 hours, rather than on presentation, highlighting the need for close monitoring and serial measurements of coagulation parameters in dogs with HS. Dogs that were hyper-coagulable had a better prognosis compared to dogs that were hypo-coagulable.

Comparison of Dogs with Septic Peritonitis due to *Spirocerca lupi* Aberrant Migration and Dogs with Other Gastrointestinal Causes of Septic Peritonitis. A Retrospective Study

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Spirocerca lupi (*S. lupi*) has a worldwide distribution. Aberrant nematode migration occurs to various organs and might cause thrombi, which obstruct the tributaries of the mesenteric arteries, causing local ischemia, intestinal segmental necrosis and subsequently peritonitis. The aim of this study was to compare clinical and clinico-pathologic parameters of dogs presenting with septic peritonitis associated with aberrant *S. lupi* migration (SL) and those with septic peritonitis of other gastrointestinal causes (SP). Records of dogs diagnosed with septic peritonitis associated with aberrant *S. lupi* migration and dogs with septic peritonitis due to other gastrointestinal (GI) causes during 2017-2020 were reviewed 46 dogs were included in this study, 21 dogs in the SL group and 25 dogs SP group. Eosinophils in the abdominal effusion were more common in the SL compared with the SP group (11/14 vs 0/15 dogs, respectively, $P=0.001$). Abdominal fluid total solids were also significantly higher in the SL compared to the SP group (5.2 g/dL, [3.0-7.0], vs. 3.4 g/dL, [2.0-5.0], respectively $P=0.03$). Median hospitalization time was significantly longer in the SL compared with SP group (3.5 days vs. 2.8 days, respectively; $P=0.001$). The Survival rate was significantly higher in the SL group compared to the SP dogs (17/19 dogs [90%], vs. 13/24 [54%], respectively; $P=0.019$). Presence of eosinophils and high TS in the abdominal effusion is highly suggestive *S. lupi* associated peritonitis. Although *S. lupi* associated septic peritonitis requires carries a favorable outcome, intensive care and long hospitalization might be necessary.

Evaluation of Blood Spinal Cord Barrier Disruption in Cases of Intraspinal Aberrant Migration of *Spirocerca lupi***

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Dogs are the definitive hosts of *Spirocerca lupi* (*S. lupi*), an endemic nematode in Israel, prevalent in tropical and subtropical areas. Aberrant migration of *S. lupi* into the spinal cord, known as intraspinal *S. lupi* (ISSL), results in acute and progressive neurological dysfunction. Empirical treatment currently in use includes frequent administration of higher dose of ivermectin for 4 weeks. This protocol seems sufficient for prevention of further migration of the nematode through the spinal cord and possibly kill it. No adverse effects associated with ivermectin toxicity have been observed in treated dogs. We therefore hypothesized that blood-spinal cord barrier (BSCB) disruption occurs focally and adjacent to the nematode's migratory tract, while the barrier remains functional at remote spinal cord segments and therefore in the brain as well, hence the lack of observed toxicity. To prove this hypothesis, we examined spinal cords removed from three dogs definitively diagnosed with ISSL. Representative cross sections from different areas relative to the nematode's migratory tract were evaluated using histological (H&E) and immunofluorescent methods. In spinal cord sections of the migration tract, histopathologic findings revealed evidence of mechanical damage and severe inflammation. Immunofluorescent analysis using MATLAB revealed increased signal of extravascular albumin co-localized with higher expression of GFAP. Expression of Iba1 was also higher in damaged segments compared to sections distant to the migratory tract. These findings support our hypothesis that increased BSCB permeability around the migrating nematode enhances the efficiency of ivermectin focally, while no adverse effect of the increased dose is observed.

Axis of Rotation of the Canine Elbow Joint

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The high complication rate of total elbow replacement in dogs may be due to failure to align implants with the axis of rotation (AOR) of the joint as seen in people. This study aimed to define the AOR in normal and diseased elbow joints in dogs. CT scans of 9 dogs' forelegs with unilateral fragmented medial coronoid process (healthy legs served as control) were converted into 3D models and uploaded into the implant design program. A system of axes was embedded in the humeral condyle of all models. Four spheres were generated using the contours of the subchondral bone of the radius, ulna, trochlea, and capitulum. A circle was generated using the depression between the capitulum and trochlea. Four axes were generated using pairs of geometric shapes. Medial and lateral images showing the exit locations of the axes were captured and imported into ImageJ. Distances from the origin of the system of axes (OSOA) to the location where each axis exited the medial and lateral cortex, of healthy and pathologic joints, were measured and compared. In normal joints, the location of the AOR was cranial and distal to the (OSOA) on both sides of the humeral condyle. The size of the area on the medial and lateral aspect of the humeral condyle was 4.5 mm x 6.6 mm (lateral) and 4.3 mm x 7.7 mm (medial), respectively. The AOR of the elbow joint can be defined relative to landmarks on the humeral condyle. Described landmarks may facilitate accurate and consistent placement of elbow implants.