

## **RICERCHE EFFETTUATE**

### **ARGOMENTI VARI**

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#### **Development and validation of a liquid chromatography–tandem mass spectrometry method for the separation of conjugated and unconjugated 17(alfa)- and 17β-boldenone in urine sample**

5th International Symposium on Hormone and Veterinary Drug Residue Analysis : held at the Province of Antwerp House Antwerp, Belgium, May 16-19, 2006 : abstract book / [s.l. : s.n., 2006]. - p 95 [Nr. Estr. 3551]

International Symposium on Hormone and Veterinary Drug Residue Analysis (5th. : Province of Antwerp House Antwerp, Belgium : May 16-19, 2006)

Natural occurrence or illegal treatment of 17(alfa)- and 17(beta)-boldenone (BOLD) presence in cattle urine is under debate within the European Union. The separation of conjugated and unconjugated forms of BOLD and the presence of related molecules, as androsta-1,4-diene-3,17-dione (ADD), appear critical points for the decision of an illegal use. Up to now, the separation of unconjugated BOLD from conjugated forms has been made by chromatographic separation of the two forms or by urine samples analysis with or without a prior step of enzymatic deconjugation. In this work a new approach of BOLD and ADD confirmatory analysis in urine is described. The separation between conjugated and unconjugated forms of BOLD was obtained by a preliminary liquid-liquid extraction of urine samples with ethyl acetate. In this step the organic phase extracts only unconjugated BOLD and ADD, while BOLD in conjugated form remain in urine phase. Afterwards the urine phase, contains conjugated BOLD, was subjected to an enzymatic deconjugation. Solid phase extraction (OASIS-HLB Waters) was used for the purification and concentration of analytes in organic and urine phases and liquid chromatography ion electrospray tandem mass spectrometry (LCMS-MS) was applied for the confirmation of BOLD and ADD, using deuterium-labelled 17β-boldenone (d3-B-bold) as internal standard. The method was validated as a quantitative confirmatory method according to Commission Decision 2002/657/CE. The results obtained show good linearity, precision and accuracy. The decision limits (CC(X), obtained were 0.43 and 0.44, 0.43 and 0.44, 0.43 ng/ml for 17α-, 17β-boldenone (unconjugated and conjugated form) and ADD (unconjugated form) respectively.

Ferrari M

#### **Differentiative potential of cardiomyocyte satellite cells and possible utilization in cardiac tissue repair**

Eur J Histochemistry. - Vol. 3 ( 2006). - p 210? [Nr. Estr. 3536]

Workshop "Skeletal Muscle Fiber: Morpho-Functional Patterns and Technical Approaches " : Urbino, Italy : October 3-6th, 2006)

One possible strategy in the utilization of cell precursors in cardiac tissue damage repair concerns satellite cells. These are localised between mature myofiber basal lamina and cell membrane, on fiber surface. They are responsible of muscle tissue maintaining, as well as of its repair and regeneration. They are generally quiescent cell progenitors, but, after activation, they generate a population of myoblasts which proliferate and differentiate, to form plurinucleate myotubes. Satellite cells are different, if compared to myoblasts, for biochemical and biological characteristics. Skeletal muscle satellite cells have been also reported to have a certain plasticity and consequent differentiation capabilities directed to different cell lineages, including myocytes. These observations, together with autologous cell utilization, addressed the interest to the possible use of differentiated satellite cells in altered cardiac tissue repair. The experiments have been carried out on ovine cells, which, after in vitro amplification, have been inoculated in the same animal, undergoing an experimentally induced cardiac lesion. The results demonstrated a scarce capability to repair the

altered tissue, probably because of an impaired integration with cardiomyocytes.

Furianello T, Caldin M, Stocco A, Tudone E, Tranquillo<sup>o</sup>M, Lubas G, Solano-Gallego L

#### **Stability of stored canine plasma for hemostasis testing**

Vet Clin Pathol. - Vol. 35 no 2 ( 2006). - p 204-207 - 14 bib ref [Nr. Estr. 3424]

*A review of the literature revealed limited information about the stability of samples for coagulation testing in dogs. Objective. The aim of this study was to evaluate the stability of individual coagulation factors, clotting timer, and other parameters of hemostasis in stored canine plasma. Methods. Citrated plasma samples were obtained from 21 dogs. Prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen concentration, and factor I, II, V, VII, VIII, IX, X, XI, and XII activities were measured on an automated coagulation analyzer with commercially available reagents. Antithrombin (AT) activity and D-dimer concentration were measured on an automated chemistry analyzer using validated kits. Samples were analyzed within 1 hour after collection (initial analysis) and once daily for 2 or 4 consecutive days following storage at room temperature (RT) or 4°C, respectively. Results: Storage time at either temperature did not have any effect on PT, factor II, V, VII, X, or XII activities, D-dimer concentration, or AT activity. In contrast, aPTT was significantly prolonged after 72 and 96 hours at CC; fibrinogen concentration was decreased after 48 hours at RT; the activities of factors VIII and IX were decreased after 48, 72, and 96 hours at 4°C; and factor XI activity was decreased after 72 hours at 4°C. Conclusions: Results suggest that storage of canine plasma for 2 days at RT does not have a significant effect on hemostasis test results with the exception of a slight decrease in fibrinogen concentration. In contrast, aPTT and factors VIII, IX, and XI were unstable in refrigerated plasma after 48 or 72 hours of storage.*

Furlanello T, Tasca S, Caldin M, Carli E, Patron C, Tranquillo<sup>o</sup>M, Lubas G, Solano\_Gallego L

#### **Artifactual changes in canine blood following storage, detected using the ADVIA 120 hematology analyzer**

Vet Clin Pathol. - Vol. 35 no 1 ( 2006). - p 42-46 - 20 bib ref [Nr. Estr. 3414]

Artifactual changes in blood may occur as a consequence of delayed analysis and may complicate interpretation of CBC data. Objective: The aim of this study was to characterize artifactual changes in canine blood, due to storage, using the ADVIA 120 hematology analyzer. Methods: Blood samples were collected into EDTA from 5 clinically healthy dogs. Within 1 hour after blood sample collection and at 12, 24, 36 and 48 hours after storage of the samples at either 4°C or room temperature (-24°C), a CBC was done using the ADVIA 120 and multispecies software. A linear mixed model was used to statistically evaluate significant differences in values over time, compared with initial values. Results: The HCT and MCV were increased significantly after 12 hours of collection at both 4°C and 24°C, and continued to increase through 48 hours. The MCHC initially decreased significantly at 12-24 hours and then continued to decrease through 48 hours at both temperatures. Changes in HCT, MCV, and MCHC were greater at 24°C than at 4°C at all time points. A significant increase in MPV and a decrease in mean platelet component concentration were observed at all time points at 24°C. Samples stored at 24°C for 48 hours had significantly higher percentages of normocytic-hypochromic RBCs, and macrocytic-normochromic RBCs, and lower platelet and total WBC counts. Conclusions: Delayed analysis of canine blood samples produces artifactual changes in CBC results, mainly in RBC morphology and platelet parameters, that are readily detected using the ADVIA 120. Refrigeration of specimen, even after 24 hours of storage at room temperature, is recommended to improve the accuracy of CBC results for canine blood samples.

Rosetti M, Frasnelli<sup>o</sup>M, Tesei A, Zoli W, Conti M

**Cytotoxicity of different selective serotonin reuptake inhibitors (SSRIs) against cancer cells**

J Exp Ther Onc. - Vol. 6 ( 2006). - p 23-29. - 30 bib ref [Nr. Estr. 3537]

Cell membrane ion transporters expression and activity are altered in cancer cells and these phenotypic alterations offer potential targets for cancer therapies. Among the therapeutic agents affecting cell membrane transporters, serotonin reuptake inhibitors (SSRIs) have been shown to have anticancer potential. In this work, we have compared two SSRIs, one very specific for serotonin reuptake transporters (paroxetine) and another which also inhibit norepinephrine and dopamine transporters (venlafaxine), for their ability to counteract growth of various murine and human cancer cell lines. We found that paroxetine has cytotoxic activity against tumor cells, both of murine or human origin in the micromolar concentration range, whereas venlafaxine has not. A neurotransmitter receptor mediated mechanism of action appears thus unlikely for SSRIs cytotoxicity on cancer cells. With ranges of SSRIs cytotoxicity on cancer cells defined, limits in their possible applicability in cancer therapy is discussed.

Stacchiotti A, Borsani E, Riccia F, Lavazza<sup>o</sup>A, Rezzani R, Bianchi R, Rodella LF

**Bimoclomol ameliorates mercuric chloride nephrotoxicity through recruitment of stress proteins**

Toxicol Lett. - Vol. 166 ( 2006). - p 168-177. - 42 bib ref [Nr. Estr. 3518]

Bimoclomol (BIM), is a stress proteins coinducer, that acts synergistically with a mild stressor to activate cytoprotective stress proteins. BIM has been successfully utilized in animal models for the treatment of various nervous, cardiac and cerebrovascular diseases. Mercuric chloride (HgCl<sub>2</sub>) induces acute renal failure in rats by a single dosage. The present in vivo study was conducted to assess the efficacy of BIM against acute HgCl<sub>2</sub> nephrotoxicity. At different times after BIM and/or HgCl<sub>2</sub> exposure we evaluated renal morphology and the localization/abundance of three stress proteins (HSP72, GRP75, HSP60) by electron microscopy immunohistochemistry and Western blot analysis. BIM delivery to rats 6 h before mercury, ameliorated damage to renal ultrastructure, with recovery of tubular and mitochondrial membranes 24 h after mercury treatment. In rats pretreated with BIM prior HgCl<sub>2</sub> exposure, HSP72 was significantly overexpressed in proximal tubules in a time-dependent manner. In contrast, the amount of GRP75 and HSP60 after BIM pretreatment were comparable to the group treated with mercury alone, but these stress proteins had translocated to the nuclei at 14 and 24 h, respectively. These novel findings suggest that BIM mitigates HgCl<sub>2</sub> nephrotoxicity in rats through the early recruitment of stress proteins in midcortical proximal tubules that are the main renal mercury-targets.

Stacchiotti A, Ricci F, Rezzani R, Li\_Volti G, Borsani E, Lavazza<sup>o</sup>A, Bianchi R, Rodella LF

**Tubular stress proteins and nitric oxide synthase expression in rat kidney exposed to mercuric chloride and melatonin**

J Histochem Cytochem. - Vol. 54 no 10 ( 2006). - p 1149-1157. - 46 bib ref [Nr. Estr. 3380]

Stress proteins such as HSP70 members (HSP72 and GRP75) and metallothionein (MT) protect the kidney against oxidative damage and harmful metals, whereas inducible nitric oxide synthase (iNOS) regulates tubular functions. A single dose of mercuric chloride (HgCl<sub>2</sub>) can cause acute renal failure in rats, its main target being the proximal tubule. Oxidative damage has been proposed as one of its pathogenic mechanisms. In this study we tested whether melatonin (MEL), a powerful antioxidant compound, is effective against HgCl<sub>2</sub> nephrotoxicity. Rats were treated with saline,

HgCl<sub>2</sub> (3.5 mg/kg), MEL (5 mg/l<9), and MEL + HgCl<sub>2</sub> and examined after 24 hr for HSP72, GRP75, MT, and iNOS by immunohistochemistry and immunoblotting. Tubular effects of the treatment were then characterized by ultrastructure. In the HgCl<sub>2</sub> group, all markers were overexpressed in convoluted proximal tubules and sometimes in distal tubules. In the MEL + HgCl<sub>2</sub> group, GRP75 and iNOS decreased in convoluted and straight proximal tubules, whereas HSP72 and MT persisted more than the saline and MEL-only groups. Tubular damage and mitochondrial morphometry were improved by MEL pretreatment. In conclusion, the beneficial effect of MEL against nephrotoxicity HgCl<sub>2</sub> nephrotoxicity was outlined morphologically and by the reduction of the tubular melatonin expression of stress proteins and iNOS. These markers could represent sensitive recovery stress proteins index against mercury.

Stacchiotti A, Rodella LF, Ricci F, Rezzani R, Lavazza°A, Bianchi R

**Stress proteins expression in rat kidney and liver chronically exposed to aluminium sulphate**

Histol Histopathol. - Vol. 21 ( 2006). - p 131-140. - 64 bib ref [Nr. Estr. 3184]

Aluminium (Al) is the third most widespread metal in the environment. It is toxic for the brain, bone and haematological system but unfortunately very little data exist for other organs. Stress proteins are induced or enhanced against metal toxicity with an essential role in the recovery of organules and other cellular proteins. This immunohistochemical study was performed to analyze the distribution of three stress proteins (HSP25, HSP72, GRP75) in rat kidney and liver orally exposed to Al sulphate daily for 3 and 6 months. Al-induced alterations were further studied by histopathology (H&E, PAS, Perl's, Masson) and ultrastructural morphometry. In the kidney: HSP25 was enhanced in proximal tubules after 6 months Al-exposure when abnormal brush borders were observed; HSP72 was induced in proximal tubules only after long Al-treatment; GRP75 was raised in midcortical area sometimes within nuclei. Furthermore, lysosomal and lipofuscins densities increased in the juxtamedullary tubules after 3 months Al exposure with respect to controls. In the liver: Perl's positive deposits and fibrosis became evident after Al treatment. HSP25 was very weak; HSP72 focal in pericentral hepatocytes at 3 months and induced also in Kupffer cells at 6 months; GRP75 diffuse in periportal hepatocytes and non parenchymal cells at 6 months. Prolonged Al exposure stimulated stress proteins strictly organ-dependently in the rat. Their distribution in kidney and liver seems related to cumulative sublethal effects induced by metal and could be a sensitive index of Al susceptibility of these organs.

Tranquillo V

**Analisi del rischio: introduzione ai metodi quantitativi nella valutazione del rischio microbico**

Osservatorio. - Vol. 9 no 3 ( 2006). - p 4-11 [Nr. Estr. 3293]

Tranquillo °V

**Analisi del rischio: introduzione ai metodi quantitativi nella valutazione del rischio microbico II parte**

Osservatorio. - Vol. 9 no 6 ( 2006). - p 11-16 [Nr. Estr. 3362]

Zanardi°G

**Revisione critica dei modelli predittivi dell'afta epizootica**

Osservatorio. - Vol. 2 no 9 ( 2006). - p 10-15. - 1 bib ref [Nr. Estr. 3233]

Zanardi°G

**L'illusione della certezza**

Osservatorio. - Vol. 9 no 1 ( 2006). - p 9-12 [Nr. Estr. 3278]

Zanardi°G, Bonazza V, Cantoni R, Stefini G, Vassena G

**WebGIS: la georeferenziazione su internet**

Medicina umana, medicina veterinaria e tutela dell'ambiente: possibili sinergie in sanità pubblica : riassunti : Istituto Superiore di Sanità Roma, Perugia 12-13 Giugno 2006 / a cura di Gaia Scavia ... [et al.]. - Roma : Istituto Superiore di Sanità, 2006. - (ISTISAN congressi ; 06/C4) p 112 [Nr. Estr. 3297]

Workshop nazionale di epidemiologia veterinaria : Perugia : Perugia 12-13 Giugno 2006)

WebGIS è una tecnologia internet per la gestione del territorio, che rappresenta lo sviluppo di un applicativo informatico, denominato Geo\_ZOO, realizzato nel 2003 dalla collaborazione tra l'Osservatorio Epidemiologico Veterinario della Regione Lombardia e la Facoltà di Ingegneria di Brescia, e messo a disposizione dei servizi Veterinari delle AASSLL lombarde allo scopo di georeferenziare i siti di interesse zootecnico in ambito locale, sul proprio Personal Computer. WebGIS, evoluzione dell'applicativo Geo\_ZOO installato in locale, consente ai Servizi Veterinari delle AASSLL di accedere tramite internet al GIS regionale per consultare le georeferenziazioni esistenti, modificarle o eseguirne di nuove, utilizzando strumenti supplementari per la gestione ordinaria e straordinaria del territorio di competenza. Ad esempio, determinare le zone di protezione e sorveglianza in caso di focolaio di malattia infettiva, calcolare la distanza tra siti georeferenziati e qualsiasi elemento presente sulla mappa, valutare i percorsi viari, estrarre automaticamente il numero di allevamenti presenti e i capi allevati in una determinata zona, avere la visione complessiva degli insediamenti sul territorio e rendersi conto della situazione territoriale, in cui si verifica un determinato fenomeno. WebGIS rappresenta un utile strumento di servizio, mirato ad aggiornare e integrare il sistema informativo territoriale regionale, naturalmente collegato all'anagrafe zootecnica e suscettibile d'implementazione, sia dal punto di vista delle informazioni sanitarie sia amministrative. Per creare il WebGIS è stata utilizzata una tecnologia software open source, che non necessita di alcuna licenza relativa agli strumenti di produzione delle mappe e di utilizzo di database. I costi hanno riguardato l'attrezzatura hardware (server WEB), l'infrastruttura di rete (collegamento ad Internet e costo di connessione) e la progettazione, sviluppo e personalizzazione dell'intero sistema. I requisiti per utilizzare i servizi offerti da WebGIS sono un collegamento ad Internet e la disponibilità di un browser per la navigazione (eg Microsoft Explorer, Mozilla, Netscape, Opera, ecc.). Le informazioni su cui si basa WebGIS sono di tipo cartografico - confini amministrativi, Carta Tecnica Regionale (CTR), ortofotocarte regionali a colori, modello digitale del terreno (orografia), georeferenziazioni dei siti zootecnici - in collegamento con le anagrafi zootecniche.

Zanardi°G, Bonazza V, Stefini G, Cantoni R

**Web gis in Lombardia: un nuovo strumento a disposizione dei servizi veterinari**

Osservatorio. - Vol. 9 no 4 ( 2006). - p 4-9. bib ref 3 [Nr. Estr. 3319]

Zanardi°G, Bonazza W, Cantoni R, Stefini G, Vassena G

**WEB-GIS Lombardy region : a tool for the health veterinary services**

1st OIE international conference : use of GIS un veterinary activies : 8-11 Ottobre 2006 Silvi Marina (TE) : Abstract book / [s.l. : s.n., 2006]. - p 109 [Nr. Estr. 3371]

OIE international conference (1. : Silvi Marina (TE) : 8-11 ottobre 2006)

Zanardi°G, Parrinello°G, Tranquillo°V, Bellini °S, Decarli°A

**An epidemic simulation model of foot and mouth disease spread applied into a high density populated area of Lombardy Region (Italy)**

Innovation : reshaping veterinary epidemiology : ISVEE XI : handbook & abstracts : 6-11 August 2006, Cairns Convention Centre, Queensland, Australia / [Queensland Australia : 2006]. - p 96 [Nr. Estr. 3313]

International Symposium of International Society for Veterinary Epidemiology and Economics (ISVEE) (11th : Queensland Australia : 6-11 August 2006)

The aim of this study was to develop a simulation model for the spread of the Foot and Mouth Disease Virus in a high-risk area like the Lombardy region, which is typical for its high territorial concentration and density of intensive herds. The availability of a simulation model allows to evaluate a priori the best control strategies. We defined a 'risk potential' as a combination of a gravitational component ( interaction between herds size and their distance) on one side and, on the other side, to some known risk factors like: Susceptibility, transmissibility, latency and a random factor, 'frailty'. All risk factors were regarded as random variables, except for susceptibility, transmissibility with a suitable distribution. The probability for a herd to get infected has been calculated assuming a logistic model for the effect of the 'risk potential' on it. We generated randomly an index-case and a number of contacts among herds in the area of interest with the index-case. New cases of infection were sampled from the upper tail of the risk distribution. In the last step the model identified the first case, then applied a control basic strategy based on restriction measures to stop contacts and on depopulation of animals in protection areas. Authors show the results of the simulations, each one providing 1000 iterations of the model, run each time for an index-case chosen taking into account the main species, the herd size and the combination of these two aspects.