

The 45th Symposium of Veterinary Medicine: Artificial Intelligence on Animal Welfare

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

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INVITED LECTURES

Applications of Artificial Intelligence for Veterinary Medicine

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This is a time of exciting opportunities in animal behavior research. Advances in deep learning, leading to the release of generic frameworks such as DeepLabCut have unleashed the potential of video-based motion tracking for almost any animal species. However, recognizing animals' internal states, such as affect and pain, remains a challenging and underexplored area due to difficulties in collecting quality data and annotating ground truth. In this study, we explore non-invasive computer vision-based methods for recognizing pain and emotions through facial expressions and body postures in various species, including cats, dogs, horses, rabbits, and sheep, using both images and videos as input. For example, by utilizing temporal information from videos, our developed models achieve accuracy rates between 76% and 95% in recognizing pain in cats. Additionally, we compare deep learning approaches with landmark-based pipelines and investigate the explainability of these models, focusing on their ability to provide understandable explanations for their predictions. We also compare the performance of the machine to human experts in the task of pain recognition in sheep, showing that in some cases machine outperforms humans when shown the same visual information.

Automatic Monitoring of Animal Behavior, Personality, and Mood

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Recent advancements in animal welfare research have increasingly focused on promoting positive welfare through continuous and unobtrusive monitoring of animals' affective states in naturalistic conditions. This shift also emphasizes the individual needs of each animal. In this study, we introduce several systems designed to achieve these objectives by deep phenotyping and measuring the unique behavioral fingerprints of individual animals, thereby providing insights into their emotional states. We highlight three specific systems tailored to different species. Firstly, the “social box,” a video monitoring system for observing groups of laboratory mice, allows for detailed analysis of their social interactions. Secondly, a tag-based system tracks the social behavior of a herd of 70 cows, providing comprehensive data on their social organization within the herd. Lastly, a large-scale monitoring system for feral cats enables the study of thousands of individuals in public-assisted research. From a computational perspective, these systems employ machine learning algorithms to automatically measure over 100 distinct behaviors for each species and infer their personality traits. Understanding the relationship between behavior and personality is crucial for accurately assessing the emotional states of animals. Our findings illustrate the potential of these advanced monitoring technologies to deepen our understanding of animal behavior and welfare, thereby paving the way for more personalized and effective welfare strategies.

SCIENTIFIC ABSTRACTS

* Best Abstract Award winner:

First *in-vitro* Isolation of the Human Relapsing Fever Spirochete *Borrelia persica* from A Dog: Genetic Characterization and Improved Cultivation Techniques

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Borrelia persica causes tick-borne relapsing fever in Israel, the eastern Mediterranean basin, and parts of Central Asia. This zoonotic disease is potentially life-threatening for humans and companion animals. Isolation and *in-vitro* cultivation of *B. persica* in culture medium is difficult and there are only two previous isolates. This

study describes the first successful cultivation of *B. persica* from an ill dog. Genetic characterization by PCR amplification and DNA sequencing of the flagellin, glycerophosphodiester phosphodiesterase, and 16S *rRNA* genes were made for both blood and culture. Additionally, real-time and conventional PCRs were performed to detect co-infections with *Ehrlichia canis*, *Babesia* spp., and *Hepatozoon* spp. The growth dynamics of *B. persica* strain HU-D03 was evaluated for long-term stability and propagation characteristics. Real-time PCR of the blood was positive for *Borrelia flaB*, *glpQ*, and 16S *rRNA* genes, and negative for other tested pathogens. Sequencing analysis of the *flaB* and 16S *rRNA*-genes showed 100% identity to *B. persica*. Rapid motility spirochetes were observed in culture with a cell density of 2×10^6 /ml three days post-sampling. The isolate was passaged every 3-4 days, reaching cell densities of up to 3×10^7 /ml over 30 passages. Cryopreservation at -80°C without cryoprotectants allowed successful growth from thawed samples up to three months after freezing. The generation time during the exponential growth phase was 14.6 hours. The new canine isolate would be helpful for research on the pathogenesis of relapsing fever and the improved cultivation and preservation methods may assist in future studies of relapsing fever replacing previously reported in-vitro methods.

Effect of Environmental Enrichment on Stress Markers in Goats

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Farm animals are predominantly exposed to stressors during their husbandry. Lately, we developed a minimally invasive method to measure stress via blood sampling to provide reliable markers for husbandry stress. Here, we aimed to evaluate the effect of brushes and stages, as an environmental enrichment to alleviate stress in goats. 36 mixed breed goats were divided into 3 groups according to farm's common physiological statuses (12 goats/group): dry, milking and does. Following 28 days of adaptation period each group was exposed to 10 days environmental treatment (brushes and stages) or not (control) at different time points; thus, each group served as its own respective control. At day 10 of each treatment blood samples were collected from each goat and group. Gene study was done via qPCR using mRNA produced from isolated leukocytes and serological studies were done via ELISA using serum. Following the enrichment treatment (compared to respective controls), we observed a reduction in serum reactive oxidative stress metabolites—AGEs in all statuses. Moreover, levels of transferrin (AGEs binding protein) and their complexes varied according to the physiological status: declined, inclined and presented a mixed image in dry, milking and does respectively. Additionally, immune stress related gene expression of cytokines: IL-6&1β and anti-oxidative proteins: lysozyme and transferrin, presented same pattern. In conclusion, reliable methodology was developed to assess husbandry stress in goats. Current enrichment produced different responsiveness depending on goats' physiological status. Hence, we recommend implementing current enrichment only in dry goats, while continue evaluating other methods.

Blood Crossmatching in Rescued Southern White-Breasted Hedgehogs (*Erinaceus concolor*)

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Rescued hedgehogs might present with severe anemia, potentially requiring blood transfusion. Finding suitable blood donors might be challenging. Positive donor-recipient crossmatch, showing agglutination or hemolysis, are indicative of incompatibility. The goal of this pilot investigation was to check potential blood crossmatching reactions in Southern white-breasted hedgehogs (*Erinaceus concolor*). While under brief isoflurane anesthesia, blood samples were collected from the cranial vena cava, from rescued, mature animals (n=16; males, 9; females, 7) using heparinized syringes. The blood was placed in tubes, centrifuged and the erythrocytes were separated and washed in saline, and then re-centrifuged. The RBC pellet and plasma were used as control, and for minor and major crossmatches. Each crossmatch was evaluated both microscopically and macroscopically. There were 78 matches in total, including 39 control matches and 39 randomized paired minor and major crossmatching procedures. All the self crossmatches were negative. Among the paired crossmatches, microscopically, 12/39 (31%) minor and 18/39 (46%) major matches were positive, of which only 1/39 minor and 9/18 major positive matches were macroscopically positive. One procedure was negative for control but showed concurrent strong major, minor and hemolysis reactions. Concurrent positive minor and major matches showed in 6/39 reactions. Positive minor matches exclusively showed in 6/21 negative major matches. This high frequency of positive agglutination reactions suggests the common presence of naturally occurring anti-erythrocyte alloantibodies in this species and high risk of acute immune-mediated hemolytic transfusion reaction. Based on these data, donor-recipient crossmatching should always be performed before administering blood products in this species.

Primary Immune-Mediated Thrombocytopenia Tentatively Diagnosed in Four Cats

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Primary immune-mediated thrombocytopenia (pIMT) is uncommon in cats, and to our knowledge, scarcely reported. pIMT is mostly diagnosed based on excluding all primary conditions potentially causing secondary IMT and observing positive response to immunosuppressive therapy. Definitive diagnosis is based on immunoassays demonstrating bound or bindable anti-platelet or anti-megakaryocytes antibodies. Here we describe four cats with proven severe thrombocytopenia, with no primary underlying diseases, tentatively diagnosed with pIMT during years 2018-2020. Cat ages ranged between 1.5 and 10 years. At presentation, cats showed pale mucous membranes, and extreme weakness to collapse. The platelet counts ranged from 5 to $11 \times 10^3/\mu\text{L}$ (reference interval, $156.4-626.4 \times 10^3/\mu\text{L}$). All cats were treated with prednisolone. In two, prednisolone was the single immunosuppressive agent, and both had recovered. Another cat, partially and insufficiently responded

to prednisolone, and thus, also received mycophenolate-mofetil, leading to remission. The remaining cat, with insufficient response to prednisolone, received in addition, over time, mycophenolate-mofetil, followed by leflunomide, due to partial response. The thrombocytopenia had improved, but the cat required long-term prednisolone therapy, eventually resulting in diabetes mellitus. In conclusion, with only four cases over the 2-year study period, pIMT was an extremely uncommon cause of thrombocytopenia in cats in our hospital. The profound thrombocytopenia at presentation posed a serious risk for life-threatening bleeding. Nevertheless, bleeding signs were absent in all cats. Prednisolone treatment alone might induce remission in some cats, but when response to prednisolone is insufficient, or with recurrence, additional immunosuppressive drugs are advised. The overall prognosis in this small cohort was good.

Clinical Findings, Prognostic Factors and Outcomes of Feline Protein Losing Nephropathy: A Retrospective Study

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Feline protein losing nephropathy (PLN) is often presumed to be primary immune-mediated glomerular disease. The disease has substantial clinical implications, however, clinical signs, clinicopathologic findings and outcome are not well described. In this retrospective study, we aimed to characterize the disease and identify prognostic factors for remission and outcome. PLN was diagnosed based on compatible clinical signs and urine protein to creatinine ratio (UPC) >2 , after exclusion of pre- and post-renal causes. Thirty-seven cats were included, of which 17 (46%) were males and 20 (54%) females. Median age was 3 years (range, 1.5-11.5). Nineteen cats (54%) survived 30 days from discharge and 16 (44%) did not. The most common clinical signs at presentation were edema (44%, 16/36) and weight loss (46%, 12/35). Serum urea ($P < 0.001$), creatinine ($P = 0.020$), phosphorus ($P = 0.004$), AST ($P = 0.016$), and CK ($P = 0.002$) at presentation were higher in non-survivors compared with survivors, while glucose ($P = 0.009$), total calcium ($P = 0.012$) and MCHC ($P = 0.046$) were lower. Treatments included prednisone (60%, 18/30), immunosuppression (47%, 18/37), antiproteinuric drugs (81%, 22/27), antibiotics (74%, 20/27) and antithrombotics (54%, 20/37). Only antithrombotics were associated with short-term survival (OR-4.2, CI95% 0.98-17.95, $P = 0.048$). Complete (UPC <0.4) or partial remission ($>50\%$ reduction in UPC) were documented in 4 (12.5%) and 10 (33%) cats, respectively, all of which survived. Estimated overall mean survival time was 520 days (CI95% 91-949). Complete remission, creatinine and administration of antithrombotics were predictors for long-term survival (856 days vs. 230 days, $P = 0.037$; OR-1.37 CI95% 1.15-1.63, $P < 0.01$; 3380 days vs. 190 days, $P = 0.006$, respectively).

Movement of the Medial Meniscus in the Stifle of Canine Cadavers with the Cranial Cruciate Ligament Intact and Cut

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In cranial cruciate ligament (CCL) deficient stifle, the medial menisci becomes the joint's primary stabilizer. Our objective was to quantify the cranial translation of the tibia, and the caudal and cranial horn of the medial meniscus relative to the femur in the intact and CCL deficient stifle. This study was performed using 12 skeletally mature hind limbs, obtained from dogs, weighing between 20-25 kg. Using a custom-designed joint-testing machine at 3 different stifle angles (90°, 120° and 135°). Three sensors were attached to the tibia, caudal and cranial horns of the medial meniscus to record their motion relative to the femur, using cranially positioned weights. Afterwards, a craniomedial arthrotomy was performed to cut CCL and repeat the tests, using identical technique. Statistical analyses were performed using a software program ($p < 0.05$). The cranial translation of the tibia, and the caudal and cranial horns of the medial meniscus significantly increased across all loads, in every stifle angle, and with the CCL cut compared to the intact stifle.

Notably, at 90°, there was significant increase in cranial translation compared to other angles. However, we did not find significant differences comparing relative translation between the structures themselves. In this study we were able to show a new technic to quantify and compare the motion of the medial meniscal horn, in a CCL intact\cut stifle. As expected, translation increased along all loads and with the CCL cut, indicating the significance of the meniscus in stabilizing the stifle joint when CCL injuries occur.