

2012

Proceedings of ANIWEL Seminar



4th and 5th of October 2012

University of Helsinki



ANIWEL Seminar 2012



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<http://aniwel.edublogs.org/>

ANIWEL Seminar 4th and 5th October 2012
University of Helsinki, Viikinkaari 11



Abstracts

Consequences of envenomation by *Vipera berus berus* in urinary protein profile in dogs

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Introduction: Between April and September every year many dogs in Scandinavia are bitten by *Vipera berus berus*, the only venomous snake in the area. Exposure to snake bite venom causes local and systemic symptoms and in severe cases can lead to death.

Aim of the study: The aim of this study was to investigate whether envenomation causes differences in urinary proteome in dogs.

Methods: Urine samples were collected from four dogs bitten by *Vipera berus berus* and treated in the intensive care unit of the Veterinary Teaching Hospital at the University of Helsinki. The inclusion criteria were a strong suspicion of viper bite no more than two days before admission and clinical signs of a viper bite. Exclusion criteria were defined as: ongoing treatment with glucocorticoids or known history of liver or kidney diseases. Urine samples from six privately owned, healthy dogs were obtained for controls. Samples were subjected to 2D DIGE analysis. Image analysis was performed with DeCyder 7.0 2D software and protein spots demonstrating statistically significant ($p < 0.05$), minimum 1.5-fold difference in average spot volume ratios between envenomed and control dogs were picked and identified with LC-MS/MS.

Results: In 2D DIGE analysis seven proteins were significantly ($p < 0.05$) over expressed in urine of dogs bitten by *Vipera berus berus* compared to control group. From those, five proteins were identified: beta-2-microglobulin (b2MG), alpha-1-antitrypsin (AAT), albumin, fetuin-B and superoxide dismutase (SOD1).

Conclusions: Results indicate that snake bite by *Vipera berus berus* alters the urinary protein profile in dogs. The identified proteins participate in immune responses, regulation of proteolysis, cysteine-type endopeptidase inhibitor activity, and oxidation-reduction processes. The excreted alpha-1-antitrypsin (AAT), beta-2-microglobulin (b2MG) and albumin are well-studied proteins associated with tubular injury. More studies are needed to understand the role and origin of fetuin-B and copper/zinc superoxide dismutase (SOD1) in dog urine after a European adder bite, and their sensitivity and specificity as potential biomarkers.



Comparative histopathology of mammary gland alterations in woman and dog

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Background: The most prevalent cancer type in women and intact female dogs are mammary tumors. Human breast cancer research needs more animal models. Dog has already been suggested for this. However, published studies have concentrated only on a limited range of lesions, and direct comparison is difficult due to differences in the respective classification systems. Our aim was to describe equivalent non-neoplastic and neoplastic mammary gland alterations in woman and female dog.

Materials and methods: We used archived formalin-fixed, paraffin-embedded human and canine mammary tissue samples. Histological comparison was performed with HE-stained tissue sections and immunohistochemical antibodies such as CK5/6, ER α , PR, Ki67, Her2, p63, SMA, calponin and E-cadherin. Furthermore, we collected epidemiological data for analysis from human (n=1935) and canine (n=723) patients for the years 2003-2005 and 1998-2011, respectively.

Results: We identified several comparable benign and malignant mammary gland alterations. These consist of lobular, tubular and ductal as well as mesenchymal of origin. Most of these are now described for the first time. The epidemiological results show also shared characteristics, such as the distribution of cases based on gender, on tumor dignity (benign or malignant), the age distribution of cases based on tumor dignity as well as the distribution of tumors based on tissue of origin (epithelial or mesenchymal). Differences were also noted, especially, in the frequencies of tumor subtypes. In the dog those with myoepithelial proliferation are more common.

Conclusion: The results indicate the female dog as a suitable model for translational research. Equivalent entities exist, but there are also canine specific lesions. Next we concentrate on the corresponding tumor subtypes, verify their IHC results with more samples and compare their molecular biological identity.



Safety Assessment of Oncolytic Vaccinia Virus in Two Laboratory Beagle Dogs

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Oncolytic viruses are a new promising treatment modality for cancer. We have previously shown that the double deleted vaccinia virus (vddd-tdTom-hCD40L) kills canine cancer cell lines in vitro and inhibits progression of canine tumor xenografts in mice. The objective of the study was to evaluate safety of vddd-tdTom-hCD40L in two beagle dogs in preparation for a phase I dose escalating study in pet dogs with incurable cancers.

Dogs received 3 times the human maximum tolerated dose of the virus as an intravenous infusion. Dog 1 received one infusion of the oncolytic virus and was euthanized 24 hours later. Dog 2 received 4 doses every 7 days and was euthanized 7 days after the last dose. Dogs were monitored for adverse events by physical exam, hematology, serum biochemistry and urinalysis according to Veterinary Cooperative Oncology Group, followed by full necropsies.

Dog 1 had grade 1 fever (39.5°C) 8 h after virus administration and grade 1 lethargy. Dog 2 had one <2 minutes long episode of grade 3 seizure 5.5 h after the first virus administration. The dose for dog 2 was subsequently reduced and no further adverse events were noticed. The only adverse event seen in laboratory tests was grade 1 increase in AFOS in Dog 1. Necropsies had no specific findings associated with virus administration.

The grade 3 seizure observed in Dog 2 may have been associated with hypersensitivity reaction induced by oncolytic vaccinia virus. In the phase 1 dose escalating study a lower dose of oncolytic vaccinia virus will be administered intratumorally. Therefore, treatment related toxicity is less likely to occur. However, the dogs in the phase I study will be carefully monitored for seizures, since the adverse event seen in this study was unexpected.



Veterinarians' empathy towards animals is associated with cattle pain scoring

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Empathy is considered desired skill among health professionals. Cattle pain treatment relies on veterinarians, therefore the relationship between veterinarians' empathy, attitudes, and education on cattle pain assessment and management was studied.

A web-based questionnaire was sent to veterinary students in preclinical and clinical phase and to veterinarians specializing in production animals. We asked questions about demographics, opinions of disbudding and pain medication (using a five point Likert scale), pain scoring of cattle conditions and procedures (using an eleven point scale).

We measured empathy in humans with an Interpersonal Reactivity Index (human IRI). Subscales related to empathy towards humans (HE) such as empathic concern, perspective taking, were reworded to measure empathy towards animals (animal IRI). We got 190 responses, and a response rate of 40 % was achieved.

The effect of education level, working experience as veterinarian, age, sex, affection to family pet, total sum of IRI, and family size on average pain scores and animal empathy scores were analyzed with linear mixed models. The difference between animal IRI and HE was analyzed with paired samples t-test.

93% of veterinarians and last year students would use sedation, local anesthetics and pain medication to treat disbudding pain. 88% completely disagreed with the statement "The calf requires no pain medication for disbudding". Respondents evaluated disbudding without pain medication as the most painful procedure.

Animal IRI was greater than human IRI and it was affected by respondents' empathy ($P < 0.001$), family size ($P = 0.004$), and personal attachment to pet ($P < 0.001$). Higher average cattle pain scoring was associated with animal IRI and age of respondents ($P < 0.005$ for both) and higher average pain scores were associated negatively with working years as veterinarian ($P < 0.05$).

The Finnish veterinary students and working veterinarians all scored higher on animal empathy than human empathy. Veterinarians' attitudes to pain treatment in animals were positive.



Follicle size at ovulation may affect fertility in the sow

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The follicle size at ovulation is known to be between 6-9 mm in the sow. Aim of the present study was to assess if this follicular size at ovulation is associated with the number of piglets born alive. Sows (n = 93) from a sow pool system with a wean-to-estrus interval of 4 to 6 days were included, if farrowed at least once. Starting at day 3 after weaning, sows were checked for standing oestrus in the presence of a boar twice a day. When in heat, ovaries were examined by ultrasound through the abdomen twice a day until ovulation. The sizes of the three largest follicles at ovulation were measured and averaged. For the analysis, a multiple linear regression model was used. Additionally to the follicle size, parity, breed, number of inseminations, the boar and whether or not the inseminator got informed about the time of ovulation, were considered as explanatory variables. Out of the variables, only the parity (4.8 ± 2.9 ; $p < 0.05$) and the follicle size at ovulation (7.3 ± 1.8 ; $p < 0.05$) was associated with the number of piglets born alive (11.1 ± 4.3). Follicles ovulating at the size of 7 to 8 mm resulted in larger live born litters than follicles ovulating at < 7 mm or > 8 mm. These results indicate that there is an optimal size of follicles at ovulation. Ovulation at too small or too large follicular size may be linked to inadequate intra-follicular or external hormonal regulatory mechanisms, e.g. LH or oxytocin, resulting into an oocyte with lower quality.



Influence of Farrowing Environment on Early Lactation Performance and Oxytocin Level in Pigs

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The present study was conducted to investigate the effect of three different farrowing environments on early lactation performance and sow plasma oxytocin level as a modulator of nursing capacity. A total of 33 animals (11 gilts and 22 sows) were allotted to three different housing environments. Treatments were: CRATE (farrowing crate, 80 × 210, with limited amount of nest building materials), PEN (loose-housed in pen, 210 × 335, with limited amount of nest building materials), and NEST (loose-housed in pen, 210 × 335, with sufficient nest building materials). All the experimental sows were confined in farrowing crate after parturition. Sow backfat thickness and body weight were measured days on -7, 0 and +7 from parturition, and their litter weights on days 0 and +7 after farrowing. Sow plasma was collected to assay oxytocin and NEFA level at 07:00 and 13:00 before feeding on days -3, -2, -1, +1, +2, +4 and +7 from parturition, through in indwelling jugular catheter. Animals were video-recorded for 24h on both d 3 and 6 to observe nursing behavior. In order to estimate colostral immunoglobulin (Ig) concentration, serum of two female piglets born from gilts was randomly collected at 12h, 24h, 48h and d7 after birth. All the data were analyzed by mixed model with repeated measures. Sow plasma oxytocin concentration before parturition was higher in the NEST ($p < .05$). However, there was no significant difference between treatments after parturition. Average duration of all nursing and successful nursing was significantly longer in the CRATE ($p < .05$ and $p < .01$, respectively), and correlated negatively with piglet weight gain ($r_s = -0.37$ and -0.43 , respectively; $p < .05$ for both). Sow NEFA levels tended to be higher in the NEST during experimental sampling period ($p = 0.07$), and correlated positively with oxytocin level ($r_s = 0.27$, $p < .001$) and negatively with sow backfat thickness and body weight ($r_s = -0.35$ and -0.30 , respectively; $p < .0001$ for both). Piglet Ig M and Ig G serum concentrations tended to be higher in the NEST ($p = 0.05$ and $p = 0.07$, respectively). In conclusion, sow plasma oxytocin concentration could be increased with abundant nest materials before parturition, and this result could lead to improve nursing performance in early lactation.



Elevation of matrix metalloproteinase -2 and -9 activities in bronchoalveolar lavage fluid of dogs with idiopathic pulmonary fibrosis

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Canine idiopathic pulmonary fibrosis (CIPF) is an interstitial lung disease carrying many similarities with human IPF. CIPF occurs spontaneously and could therefore be a better model for IPF than experimental murine models. IPF is characterized by deposition of extracellular matrix (ECM) into lung parenchyma. Matrix metalloproteinases (MMPs) are enzymes which remodel ECM. Therefore, we investigated gelatinolytic MMP-2 and -9 activities in dogs with CIPF, dogs with chronic bronchitis (CB) and healthy control dogs. Dogs with CIPF (n=19), CB (n=11), and controls (n=12) underwent thorough examinations including bronchoscopy and high-resolution computed tomography (HRCT). CIPF was verified by histopathology in 11/19 dogs. MMP activities were analyzed quantitatively from bronchoalveolar lavage fluid (BALF) by gelatine SDS-page zymography. ProMMP-9 activities were higher in IPF (median 0.48, interquartile range 0.12-1.12) relative to CB (0.09, 0.02-0.12), $p=0.004$, or controls (0, 0-0.05), $p<0.001$. ProMMP-9 activities were higher in CB than in controls, $p=0.024$. Additionally, proMMP-2 activities were elevated in CIPF (0.03, 0-0.06) compared with CB (0, 0-0), $p=0.007$, or controls (0, 0-0.01), $p=0.013$, but did not differ between CB and controls. Active MMP-9 and MMP-2 were only detected in CIPF dogs (5 dogs and 1 dog, respectively). We demonstrated that BALF gelatinolytic activity in CIPF is characterized by the overexpression of both MMP-9 and MMP-2. Consequently, these enzymes may play an important role in ECM remodeling characteristic for the disease. Our results suggest that the gelatinolytic activity in CIPF resembles that of human IPF. Therefore, CIPF could possibly serve as a model for some aspects of human IPF research.

This abstract will be presented as a poster at the 17th International Colloquium on Lung and Airways Fibrosis (ICLAF) in Modena 1st of October.



The effects of post partum ketoprofen on feeding behaviour and its consequences in multiparous sows

Elina Viitasaari, Laura Hänninen, Mari Heinonen, Marja Raekallio, Olli Peltoniemi, Anna Valros

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Farrowing is painful and may cause protracted inactivity. This may lead to vicious circle of discomfort and poor appetite, thus impairing the maintenance of body condition, which can lead to shoulder ulcers and prolonged constipation.

We performed a double blinded, randomized study on 40 sows. Half of the sows received ketoprofen (NSAID) at dose of 3 mg/kg intramuscularly for 3 consecutive days post partum and the other half saline as placebo (PLACEBO). Feeding behaviour was assessed by the feed left in the trough and scored as either normal (trough is empty) or feed refusal (feed left in a trough). Body condition score (BCS) was measured on day 0, day 14 and at weaning with a 5 step scale (1=thin, 5=fat). The constipation and occurrence of shoulder sores were followed daily from day 0 to day 7. Constipation was scored in a 3 step scale (0=no faeces, 2=normal faeces) and shoulder sores were scored in a 4 step scale (0=intact skin, 3=severe or large wound). The effect of treatment on the first day of feed refusal, number of constipation days and occurrences of shoulder sores were studied with ANOVA. BCS was studied respectively taking repeated measures into account.

Feed refusal occurred later in NSAID than PLACEBO sows (8.3 ± 0.6 vs. 3.6 ± 0.6 , $p < 0.05$). NSAID sows showed tendency to maintain BCS better than PLACEBO sows (2.9 ± 0.1 vs. 2.5 ± 0.1 $p = 0.05$). BCS changed during lactation differently. At farrowing NSAID vs PLACEBO was 3.0 ± 0.2 vs. 2.8 ± 0.2 $p > 0.05$, day 14 2.9 ± 0.1 vs. 2.4 ± 0.2 $p < 0.05$ and weaning 2.8 ± 0.2 vs. 2.3 ± 0.2 $p > 0.05$. Shoulder sores appeared sooner for PLACEBO than NSAID sows (6.0 ± 0.5 vs. 4.4 ± 0.5 days, $p < 0.05$). NSAID sows showed less constipation days than PLACEBO sows (5.7 ± 0.2 vs. 6.5 ± 0.3 $p < 0.05$).

Ketoprofen treated sows maintained good appetite longer and their BCS tended to be higher until weaning than placebo treated sows. Ketoprofen treatment delayed shoulder sore appearance and reduced the number of constipation days.

This abstract was presented as a poster at the 46th Conference of International Society for Applied Ethology 2012 in Vienna.





ANIWEL Seminar 2012



ANIWEL SEMINAR 2012

ANIWEL

Helsinki, Viikki, Viikinkaari 11

4-5.10.2012



1 ects (2 ects with presentation)

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| Thu 4.10. Info center 511 | | |
| 9.30 | Coffee | |
| 9.45 | Opening | What is ANIWEL? Director of ANIWEL Graduate school Outi Vainio |
| 10-11 | Plenary +discussion 1h | Designing clinical trials so that the publication process runs smoothly, Editor of Livestock Science Dominiek Maes |
| Break 15min | | |
| 11.15-12.15 | Plenary +discussion 1h | University portfolio in nutshell, Mia Ruohoniemi Faculty of Veterinary Medicine |
| Lunch break 1h | | |
| Info center Hall 4 | | |
| 13.15-14.45 | Student presentations 6 á 15 min | Mari Palviainen Vivi Deckwirth Karoliina Autio Ingela Wikman Stefan Kothe Jinhyeon Yun |
| 14.45-15.00 Break & posters | | |
| 15.00-16.30 | Workshop | Discussions about methods by students, Marja Raekallio |
| 17-20 | Social program Aleksanterink. 7 | Think corner, possibility to attend panel discussion "Ekologinen intiaani" or enjoy wine |
| Fri 5.10. Info center Hall 4 | | |
| 8.45-10.15 | Plenary +discussion 1.5h | Which test should I use? Introduction to statistics, Jari Metsämuuronen |
| Break 15min | | |
| 10.30-11.00 | Plenary +discussion 30min | Storing, sharing and managing data, Viikki campus library, R. Iivonen / M. E. Kuusniemi |
| 11.00-12.30 | Plenary +discussion 1.5h | The Research of Departments in Veterinary Faculty L. Hänninen, M-L Hänninen, S. Sankari, T. Taira, |
| Lunch break 1h | | |
| 13.30-15.30 | Workshop 2h | Psychological perspectives to the process of writing an article, Maaret Wager |
| 15.30-16.00 | Award ceremony | |