

## Concurrent Session 1

### Clinical Immunology/Immunopathology

Chairs: Eliane Marti, Miroslav Toman

Room 242

Tuesday, September 5<sup>th</sup>  
11.00-12.30

**CSI-01 Cytokine and chemokine gene expression in canine and feline immune-mediated disease**

Day M.J.

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Key to the pathogenesis of immune-mediated disease are the cytokines and chemokines which regulate the nature and tissue localization of aberrant immune responses. Investigation of these mediators in dogs and cats has been hampered by lack of specific reagents, and only recently have relevant antisera become available. Because of this, studies of cytokines and chemokines in these species have largely been undertaken by measurement of gene transcription within tissue or cultured cells. Early investigations employing RT-PCR with semi-quantitative, gel-based read-out have given way to the application of quantitative, real-time RT-PCR methodology. This presentation reviews studies conducted by the author's laboratory that apply these methods to investigation of spontaneously arising canine and feline immune-mediated diseases. Two selected examples are briefly summarized in this abstract:

**CANINE INFLAMMATORY ENTEROPATHIES**

Investigations of mRNA expression in duodenal biopsies collected from dogs with inflammatory enteropathy show no difference in the level of any transcript (IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-18, IFN $\gamma$ , TNF $\alpha$ , TGF $\beta$ ) compared to controls. There was no correlation between cytokine mRNA and histopathological severity or type of inflammation.

**CANINE EOSINOPHILIC BRONCHOPNEUMOPATHY**

EBP is an asthma-like disease characterized by mucosal infiltration of eosinophils and CD4+ TCR $\alpha\beta$ + cells. Expression of mRNA encoding IL-4, IL-5, IL-6, IL-10, IL-12p40, IL-13, IL-18, IFN $\gamma$ , TNF $\alpha$ , TGF $\beta$ , eotaxin-2, eotaxin-3, MCP-1, MCP-2, MCP-3, MCP-4, RANTES and CCR3 has been examined. There were no significant differences between EBP and control biopsies with respect to cytokines, but EBP samples had greater expression of eotaxin-2 and -3 and MCP-3 genes, and less expression of mRNA encoding RANTES.

**CSI-02 Susceptibility to canine diabetes mellitus is associated with MHC class II polymorphism**

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Despite the relatively common occurrence of insulin-dependent diabetes mellitus (IDDM) in dogs, very little is known of its aetiology and pathogenesis. IDDM occurs spontaneously in dogs and is thought to have an autoimmune component and to be a model of human latent autoimmune diabetes of adults (LADA). Some dog breeds (e.g. Samoyed) are particularly predisposed, whereas others (e.g. Boxer) are highly resistant. Susceptibility to Type I diabetes in man strongly associated with genes in the Major Histocompatibility Complex (MHC), particularly the MHC class II polymorphisms. A cohort of 529 case and 1047 control dogs were genotyped for canine MHC (DLA) class II genes, using sequence based typing. Three DLA haplotypes DRB1\*009/DQA1\*001/DQB1\*008, DRB1\*015/DQA1\*0061/DQB1\*023 and DRB1\*002/DQA1\*009/DQB1\*001, were found at significantly increased frequency in diabetic cases compared to controls. One DLA-DQ haplotype, DQA1\*004/DQB1\*013, was significantly reduced in diabetic cases. Further analysis revealed that DQA1 alleles carrying an arginine at codon 55 in exon 2 of the DQA1 chain were at increased frequency in diabetic dogs. These results add further evidence that canine IDDM

may share a similar aetiology and pathogenesis to the human disease. To our knowledge this is the first report of a comparative study of MHC and diabetes in a non-rodent species. Since no laboratory model of LADA exists, and dogs and humans share similar environments, further research into canine diabetes is warranted.

### **Identification of novel autoantigens using a proteomic approach**

Deeg C.A.\*, Pompetzki D., Raith A., Hauck S., Amann B., Goebel T., Stangassinger M., Ueffing M., Kaspers B.

CSI-03

The goal of our work was the identification of autoantigens involved in a spontaneously occurring autoimmune disease, including as of yet undetected, novel autoantigens. This is relevant because the development, progression and recurrence of autoimmune diseases are frequently driven by a group of participatory autoantigens. We identified and characterized novel autoantigens by analyzing the autoantibody binding pattern from horses affected by spontaneous recurrent uveitis (ERU) to the retinal proteome. The detected proteins by ERU sera were identified by mass spectrometry. As expected, marked positive reactions to the well-characterized autoantigens S-antigen and Interphotoreceptor-retinoid binding protein were detected. Two additional proteins were selectively bound by autoantibodies of ERU cases (8/35), which we identified as recoverin and cellular retinaldehyde binding protein (cRALBP). The uveitogenicity of recoverin had been already demonstrated in the Lewis rat. Since cRALBP is a novel autoantigen not previously described, we further characterized this protein as autoantigen. In a large scale screening, 29% of ERU cases (84/289) demonstrated a positive autoantibody reaction to cRALBP. Lymphocytes from six out of 20 horses with spontaneous uveitis showed a positive reaction to in vitro stimulation with human cRALBP. Additionally, we tested the uveitogenic potential of cRALBP on Lewis rats and unaffected horses and compared the ensuing pathology to spontaneous ERU cases. cRALBP induced uveitis with an incidence of 89% in Lewis rats and a 100% in horses. Our findings suggest that the proteomic approach for autoantibody profiling of various autoimmune diseases is a convenient and effective tool for detecting autoantigens. These results are significant for understanding the pathogenesis underlying autoimmune diseases, as well as the discovery of pre-clinical disease markers. This work was supported by DFG grants DE 719/1-5 and SFB 571.

### **Effects of somatic cloning on the immune response in cattle**

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CSI-04

Somatic cloning in cattle is associated with heavy perinatal loss. In our laboratory, about 25% of the calves die before 2 months of age. We reported earlier on thymic atrophy resulting from nuclear transfer (Chavatte-Palmer et al., Cloning and Stem Cells 2004;6:92) and have further diagnosed pathological events occurring in clones including death due to benign infections and thymic atrophy (Renard et al., The Lancet 1999;353:1489). This led us to investigate the immune function of apparently normal bovine clones.

First, 17 bovine clones (NT) aged 15 days to 5 years and originated from 4 different genotypes, and 17 contemporary controls (C) were used. They were allotted to one of 3 groups: 1 (< 2 mo, N = 4NT and 6C), 2 (3-9 mo, N = 7NT and 5) and 3 (1.5-5 yr, N = 6NT and 6C). PMBCs were collected, labeled with leukocyte subset markers (CD2, CD3, CD4, CD8, CD14, CD11b, CD25, CD45RO, P46,  $\gamma\delta$ , PanB, MHC1, MHC2) and analyzed by 2 or 3-color flowcytometry. In a second part, 6 NT and 6 C aged 8-9 mo were vaccinated with 10 mg ovalbumin in alum to compare antibody and cellular responses.

Cell subset proportions were not different between NT and C groups of all age classes. There was

no difference between groups for antibody response to vaccination, but Tcell restimulation with ovalbumin after immunization and non-specific stimulation with PHA was significantly lower in clones ( $P < 0.05$ ). These results show that leukocyte populations (lymphocyte and myeloid subsets) are normally represented in apparently healthy clones but that clones may have a reduced capacity to mount an immune response against newly encountered antigens like ovalbumin. Abnormal immune function may be a long term effect of cloning and the factors involved need to be investigated.

### **CS1-05 The role of T cells in the development of insect bite hypersensitivity in Icelandic horses**

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Insect bite hypersensitivity (IBH) is summer recurrent allergic skin disease, affecting horses hypersensitive to bites of midges and black flies. IBH is frequent in Icelandic horses exported to mainland Europe, it does not occur in Iceland due to absence of midges. Icelandic horses exported as adults to mainland Europe (1<sup>st</sup> generation) have a > 50% incidence of IBH. In contrast, their offsprings (2<sup>nd</sup> generation) born and living in mainland Europe have a < 10% incidence, although being also exposed to the allergen. We determined whether PBMC of healthy and allergic 1st and 2nd generation horses stimulated ex vivo with allergen extract or polyclonally show a bias towards a Th1 or Th2 reaction. Stimulation was performed either in summer (allergen exposure), or winter (allergen-free interval). The proportion of cells producing interferon- $\gamma$  (IFN- $\gamma$ ; Th1 cytokine) or IL-4 (Th2 cytokine) was measured by flow cytometry. Cytokine expression was also assessed by quantitative real-time RT-PCR (IFN- $\gamma$ , IL-4, IL-5 and IL-13). Both IBH and origin of birth (Iceland) were associated with a Th2-dominated reaction, as revealed by polyclonal or allergen-specific stimulation, but not by stimulation with an irrelevant antigen. This difference was seen in summer (allergen-exposure) but far less clearly in winter. The observation that 2<sup>nd</sup> generation horses showed a suppression of the IL-4 production was consistent with higher levels of IL-10 in 24 h culture supernatants from these horses. Overall, the study shows that the IgE-mediated allergy is associated with a bias towards a Th2 over a Th1 response and points to regulation of IL-4 production in the 2<sup>nd</sup> generation horses, despite allergen exposure.

### **CS1-06 Identifying the genetic basis for fell pony syndrome**

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Fell ponies are a rare breed of horse mainly found in the upland areas of the UK. Fell pony syndrome is a severe immunodeficiency which leads to the death of all affected animals. It is a disease which becomes apparent 4-8 weeks after birth with clinical signs indicative of non-regenerative anaemia and severe immunodeficiency. Laboratory studies have shown that the anaemia is profound (PCV < 5) and not autoimmune. The immunodeficiency is due to an absence of mature B cells with a consequent lack of production of immunoglobulins (all classes) into the circulation. Foals succumb to infections soon after the maternally-derived antibody disappears from their circulation and die within 2 weeks. All the evidence from family and stud book analyses has indicated that the syndrome is an autosomal recessive disease. It is considered that there is a very high carrier rate (greater than 50%) in the Fell pony population in the UK and that circa 10% of foals born each year are affected and die. A panel of microsatellite markers was used to map the position of the gene involved. A homozygosity mapping approach was taken, which detects the disea-

se locus by virtue of the fact that the chromosome region will preferentially be homozygous by descent in affected individuals (Lander and Botstein, 1987, Science 236 1567-1570). We have identified two adjacent markers which display this preferential homozygosity in affected foals, and indicate a chromosome region with several obvious candidate genes whose actions are crucial in both the immune response and haemopoiesis. These exciting leads are currently being investigated, and up to date results will be presented. The identification of the genetic alteration causing this disease will enable a carrier test to be developed. This will allow the elimination of the disease from the Fell pony population by avoiding carrier-carrier breeding.

### **Lymphocyte activity and subsets distribution in selected diseases in dogs**

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Changed blood lymphocyte counts and activity have been described in association with canine diseases, e.g. in German shepherd deep pyoderma, systemic lupus erythematosus, leishmaniasis etc. They document immunosuppression concurrent to infectious diseases, or on the other hand they characterize secondary immunodeficiency that may facilitate subsequent infection. These parameters are of course essential for characterization of primary immunodeficiency or leukemias. The aim of the present study was to summarize existing knowledge and to add our experience including methods of assessment of minority lymphocyte subpopulations (NK cells,  $\gamma\delta$ T lymphocytes, double-positive lymphocytes). We detected a considerable immunosuppression in bitches affected by pyometra primarily characterized by decreased lymphocyte activity, whereas changes in respective lymphocyte subpopulation ratios were not marked. After hysterectomy with antibiotic treatment, the ratios soon returned to normal without necessity to stimulate the immune system. We confirmed that immunosuppression is concurrent to German shepherd deep pyoderma. Besides the known facts concerning decreased lymphocyte activity and changed ratios of lymphocyte subpopulations CD4+, CD8+ and CD21+, we detected changes in CD3-CD8+ (NK) cells, also in healthy German shepherds. Lymphocyte activity and subset distribution changes were less marked in the other dog breeds affected by deep pyoderma. Immunosuppression manifested by decreased lymphocyte activity was also concurrent to chronic renal failure and its extent was related to the degree of renal disease. Diagnostic relevance of changes in lymphocyte counts and activity is discussed as well as the potential use of these methods in clinical practice. Supported by grant MZE 0002716201.

CSI-07

### **Effects of two different surgical approaches on cellular stress response in cows with left abomasal displacement**

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Introduction: Various cytokines mediate the inflammatory reaction of the body during surgical interventions. Following invasive abdominal surgery the cytokines Interleukin-1 (IL-1) and Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) are the first that are released from the traumatized tissues. Subsequently, these cytokines stimulate the production of further cytokines and by this means diminishes surgery-induced stress. Alterations in the cytokine pattern after surgery can generally point on possibly arising post-surgical complications. The Heat Shock proteins (Hsp) are useful indicators for cellular stress, since they maintain cellular homeostasis, protect the cells from different stress stimuli and exert influence on the immune system. In case of additional stress, patients who underwent invasive surgery are not able to elicit an adequate Hsp70-response, resulting in a disturbed immune defense and increased infection rate. The present study investigates the cytokine expression and cellular stress response following laparoscopic and laparotomic surgery

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on cows with abomasal displacement. Methods: Cows admitted to the clinic for cattle diseases with abomasal displacement were included in this study. Surgery was carried out either by omentopexy (Hannover method) or laparoscopy (Janowitz method). Samples of venous blood were drawn from each cow, immediately before and after surgery as well as 2, 4, 6, 24 and 48 hours later, respectively. Different expressions of Hsp70 and selected cytokines (IL-1, IL-2, IL-4, IL-6, IL-10, TNF- $\alpha$ ) were calculated using Real Time-PCR. Conclusion: Comparison between laparoscopic and laparotomic techniques showed significant differences in cytokine and Hsp gene-expression, indicating increased cell.

CSI-09

### **Establishing of a bovine liver cell system as model for fatty liver degeneration in cows**

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Introduction: The period around calving forms the most vulnerable time in the life of a dairy cow, characterized by an increased prevalence of metabolic and infectious diseases. Most of these disorders originate from defective liver function due to disturbances of its glucose, fat and protein metabolism or an insufficient generation of an inflammatory response. However, the mechanisms underlying the defective liver function in dairy cows are not completely understood yet, mainly due to the inaccessibility of the bovine liver in vivo. Methods: A part of the liver from a freshly slaughtered cow is used in a perfusion system to isolate and culture primary bovine hepatocytes for in vitro studies on the effect of systemic stimuli or drugs which specifically strain or alleviate the cellular stress response of liver cells. Before and after stimulation samples of the cells were taken, from which total RNA is isolated and transcribed into cDNA. Different expression of Hsp70 and IL-1 $\beta$ , IL-6, IL-10, INF- $\gamma$  and TNF- $\alpha$  is calculated against house-keeping genes using Real Time-PCR. Results: The new established liver perfusion technique delivered viable hepatocytes which survived in culture for two weeks. After being exposed to different stimuli (heat, NEFA's, keton bodies) the hepatocyte RNA was isolated for further analysis. Conclusion: By using the in vitro-experiments on bovine hepatocytes we expect to gain insights into the genetic and molecular basis of the cellular stress response in the bovine liver and to contribute to the reduction of animal experiments.

CSI-10

### **Association of canine hypothyroidism with a common Major Histocompatibility Complex DLA class II allele**

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The Major Histocompatibility Complex class II genes are central to the regulation of adaptive immunity including response to vaccination, infection, autoimmunity and susceptibility to malignancies in all species studied to date. Variation in immunity correlates strongly with polymorphisms observed in these genes. The dog is a major companion animal and suffers from a range of immune mediated conditions including a lymphocytic thyroiditis which has many similarities to Hashimoto's thyroiditis in man. We have recently reported an association in Doberman

Pinschers between canine hypothyroidism and a rare DLA class II haplotype, that contains the DLA-DQA1\*00101 allele. We now report a further series of 173 hypothyroid dogs in a range of breeds where a significant association with DLA-DQA1\*00101 is shown. Several breeds that do not normally carry this DLA allele within their populations are not represented in our disease group. Thus breed susceptibility may be related to the frequency of DLA-DQA1\*00101 within each breed.

### **Treatment of fibrosarcomas in dogs by local application of recombinant human IL-2 – a clinical approach – results after 5 years**

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Fibrosarcomas are malignant tumours occurring in several animals, especially dogs and cats. The tumours rarely metastasize in dogs but recidives occur frequently. If post-surgery histo-pathological examination reveals tumour cells at the border of the excision, recidives are almost inevitable. Interleukin 2 (IL-2) has been approved as systemic drug against very aggressive tumours in humans. The rate of severe side effects in systemic application, however, is very high. The local application of IL-2 has been evaluated in human medicine – an approach that allows a higher local concentration without any severe side effects. In this study, the use of local IL-2 treatment against minimal rest tumours was evaluated, using recombinant human IL-2 in cases of dog fibrosarcomas. Since 1999, 10 animals were treated locally post operationem several times at growing intervals with IL-2. In four cases, recidives were palpable prior to onset of treatment. In four cases, histopathological examination of the tumours indicated the existence of tumour rest cells at the site of surgery. Animals were monitored for 12-60 months. Five animals had to be euthanized due to another disease 6-48 months after therapy. The minimal tumour recidive that was treated in this case had undergone complete regression by that time. Only in one case a recidive was seen and this underwent complete regression after another treatment with IL-2. All animals are clinically healthy and tumour free up to date. Accordingly, IL-2 is a most valuable support in the therapy of fibrosarcomas in dogs.

CSI-11

### **Autoantigen identification in canine DCM**

Buse C.B.\*, Hauck S.M., Schoeffmann S., Stangassinger M., Deeg C.A.

Dilated cardiomyopathy (DCM) is a frequent spontaneous disease in large dogs which often leads to sudden death. In most cases, the aetiology of the disease is unknown. DCM is also known as a severe, life-threatening heart disease in humans. DCM is speculated to be caused by autoimmune reactions to myocardial proteins in a certain percentage of DCM cases in man. In our work we analysed the autoimmune responses of dogs with DCM to muscle-derived autoantigens. For this purpose we separated six tissue proteomes (skeletal muscle, smooth muscle, left and right atrial and ventricular cardiac muscle, respectively) by 2D-gel electrophoresis and transferred it on Western blot membranes. On these blots we screened the autoantigen detection pattern of sera from 61 DCM dogs and 31 controls. Autoantibody binding was visualized by an anti-dog-IgG-POD antibody and ECL detection. Comparison of the antibody binding profile from DCM dogs versus healthy controls revealed that the sera of DCM dogs bound to more proteins than the sera of controls in all studied tissues (heart muscle, skeletal muscle and smooth muscle). Differential analysis of diseased versus control binding patterns produced a set of proteins selectively bound by canine DCM cases. These proteins were subsequently identified by mass spectrometry (MALDI-TOF-TOF). The results were then verified using purified candidate proteins in indirect

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enzyme linked immuosorbent assays (ELISA). Two-dimensional Western blotting combined with mass spectrometry enabled us to identify possible autoantigens playing a role in the pathogenesis of DCM in dogs. The identification of a large percentage of autoantibody positive canine DCM cases points to a crucial role of autoimmune reactions in these cases.

### **CSI-13 Antirabies vaccine protection in 2, 4 and 6 month old calf born from antirabies non vaccinated, prime vaccinated and revaccinated females**

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One of the measures for the large animals rabies control is the antirabies vaccine indicated for animals 3 month old or more. Considering the interference of colostral antibodies in antirabies vaccination the aim of this work was to evaluate the protection induced by antirabies vaccine in 2, 4 and 6 month old calf born from rabies non vaccinated, first vaccinated and revaccinated females. Serum neutralizing antibodies titers were evaluated in females and calves 24 h after the birth as well as in calves 15 days after the first vaccine and the booster vaccine and, also, monthly during a period of 12 months. Titers of colostral antibodies were higher in calves born from revaccinated females at the first month of the birth, gradually decreasing presenting at the 4th month median titers superior to 0.5 UI/mL. Greater percentage of animals considered to be protected (77.8%) 15 days after the first vaccine was observed in 2m old vaccinated calves born from nonvaccinated females. After the booster 100, 50 and 90%, respectively, of protection was obtained from non vaccinated, vaccinated and revaccinated females. The range of protection was 0 to 33.3% after the first vaccine in 4 m old and 40 to 50% in 6 m old vaccinated calves but this percentage was 100% for all groups 15 days after the booster vaccination. No significant difference was observed after the booster for all groups independently of the maternal status. The results demonstrated the viability of antirabies vaccine in calves 2 m old but reinforce the need of antirabies booster vaccination for a effective protection. Financial support by FAPESP.

### **CSI-14 Effector cells in equine allergy – Blood derived monocytes participate in IgE driven immune responses**

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Insect bite hypersensitivity (IBH) in horses is widely accepted as a type I allergy requiring the generation of allergen-specific IgE. Among other cell types, monocytes and their differentiated forms can modulate this adaptive immune response. Here we investigated the phenotypic endowment of PBL with the relevant, allergy-mediating isotype, IgE, as sensitized in vivo. Furthermore, the horses were functionally examined towards their atopic status (IBH affected or not) in means of an ex vivo histamine liberation assay (HRT). Of all tested horses (n = 21), nine horses showed reactivity towards insect allergens (i.e. dose dependent histamine release after allergen challenge). Regardless of their atopic status, all horses revealed a similar histamine release after incubation with anti IgE antibodies. For this, the expression of FcεRI on basophils and mast cells is obligatory. In horses the overall percentage of IgE positive cells from atopic donors was not different to healthy horses (1.39 ± 0.36%). Flow cytometrically, IgE+ cells were found in all leukocyte populations, with 0.43 ± 0.43% for PMN, 9.03 ± 3.52% for monocytes and 0.26 ± 0.25% for other MNC, respectively. In contrast to humans, the only detectable leukocyte population showing a constitutive endowment with IgE are monocytes. Furthermore, no difference between allergic and healthy horses could be shown, though up to 70% expression of FcεRI on monocytes from human

atopic donors can be found – but almost no binding of IgE in vivo. These striking differences between horses and men suggest that the contribution of monocytes to equine allergy is more evident.

### **Multiplex xMAP™-technology as a tool for analysis of bovine markers of inflammation and infection**

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In clinical cases of calf diarrhoea or respiratory problems an indication on the temporal phase of the inflammatory response and the origin of infection can be of great importance. The xMAP™-technique could be used to achieve such information. Proinflammatory cytokines, e.g. IL-1 $\beta$ , activates the immune system and are mainly produced in early stages of inflammation. They induce production of acute phase proteins, e.g. serum albumin A (SAA), which have more long lasting effects in the inflammatory response than cytokines. By measuring IL-1 $\beta$  and SAA a time perspective of the inflammation is gained. To determine if the invading pathogen is of viral or bacterial (Gram-negative) origin IFN- $\alpha$  and lipopolysaccharide (LPS) can be used as markers. We have evaluated the xMAP™-technique for simultaneous detection of bovine IL-1 $\beta$ , SAA, IFN- $\alpha$  and LPS. Antibodies against the analytes were coupled to microspheres with different fluorescent signatures. In order to decide the amount of analyte, a fluorophore-conjugated antibody was added to the samples. Singleplex xMAP™-assays performed in buffer provided detection limits (DL) of 80 pg/mL, 4 ng/mL, 10 pg/mL and 1.5 ng/mL for IL-1 $\beta$ , SAA, IFN- $\alpha$  and LPS, respectively. Multiplex xMAP™-assays performed in buffer showed that the DLs of IL-1 $\beta$ , SAA and IFN- $\alpha$  remained stable, while the DL of LPS was elevated compared to the singleplex assay. Preliminary studies on clinical samples showed that IFN- $\alpha$  and IL-1 $\beta$  could be detected in lung fluid from calves experimentally infected with bovine respiratory syncytial virus. More studies on clinical samples will be performed to further evaluate the method.

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### **Rapid kit for the complement fixation test to detect antibodies induced by rough-type *Brucella* spp. preliminary results**

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This kit is suitable for rapid detection in animal and human sera of complement-fixating antibodies induced by *Brucella* rough strains, such as *B. abortus* RB51 or *B. ovis*. *Brucella* rough strains can not be used as antigens for Complement Fixation Test (CFT) because of the strong anti-complementary activity. This kit uses the RB51/AC antigen from *B. abortus* RB51, deprived of anti-complementary activity. The kit provides: guinea-pig complement, hemolysin stock solution, sheep erythrocytes, RB51/AC antigen, bovine anti-R serum, bovine negative serum. Kit reagents can be used at working dilutions, indicated on the label, without preliminary testing and they can be stored for a long time. The efficiency of this kit was evaluated by testing 20 cattle vaccinated with one dose of RB51 vaccine and 10 buffaloes vaccinated with one or two doses of RB51. The CFT results were compared with the vaccination status of animals and the concordance, specificity, sensitivity, positive and negative predictive values of the kit were determined. Eight naturally *B. ovis*-infected sheep were also tested. Following results were obtained: Concordance: cattle 96.7%; buffaloes 93.7% to 100%; sheep 100%. Sensitivity: cattle 95.0%; buffaloes 90% to 100%; sheep 100%. Specificity: cattle 100%; buffaloes 100%; sheep 87.5%. Positive predictive value: cattle 100%, buffaloes 100%; sheep 88.9%. Negative predictive value: cattle 90.9%; buffaloes

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85.7% to 100%; sheep 100%. These data indicate that this kit is able to detect RB51-vaccinated cattle and buffaloes and *B. ovis*-infected sheep with high specificity and sensitivity. It can be used to identify RB51-vaccinated animals according to the European Community Council Decision (15 July 2002).

### CSI-17 **The association of CTLA4 to canine diabetes: a breed-based analysis**

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Cytotoxic T-lymphocyte-associated protein 4 (CTLA4) encodes a protein which transmits an inhibitory signal to T-cells and thus represents an important regulatory molecule controlling T-cell proliferation. Mutations in this gene have been associated with a number of diseases including insulin-dependent diabetes mellitus, also known as Type 1 diabetes (T1D). T1D is a complex genetic disease in which the insulin-producing  $\beta$ -cells of the pancreas are progressively destroyed, leading to insulin deficiency. This cellular destruction is preceded by pancreatic infiltration of T-lymphocytes. Canine diabetes presents and is treated in the same way as human T1D. It affects around 0.5% of the canine population, with some breeds showing marked susceptibility (Samoyed, odds ratio = 17.3) while others do not present with the disease (Boxer, odds ratio = 0.07). We genotyped a diabetic cohort (n = 489) containing 20 pedigree breeds and a group of crossbreeds, for 15 single nucleotide polymorphisms (SNPs) in the CTLA4 gene. All cases had breed-matched controls and entire females were excluded from the dataset. Control populations were checked for Hardy-Weinberg compliance. Allele frequencies were compared between controls and cases using  $\chi^2$ , and haplotype analysis using an association score test. We found an association between a rare haplotype showing a significant association to disease status. This haplotype was not found in the controls, hence, could be indicative of susceptibility to T1D.

### CSI-18 **TNF $\alpha$ and HMGB1: Two cytokines with different roles in canine sepsis**

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The immunopathology of sepsis is characterized by a systemic inflammatory response mediated by an early release of tumor necrosis factor alpha (TNF $\alpha$ ). In contrary to TNF $\alpha$  the recently identified cytokine High Mobility Group Box 1 (HMGB1) is supposed to be a late mediator. To examine the role of these two cytokines in canine sepsis we established novel sensitive detection systems and tested two commercially available substances for the neutralization of canine TNF $\alpha$  in vitro. Cloning and expression of canine TNF $\alpha$  and the TNF $\alpha$ -R1 (P60) was performed in eukaryotic expression systems. The bioactivities of rcanTNF $\alpha$  and P60 were determined by the ability of the P60 to neutralize the cytotoxic effect of rcanTNF $\alpha$  in the WEHI bioassay. Using the P60 as capture reagent combined with a commercially available anti-canine TNF $\alpha$  biotin conjugate a novel sensitive ELISA could be established. To examine possible crossreactive activities of Infliximab and Etanercept, their ability to neutralize canTNF $\alpha$  was tested in the WEHI bioassay. Thus we could show that Etanercept but not Infliximab can neutralize canTNF $\alpha$ . We next collected plasma from 80 dogs with diagnosed sepsis and quantified TNF $\alpha$ . Interestingly the TNF $\alpha$  levels were not elevated in these dogs. For this reason we furthermore established a Western-blot

to detect HMGB1 using a polyclonal antiserum raised against procaryotically expressed recombinant HMGB1. HMGB1 could be detected as opposed to negative control sera. In conclusion, the established assays are important tools to characterize the immunopathogenesis of canine sepsis and will lead to improved diagnosis and therapy.

### **Effect of surgical therapy of pyometra on immunological parameters in bitches**

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The aim of this study was to test if surgical therapy of pyometra leads to improvement of altered immunological parameters in bitches. Thirteen bitches with pyometra confirmed by ultrasonography (experimental animals) as well as 12 healthy bitches (controls) were used in the study. Blood count and *in vitro* lymphocyte activity measured by the lymphocyte transformation test were evaluated in the bitches. Ovariohysterectomy in combination with administration of antibiotics (Synulox RTU a.u.v. inj., Pfizer, 15 mg/kg b.w.) was performed in experimental animals and the examination was repeated in these animals on day 7 after surgery. Leukocytosis and neutrophilia (7 bitches) as well as leucopenia (2 bitches) were detected in experimental animals before therapy. Independently on number of leukocytes, activity of lymphocytes in experimental animals before therapy was lower compared to controls: PHA 40 (2087 ± 2333 cpm vs. 7165 ± 3339 cpm,  $p < 0.05$ ); PWM 10 (1126 ± 1007 cpm vs. 4055 ± 2331 cpm,  $p < 0.05$ ); ConA 10 (2077 ± 1873 cpm vs. 10255 ± 6399 cpm,  $p < 0.01$ ) and ConA 2.5 (2877 ± 2744 cpm vs. 10781 ± 4399 cpm,  $p < 0.01$ ). The parameters were not different in experimental animals compared to controls on day 7 after therapy. The results showed improvement of immunological parameters in bitches with pyometra within 7 days after surgical therapy.

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### **Cytokine mRNA expression in skin biopsies of horses affected with insect bite hypersensitivity**

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Insect bite hypersensitivity (IBH) is an allergic dermatosis of the horse, caused by bites of insects (*Culicoides* spp.). Various studies have demonstrated that IgE-mediated reactions are often involved in IBH. However, some studies suggest that type IV hypersensitivity reactions are also involved in the pathogenesis of IBH. The purpose of our study was to investigate the cytokine micro-milieu in IBH lesions by comparing the mRNA expression of Th1 and Th2 cytokines in skin biopsies of acute and chronic IBH lesions with skin biopsies from healthy horses. Expression of the Th2 cytokines IL-5, IL-4, IL-13 and of the Th1 cytokine IFN- $\gamma$  was determined by quantitative real time RT-PCR in punch biopsies from lesional skin of 9 IBH affected horses during the summer. Based on histopathological findings, IBH-biopsies were subdivided into acute (N = 12) or into chronic lesions (N = 6). Seventeen skin biopsies from 10 horses without IBH served as controls. Samples were normalised to 18s rRNA. Acute IBH-lesions contained significantly more IL-4, IL-5, and IL-13 mRNA than healthy skin. Furthermore, acute lesions contained significantly more IL-5 and IL-13 mRNA than chronic IBH-lesions. Chronic IBH-lesions contained more IL-4 ( $p = 0.06$ ) than healthy skin. For the other cytokines there was no significant difference between chronic lesions and healthy skin. There were no significant differences for IFN- $\gamma$  mRNA expression between all three groups of skin biopsies. These results indicate that Th2 cytokines

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play an important role in the pathogenesis of IBH. Even in chronic IBH-lesions, there is no indication of a Th1-bias, as has been described in chronic atopic dermatitis in human patients.

### **CS1-21 UK DNA archive for companion animals**

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Many immunological disorders have a genetic basis. To perform genetic analysis of disease in animal populations, it is necessary to screen large numbers of healthy and diseased animals. This may present a problem for individual research groups as locally, case numbers can be low. By creating a national archive, it is more likely that sufficient samples for each disease will accrue quickly. Genetic research into complex conditions will only proceed effectively if DNA samples are available from animals where their disease has been accurately diagnosed and documented. The UK DNA Archive for Companion Animals ([http://pcwww.liv.ac.uk/DNA\\_Archive\\_for\\_Companion\\_Animals/](http://pcwww.liv.ac.uk/DNA_Archive_for_Companion_Animals/)) was formed in 2003 as a collaboration between the Veterinary Faculty's at the Universities of Liverpool, Bristol, Cambridge, London, Glasgow and Edinburgh and the University of Manchester. This provides a biological resource to assist veterinary research scientists' study the genetic basis of a wide range of diseases (many of which are immune mediated) in dogs, cats and horses. Samples are sent to Manchester where staff are co-ordinating this venture and banking extracted DNA. Samples will only be banked with the animal owner's permission and written informed consent. DNA is only released to bona fide investigators following an application and assessment procedure and proof of ethical approval for the intended study. Samples are presently being collected on 22 canine, 3 equine and 4 feline diseases. All samples are processed under ISO 9001/2000 quality standards and stored at -800 °C as normalised DNA in 2D bar-coded tubes. The archive has nearly 6000 samples to date, providing a valuable tool for veterinary scientific research. We invite all interested parties to submit samples or to request DNA for their own research

### **CS1-22 Expression of MHC class I in the placenta of bovine somatic clones**

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A major limitation for the development of somatic cloning in cattle is the low efficiency for producing live offspring since, with 5% to 25% of the reconstructed embryos reaching full-term development, with variation between the genotypes. Hill et al. (Biol Reprod 2002; 67:55-63) reported that early foetal losses (1<sup>st</sup> trimester) were due to inappropriate expression of trophoblast Major Histocompatibility Complex class 1 (MHC1) antigens based on immunochemistry results obtained with one antibody, on the placenta of clones originating from only one genotype. This abnormal expression was present in all clones, with more cells expressing MHC1 in clones with growth retardation. To verify this hypothesis, MHC1 expression was studied throughout gestation in bovine clone placentas originating from several genotypes.

Placentas were collected at Day32, just after implantation, Day 62, Day 180, Day 260 and term (Day 280) in clones from 3 different genotypes (N = 8) and in controls obtained by artificial insemination (N = 6). Four different monoclonal antibodies that recognize different determinants of MHC1 molecules were used (ILA-A88, IL-A19, H58A, anti-b2microglobulin). Results showed MHC1 expression in the maternal tissue but not on the fetal side, regardless of group, gestational age or fetal growth.

In conclusion, these results cast doubt over the hypothesis saying that the observed fetal losses

would be the result of an immunologic rejection due to inappropriate expression of some placental MHC1 genes. It must be explored, however, if immunological deficiencies reported in some clones after birth may be related to an abnormal expression of some non-classical MHC1 genes.

### **The efficacy of a diet rich in omega-3 fatty acids in the control of canine atopic dermatitis**

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**Introduction:** Canine atopic dermatitis (AD) is a genetically predisposed, inflammatory and allergic disorder with characteristic clinical features. It is associated most commonly with IgE antibodies to environmental allergens. Disease management focusing on factors of direct influence on the inflammatory response may include the use of n-3 fatty acids. It was our goal to study the effect of a commercial diet rich in omega-3 fatty acids on clinical signs in dogs with AD and on the occurrence of different n-3 and n-6 metabolites as potential modulators of inflammation. **Methods:** Twenty eight dogs with AD were enrolled in a randomised, controlled study, receiving either a diet rich in omega-3 (n-6: n-3 ratio of 5:1) or a control diet (n-6:n-3 ratio of 20:1) for 12 weeks. The severity of clinical signs was scored; serum, plasma and PBMC were isolated, and non-lesional and lesional skin biopsies were taken for fatty acid and eicosanoid analysis at weeks 0 and 12. **Results:** Preliminary analysis of clinical scores showed that after both diets animals improved significantly. Although improvement seemed to be stronger for one of the diets this difference was not significant. **Conclusion:** It is hypothesized that the fatty acid composition of the different samples and eicosanoid presence in serum together with the subsequent decoding of the groups may clarify the clinical results observed so far.